申报	系列：教师系列 教学科研并重型
	专业：林木遗传育种
	职称：副教授

业绩成果材料

（申报人的业绩成果材料包括论文、科研项目、获奖以及其他成果等）

单　位（二级单位）林学与风景园林学院

姓　名　　李青粉

材料核对人：　　单位盖章：

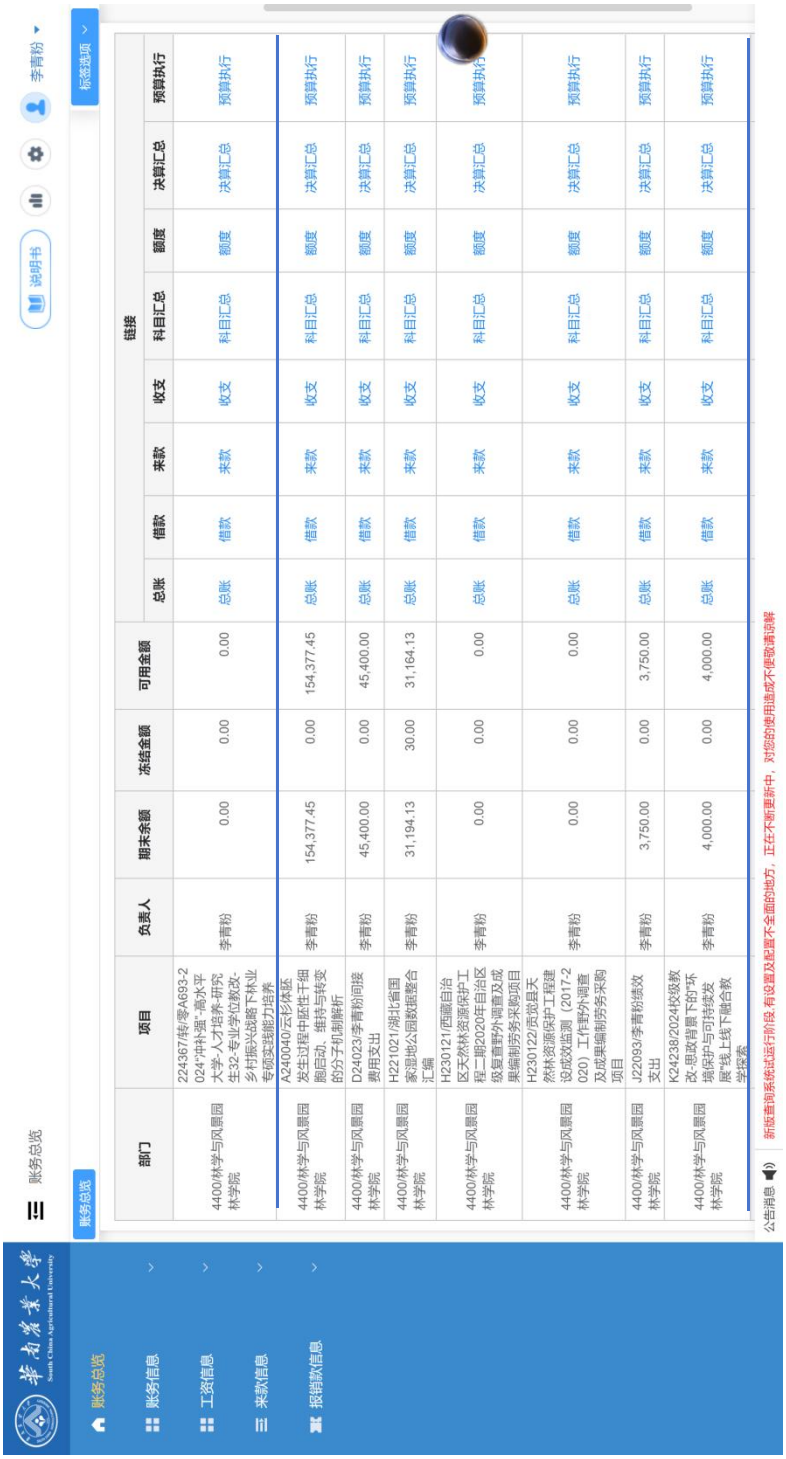
核对时间：

华南农业大学制

目录

一、教学研究业绩

1. 教学研究项目



部门	项目	负责人	期末余额	冻结金额	可用金额	链接								
						总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	224367/韩零A693-2024"冲补湿"-高水平大学-人才培养-研究生32-专业学位教改-乡村振兴战略下林业专硕实践能力培养	李青粉	0.00	0.00	0.00	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	A240040/云杉体胚发生过程中脂性干细胞启动、维持与转变的分子机制解析	李青粉	154,377.45	0.00	154,377.45	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	D24023/李青粉间接费用支出	李青粉	45,400.00	0.00	45,400.00	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	H221021/湖北省国家湿地公园数据整合汇编	李青粉	31,194.13	30.00	31,164.13	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	H230121/西藏自治区天然林资源保护工程二期2020年自治区级复查野外调查及成果编制劳务采购项目	李青粉	0.00	0.00	0.00	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	H230122/贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制劳务采购项目	李青粉	0.00	0.00	0.00	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	J22093/李青粉绩效支出	李青粉	3,750.00	0.00	3,750.00	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行
4400/林学与风景园林学院	K24238/2024校级教改-思政背景下的"环境保护与可持续发展"线上线下融合教学探索	李青粉	4,000.00	0.00	4,000.00	总账	借款	来款	收支	科目汇总	额度	决算汇总	预算执行

1.1 华南农业大学 2024 年度校级教学改革项目

思政背景下的“环境保护与可持续发展”线上线下融合教学探索与实践

华南农业大学2024年度校级教学改革项目立项名单

序号	项目编号	项目类别	单位	项目名称	负责人	项目组成员
3	JG2024003	重点项目	林学与风景园林学院	数智驱动：AI赋能的 林木育种学课程教学模式创新研究	周玮	陈晓阳、张俊杰、骈瑞琪、欧阳昆唏
17	JG2024017	重点项目	林学与风景园林学院	新工科背景下风景园林设计表达课程教学改革研究	刘京一	陈崇贤、夏宇、潘建非、李梦然
21	JG2024021	重点项目	林学与风景园林学院	新农科引领下林学一流专业改造提升路径探索与实践	刘效东	何茜、苏艳、李雪
46	JG2024046	一般项目	林学与风景园林学院	新工科背景下基于OBE的风景园林 专业美育教学研究	常博文	李静、刘小蓓、冼丽铧、胡志杰
51	JG2024051	一般项目	林学与风景园林学院	思政背景下的“环境保护与可持续发展” 线上线下融合教学探索与实践	李青粉	邓成、黄小玲
61	JG2024061	一般项目	林学与风景园林学院	《森林经理学》课程体系教学改革研究与实践	林娜	陈世清、李玉玲、王本洋
101	JG2024101	自筹项目	林学与风景园林学院	“互联网+”背景下《高尔夫球场管理》课程思政实践研究	钟天秀	欧阳昆唏、黄小玲
102	JG2024102	自筹项目	林学与风景园林学院	基于思政视域和应用场景的产教融合式《测树学》课程教学体系探索研究	邓成	李青粉、王本洋、先锋
116	JG2024116	自筹项目	林学与风景园林学院	高校学生会组织功能型团支部建设的探索与实践	喻凯	陈锐帆、王国辽、陈刚、解加米

关于公示2024年度校级本科教学质量与教学改革工程项目拟立项名单的通知

来源单位及审核人： 编辑： 审核发布：本科生院（招生办公室） 发布时间：2024-07-29

各学院、部处、各单位：

根据《关于开展2024年度校级本科教学质量与教学改革工程项目申报工作的通知》精神，经项目负责人申报、所在单位推荐和学校组织专家评审等程序，拟立项“基于大湾区新能源汽车人才需求的智能网联技术课程改革”等121个项目为2024年度校级教学改革项目；拟立项“涉外法治人才培养实验班”等36个项目为2024年度校级质量工程项目；根据学校年度人才培养工作重点及本年度申报项目质量，拟立项“‘长基计划’下新文科历史学课程体系的改革与实践”等11个项目为2024年度“长基计划”教学质量和教学改革工程专项项目（经费由“长基计划”专业专项资金资助）。教改招标项目因不符合立项指南要求，不予立项。现予以公示，具体名单见附件。

公示期自2024年7月29日至8月3日。如有异议，请在公示期内以书面方式提交（附必要的证据材料，并署真实姓名），未署真实姓名或逾期者不予受理。

附件：1.华南农业大学2024年校级教学改革拟立项名单.xlsx

2.华南农业大学2024年校级质量工程拟立项名单.xlsx

3.华南农业大学2024年度校级“长基计划”教学质量和教学改革工程专项拟立项名单.xlsx

联系人：孙齐胜

电　　话：85288020（15192666549）

地　　址：行政楼329

邮　　箱：gjyj@scau.edu.cn

附件1

华南农业大学2024年度校级教学改革项目拟立项名单

序号	项目类别	单位	项目名称	负责人	职称
1	重点项目	工程学院	基于大湾区新能源汽车人才需求的智能网联技术课程改革	吴伟斌	教授
2	重点项目	动物科学学院	基于实践创新能力培养的《养猪学》课程教学改革研究	洪林君	副教授
3	重点项目	林学与风景园林学院	数智驱动：AI赋能的 林木育种学课程教学模式创新研究	周玮	副教授
4	重点项目	公共管理学院	融合人工智能的土地资源管理“新文科”课程教学改革与实践	刘轶伦	副教授
5	重点项目	党委学生工作部（党委研究生工作部）	基于新时代就业形势的生涯教育课程本土化探索与改革	卢小圣	讲师
6	重点项目	农学院	农学类专业课线上线下混合式教学模式探索与实践——以耕作学课程为例	王小龙	副教授
7	重点项目	工程学院	基于OBE理念的工程伦理教学体系研究与实践	张亚莉	副教授
8	重点项目	食品学院	新农科背景下《研究方法与论文写作》的实践教学改革与探索	苗建银	副教授
9	重点项目	材料与能源学院	新农科背景下一流农林院校《材料物理》课程教学改革与实践	张学杰	副教授
10	重点项目	经济管理学院	基于社会情绪和创新能力提升的课程教学改革——组织行为学为例	徐峰	副教授
11	重点项目	图书馆	新媒体环境下知识产权素养微课程教育模式探索与实践	程燕锋	副研究馆员
12	重点项目	食品学院	面向行业应用及创新人才培养的《酿造工艺学实验》改革研究	蹇华丽	副教授
13	重点项目	植物保护学院	“双一流”背景下本科生拔尖创新人才培养体系创新研究——以植物保护专业为例	崔紫宁	研究员
14	重点项目	动物科学学院	《动物营养学》课程思政建设与教学模式改革	谭成全	副教授
15	重点项目	马克思主义学院	整体性比较视角下高校“四史”教育之党史课程内容体系构建研究	王景奇	副教授
16	重点项目	资源环境学院	基于虚拟仿真技术的环境工程专业“新工科沉浸式实践教学”模式探索	梁瑜海	副教授
17	重点项目	林学与风景园林学院	新工科背景下风景园林设计表达课程教学改革研究	刘京一	副教授
18	重点项目	数学与信息学院、软件学院	产教融合背景下多元协同培育创新型人才模式探索与实践——以管理科学类专业为例	韦婷婷	讲师
19	重点项目	电子工程学院（人工智能学院）	基于知识图谱的《数字电子技术》课程思政建设与实践	赵文锋	副教授
20	重点项目	基础实验与实践训练中心	基于“互联网+”的新工科工程训练云平台构建研究	温威	实验师
21	重点项目	林学与风景园林学院	新农科引领下林学一流专业改造提升路径探索与实践	刘效东	副教授
22	重点项目	资源环境学院	新农科建设背景下课程思政教学模式 探索与革新——以《土壤学》为例	李博	副教授
23	重点项目	数学与信息学院、软件学院	课程思政视域下的《高等代数》课程建设的探索与实践	陈羽	副教授
24	重点项目	信息网络中心	以新质人才培养为导向的高校“AI+智慧教学”创新模式构建研究与实践	江晓庆	高级工程师
25	重点项目	水利与土木工程学院	《工程力学》课程思政的建设与实践	卢玉华	讲师
26	重点项目	农学院	国际化背景下基于“双一流”建设的作物学本科生全英课程教学模式研究—以《生物信息学》为例	张慧	副教授
27	重点项目	水利与土木工程学院	基于项目式教学的《建筑物理》教学改革	金玲	副教授
28	重点项目	海洋学院	基于新质生产力的水产养殖学专业实践教学体系构建与实践	孙际佳	高级实验师
29	重点项目	园艺学院	基于创新能力培养的教学模式探索—以新农科课程“干花创意设计”为例	陈国菊	教授
30	重点项目	生物质工程研究院	“双碳”背景下的《生物质复合材料加工方法》课程教学改革与实践	郝笑龙	副教授

序号	项目类别	单位	项目名称	负责人	职称
31	重点项目	植物保护学院	《农业植物病理学教学实习》思政设计与实践	纪春艳	讲师
32	重点项目	生物质工程研究院	“双一流”背景下农科《有机化学》 教学模式的改革研究与实践	李鑫	教授
33	重点项目	材料与能源学院	新农科建设背景下《无机及分析化学》课程思政建设与实践	刘威	副教授
34	重点项目	艺术学院	数字人文视域下思政元素融入农林院校美育课程的创新实践研究	李春阳	讲师
35	重点项目	材料与能源学院	思政教育提高《物理化学》课程教学质量研究	杨思源	副研究员
36	重点项目	外国语学院	英语专业产教融合新路径探索：基于社会认知理论的“英语+行业”虚拟教研室建设	杨敏	讲师
37	重点项目	艺术学院	“立德树人”视域下涉农高校艺术类专业专业课程思政实现路径研究	石娟娟	副教授
38	重点项目	党政办公室（研究室）	“以学生为中心”的高效课堂评教指标体系建构研究	郭燕锋	助理研究员
39	一般项目	公共管理学院	导学讲评式教学在《公共行政学》课程中的运用研究—基于典型案例	曾小龙	副教授
40	一般项目	数学与信息学院、软件学院	以大学生计算机设计大赛促进学生创新能力培养研究	张连宽	副教授
41	一般项目	材料与能源学院	AIGC技术在家具设计与工程专业课教学中的应用研究	宋杰	讲师
42	一般项目	外国语学院	数智时代大学英语课程思政 多模态教育路径研究	赵勇	讲师
43	一般项目	数学与信息学院、软件学院	元认知导向的数据结构教学设计研究与实践	司国东	副教授
44	一般项目	人文与法学学院	立体性与多元化——基于融媒介视野下的《红楼梦》传统课程教学的变革与创新	梁颖稚	讲师
45	一般项目	马克思主义学院	“问题链+小组研学”模式在思政课教学中的运用研究——以《中国近现代史纲要》为例	汤丽莉	讲师
46	一般项目	林学与风景园林学院	新工科背景下基于OBE的风景园林 专业美育教学研究	常博文	讲师

序号	项目类别	单位	项目名称	负责人	职称
47	一般项目	资源环境学院	学科竞赛驱动下的GIS实践与创新型人才培养模式研究	吴小芳	副教授
48	一般项目	园艺学院	神秘的一片树叶—凤凰单丛加工仿真实验	刘少群	研究员
49	一般项目	水利与土木工程学院	与乡村建设实践相结合的《建筑设计II》课程教学内容体系改革与建设	许媛媛	讲师
50	一般项目	电子工程学院 （人工智能学院）	《光纤传感技术》课程知识图谱的建设	曾应新	讲师
51	一般项目	林学与风景园林学院	思政背景下的“环境保护与可持续发展” 线上线下融合教学探索与实践	李青粉	讲师
52	一般项目	公共管理学院	TBL教学模式在“小组工作”课程中的应用	曾永辉	讲师
53	一般项目	基础实验与实践训练中心	遗传学实验课程思政建设模式的探索与实践	李亚娟	高级实验师
54	一般项目	资源环境学院	基于角色互换的《肥料工艺与肥料资源利用》教学优化研究	孙少龙	教授
55	一般项目	兽医学院	结合大湾区在线开放课程的《兽医寄生虫学》教学改革与实践	元冬娟	副研究员
56	一般项目	园艺学院	《茶树育种学》“四位一体”混合式教学体系的构建与实践	晏嫦妤	助理研究员
57	一般项目	图书馆	面向大学生创新创业能力培养的图书馆服务研究	史艳丽	副研究馆员
58	一般项目	外国语学院	“双一流”背景下农业高校大学公共英语教学资源建设与教学模式研究	夏妙月	副教授
59	一般项目	兽医学院	强化生物安全理念的《兽医传染病学实验》课程实践探索	沈雪娟	实验师
60	一般项目	农学院	“知农爱农”核心素养导向的《主要农作物识别》课程设计与实践	程雄	助理研究员
61	一般项目	林学与风景园林学院	《森林经理学》课程体系教学改革研究与实践	林娜	讲师
62	一般项目	农学院	教育数字化背景下AI赋能新农科专业教学模式探索与实践——以《试验统计学实验》课程为例	布素红	讲师

1.2 2024 年华南农业大学研究生教育创新计划项目-专业学位研究生实践教学资源建设与培养模式改革研究项目

乡村振兴战略下林业专业硕士实践能力培养模式改革创新探究

2025/7/12 00:03 关于2024年华南农业大学研究生教育创新计划项目拟立项名单的公示

华南农业大学 South China Agricultural University | 研究生院

全文检索 搜索

首页 院部概览 招生考试 学位工作 培养管理 导师队伍

关于2024年华南农业大学研究生教育创新计划项目拟立项名单的公示

发布者：研究生院 发布时间：2024-04-16 浏览次数：

关于2024年华南农业大学研究生教育创新计划项目拟立项名单的公示

各有关单位：

根据《关于开展2024年华南农业大学研究生教育创新计划项目申报工作的通知》要求，研究生院组织开展了2024年校级研究生教育创新计划及专业学位研究生创新型实践教学改革研究项目立项申报工作。经个人申报、单位推荐、形式审查和专家评审程序，现将拟立项名单予以公示。

公示时间为2024年4月16日至4月22日。公示期内如有异议，请实名向研究生院反映。

联系人：潘科

电话：85280189

2024年

2024年华南农业大学研究生教育创新计划项目拟立项名单

项目类别	序号	合作单位	学科领域	校内负责人	依托单位
（一）联合培养研究生示范基地	1	广东长隆集团有限公司	兽医、畜牧等	开文宝	兽医学院
	2	广东省农业科学院作物研究所	作物学	冯发强	农学院
	3	中国水稻研究所	作物学	王少奎	农学院
	4	广州华农大智慧农业科技有限公司	计算机科学与技术	王春桃	数学与信息学院
	5	东莞植物园	园艺	赵杰堂	园艺学院
	6	佛山市铁人环保科技有限公司	农业资源与环境、资源利用与植物保护	陈火君	资源环境学院
	7	广州市海珠湿地科研宣传教育中心	风景园林	李辉	林学与风景园林学院
	8	广东东图规划科技有限公司	公共管理学	史传林	公共管理学院
	9	南方海洋科学与工程广东省实验室（湛江）	计算机技术	黄琼	数学与信息学院
	10	广州市微生物研究所集团股份有限公司	食品科学与工程	方祥	食品学院
项目类别	序号	课程名称	课程类型	负责人	所在单位
（二）一般性全英文课程建设项目	1	现代汽车新技术	专业选修课	肖博一	工程学院
（三）课程	1	农业机器人	专业选修课	王红军	工程学院

https://yjsy.scau.edu.cn/2024/0416/c208a372119/page.htm 1/3

思政建设项目	2	农产品安全生产技术与应用	专业学位课	刘婕	植物保护学院
	3	高级作物栽培分子生理（全英）	专业选修课	张慧	农学院
	4	农业生态与可持续耕作制度	专业选修课	王小龙	农学院
	5	生物组学大数据分析	专业选修课	张群洁	农学院
	6	兽医临床实践	专业选修课	苏荣胜	兽医学院
	7	现代知识产权与保护	专业选修课	刘涛	食品学院
	8	风景资源与旅游规划	专业选修课	林敏慧	林学与风景园林学院
	9	管理研究方法论	专业学位课	陈灿	经管学院
	10	金融科技	专业选修课	莫易娴	经管学院
	11	领导科学专题	专业学位课	唐斌	公共管理学院
	12	研究生心理素养与幸福人生	公共选修课	林媛	党委学生工作部
（四）示范课程理论课建设项目	1	食品加工新技术研究与新产品研发专题	专业选修课	杜冰	食品学院
	2	高级水产动物营养与饲料学	专业学位课、专业选修课	甘炼	海洋学院
	3	现代管理学	专业学位课	郭萍	经济管理学院
	4	公共预算与财政管理	专业学位课	武玉坤	公共管理学院
	5	农学概论	专业选修课	谢萍	人文与法学学院
	6	生物组学大数据分析	专业选修课	张群洁	农学院
（五）研究生在线开放课程建设项目	1	工程伦理学	公共学位课	李高扬	水利与土木工程学院
	2	有限元与ANSYS	专业选修课	胡圣荣	水利与土木工程学院
	3	森林灾害防控技术及应用	专业选修课	单体江	林学与风景园林学院
项目类别	序号	教材名称	学科领域	负责人	所在单位
（六）高水平研究生教材建设项目	1	智慧农业：关键技术与应用	计算机科学与技术	黄栋	数学与信息学院
	2	深度学习基础与应用实践	计算机科学与技术	彭红星	数学与信息学院
	3	水果产后处理技术与装备	农业工程	段洁利	工程学院
	4	岭南建筑与聚落防灾	建筑学	周韡馨	水利与土木工程学院
	5	植物线虫方法学实验教程	植物保护	文艳华	植物保护学院
	6	公共管理心理学	公共管理	贾海薇	公共管理学院
项目类别	序号	项目名称	学科领域	负责人	所在单位
（七）专业学位研究生实践教学资源建设与培养模式改革研究项目	1	基于专题驱动的《智慧农业理论与实践》教学方法探索	农艺与种业	张雷	农学院
	2	乡村振兴战略下林业专业硕士实践能力培养模式改革创新探索	林业	李青粉	林学与风景园林学院
	3	智慧养殖研究生专项建设与实践	畜牧	闫希亮	动物科学学院
	4	低碳农业背景下《生态环境材料学》交叉课程体系的构建	环境科学	朱雁平	资源环境学院
	5	“三位一体”协同育人创新型乡村振兴人才培养模式探究	水产、渔业发展	赵会宏	海洋学院
	6	咸淡水水域环境的养护与治理教学课程改革	水产、渔业发展	王俊	海洋学院
	7	产教融合食品工程专业学位研究生培养的导向性改革与实践	食品工程	黎攀	食品学院
	8	科技赋能农业专业硕士班级培养模式	农业工程	岳学军	电子工程学院（人工智能学院）

	9	专业硕士学位论文质量提升策略探究	农林经济管理	贺梅英	经济管理学院
	10	基于国际视野的“双一流”涉农高校金融专业硕士培养模式改革研究	金融学	董莹	经济管理学院
	11	参与式案例教学在公共管理硕士培养的应用及创新	公共管理	吴彦	公共管理学院
	12	基于SWOT分析的专业学位研究生教育高质量发展策略研究—以华南农业大学MPA教育为例	教育管理	宋星洲	公共管理学院
	13	新时代法硕课程思政融入研究----以华南农业大学法硕课程教学为例	法学	林友	人文与法学学院
	14	AI时代MTI翻译硕士的智能化实践教学改革研究	英语笔译	陈喜华	外国语学院
	15	双创驱动下艺术硕士专业工作室人才培养模式研究与实践	艺术学	盘湘龙	艺术学院
	16	农业数字化背景下高校研究生知识产权素养培育的创新模式研究	图情信息	刘洋	图书馆
（八）专业学位研究生课程案例库建设项目	1	面向智慧农业的《数字图像处理》课程案例库建设	计算机应用技术	崔金荣	数学与信息学院
	2	生物特征识别案例库	人工智能、新一代电子技术	代芬	电子工程学院（人工智能学院）
	3	工商管理专业学位研究生（MBA）课程案例库	工商管理	杨学儒	经济管理学院
	4	投资银行学案例库	金融学	董莹	经济管理学院
	5	数智时代财务管理教学案例库	会计学	周小春	经济管理学院
	6	《农业政策学》案例库	农林经济及管理	彭东慧	经济管理学院
	7	《管理研究方法论》课程农业管理案例库	农业管理	陈灿	经济管理学院
	8	公共部门网络舆情治理创新实践案例库	公共管理	赵国洪	公共管理学院
	9	模拟法庭实践教学案例库	法学	刘万洪	人文与法学学院
	10	关照人工智能技术发展的《中英语言对比与翻译》课程教学案例库建设	英语笔译	李舸	外国语学院

华南农业大学文件

华南农研〔2024〕10号

关于公布2024年华南农业大学研究生教育创新计划立项项目的通知

各学院、部处、各单位：

为深入实施研究生教育创新计划，进一步提高人才培养质量，结合高水平大学建设的有关工作，学校开展了2024年华南农业大学研究生教育创新计划项目的申报工作。

经个人申报、单位推荐、形式审查、专家评审和校内公示等程序，确定广东长隆集团有限公司等10个联合培养研究生示范基地，《现代汽车新技术》1个一般性全英文课程建设项目，《农业机器人》等12个课程思政建设项目，《食品加工新技术研究与新产品研发专题》等6个示范课程理论课建设项目，《工程伦理学》等3个研究生在线开放课程建设项目，《智慧农业：关键技术与应用》等6个高水平研究生教材建设项目，《基于专题驱动

的<智慧农业理论与实践>教学方法探索》等16个专业学位研究生实践教学资源建设与培养模式改革研究项目，以及《面向智慧农业的<数字图像处理>课程案例库建设》等10个专业学位研究生课程案例库建设项目，共64个项目为2024年华南农业大学研究生教育创新计划立项项目（详见附件），现予公布。

各项目负责人应按照学校高水平大学建设专项资金的有关管理办法，合理使用项目经费，按照项目既定研究周期，严格执行研究计划，扎实推进研究工作，确保按时完成研究任务，实现预期目标。

附件：2024年华南农业大学研究生教育创新计划项目立项名单

华南农业大学

2024年4月25日

（联系人：潘　科，电　话：85280189）

公开方式：主动公开

华南农业大学党政办公室　　2024年4月26日印发

2. 教学改革论文

2.1 检索证明

SCAULIB202519489

检索证明

根据委托人提供的论文材料，委托人**华南农业大学林学与风景园林学院 李青粉** 2 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因子	中科院大类分区
1	林学专业本科生创新创业课程教学改革	创新创业理论研究与实践 出版年：2024 出版日期：2024 年 8 月 22 日 卷期：7 14 页码：41-44 文献号： 文献类型：教改论文	1	普刊类	华南农业大学	CNKI	无	无
2	乡村振兴背景下加强林学专业人才培养的思考	科教导刊（电子版） 出版年：2024 卷期： 页码：- 文献号： 文献类型：	1	普刊类	华南农业大学	万方数据库	无	无

说明：论文等级和中科院大类分区按《华南农业大学学术论文评价方案（试行）》划分。

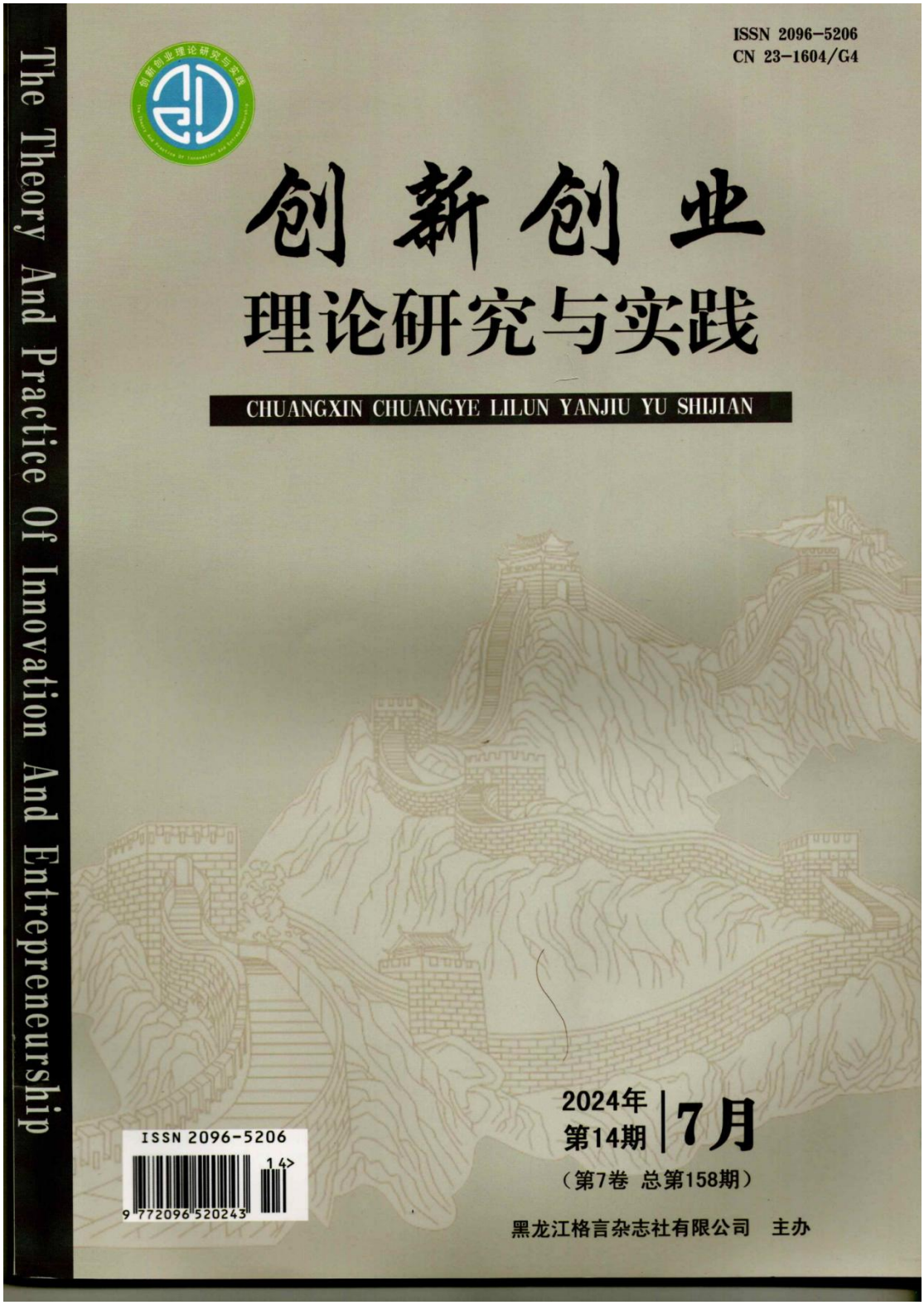
报告免责声明:如未盖章,报告无效

检索员：卢炳卫

华南农业大学图书馆

2025-07-19

2.2 林学专业本科生创新创业课程教学改革



目次

Contents

创新创业理论研究与实践 2024年7月第14期

理论研究

教学革新

教育改革与发展

林学专业本科生创新创业课程教学改革

李青粉，邓成
（华南农业大学 林学与风景园林学院，广东广州 510642）

摘要：林学专业本科生创新创业教育对促进大学生知识转化、加强农林院校与外部社会的交流合作以及解决林学专业大学生就业问题等都具有重要意义，但目前高等农林院校林学专业本科生创新创业课程教学体系尚不完善。该文针对林学专业本科生创新创业课程教学中存在的主要问题，针对性地提出了相关改革措施和对策建议，以期为农林院校更好地开展林学专业创新创业人才培养提供参考和借鉴。

关键词：林学专业；本科生；创新创业；课程设置；教学改革；人才培养
中图分类号：G647.38；S7-4　　**文献标识码：**A　　**文章编号：**2096-5206（2024）07（b）-0041-04

Exploration of the Teaching Reform of Innovation and Entrepreneurship Course for Undergraduate Students Majoring in Forestry

LI Qingfen, DENG Cheng
(College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou Guangdong, 510642, China)

Abstract: Innovation and entrepreneurship education for undergraduate students majoring in forestry is of great significance in promoting the knowledge transformation of college students, strengthening communication and cooperation between agricultural and forestry universities and external society, and solving the employment problem of undergraduate students majoring in forestry. However, the current teaching system of innovation and entrepreneurship courses for undergraduate students majoring in forestry in higher agricultural and forestry universities is not yet perfect. In response to the main problems in the teaching of innovation and entrepreneurship courses for undergraduate students majoring in forestry, relevant reform measures and countermeasures are proposed, in order to provide reference and reference for agricultural and forestry universities to better carry out the cultivation of innovation and entrepreneurship talents in forestry.

Key words: Forestry major; Undergraduate; Innovation and entrepreneurship; Curriculum setting; Teaching reform; Personnel training

近年来，随着科技发展和产业变革，创新创业已成为经济社会发展的强大推动力。为了深入实施创新驱动发展战略，进一步激发社会的创造力和市场活力，推动各行各业创新创业高质量发展，国务院于 2018 年 9 月印发了《关于推动创新创业高质量发展打造“双创”升级版的意见》，大力倡导自主创新、科学发展。现今，创新创业已成为国家发展的动力之源[1]。

要实现自主创新，关键在于培养有创新创业精神的人才。高等院校作为培养人才的重要基地，肩负着为国家培养创新创业高素质人才的重任[2]。按照国家有关要求，结合地方经济社会发展，各类高等院校已广泛开展了大学生创新创业教育，近年来，大学生的创新创业热情也持续高涨。但是由于不同专业的特点和产业性质不同，高等院校中不同专业的创新创业教育发展并不平衡。计算机、软件工程、经济管理、市场营销等专业在很多高校中已经建立了较为完善的创新创业教育教学体系，而农林类专业由于受资源特点、传统观念以及政策机制等因素的限制，起步相对较晚、基础比较较弱，目前还尚未形成完善的创新创业教育体系[3]。

我国是农业大国，林业既是国家的公益性事业，也是基础性产业，在国民经济和社会可持续发展中具有重要地位和作用[4]。近年来，随着生态文明建设和乡村振兴战略的实施，国家开始从政策和资金方面为林业提供较大的倾斜和支持，林业迎来了快速发展的良好机遇，同时也对林业高级专业人才的创新创业能力和素质提出了更高的要求，具备较强创新创业意识、精神的高素质林业人才能更好地适应现代林业发展的需要[5]。但目前，各农林院校在林学专业创新创业人才培养的课程设置和教学实施

基金项目：华南农业大学林学与风景园林学院 2021 年度校级教改项目“林学专业课程思政改革初探”（5）。
作者简介：李青粉（1987—），女，河南濮阳人，博士研究生，讲师，研究方向：林学专业教学和科学研究。
通信作者：邓成（1981—），男，湖南邵阳人，博士研究生，讲师，研究方向：林学专业教学和科学研究，电子邮箱：dengcheng6188@126.com。

过程中还存在一些问题。为更好地满足经济社会发展对林学专业创新创业人才的需求，本文深入分析了目前农林院校林学专业创新创业课程教学存在的主要问题，并针对性地提出了相关改进措施和建议。

1 林学专业本科生创新创业课程教学基本情况

在高等院校的创新创业教育中，其课程设置和教学实施过程对教学的最终效果起着至关重要的作用。2015 年，国务院在《关于深化高等学校创新创业教育改革的实施意见》中明确要求各高校建立创新创业课程。2018 年，国务院在《关于推动创新创业高质量发展打造“双创”升级版的意见》中进一步要求各高校将创新创业教育和实践课程纳入必修课体系[6]。为了充分发挥大学生创新创业的价值并让学生能够学以致用，各高等院校均根据教育部的相关要求，结合自身实际情况，开设了本科生创新创业的有关课程，积极营造良好的创新创业氛围，提供创新创业环境，培养大学生的创新精神、创业意识和创新创业实践能力，以期帮助大学生更好地促进社会发展。

同其他专业一样，各农林院校的林学专业本科生也都开设了创新创业课程，经过调查统计发现：目前大部分农林院校都将创新创业课程作为通识教育课程的一部分，其课程数量一般在 1~2 门，多为必修课，而课时量一般在 16~32 学时，学分一般为 1~2 个学分。课程内容以学生创新创业基本知识和注意事项为主，有关学生价值观和个人品质教育等思政方面的内容较少[7]；课程教学方式大多是以课堂理论讲授为主，实践课程相对较少，课程考核多以理论考试或提交创业计划书等课程作业为主[6]；任课教师多由学生辅导员、学校就业部门工作人员和部分专业教师担任，其中辅导员占了较大比例[8]。

尽管各农林院校已建立了林学专业本科生的创新创业课程体系，并开展了多年的创新创业教育管理实践，但目前，在教育教学过程中还存在一些不足，在课程设置和教学实施中还存在一些问题，剖析这些问题，并针对性地加以解决，对于提高农林院校创新创业课程的教学质量，从而更好地为我国的生态文明和林业现代化建设提供强有力的人才保障和智力支持，具有重要意义。

2 林学专业本科生创新创业课程教学中存在的主要问题

2.1 课程学时较少，开设时间不合理

部分农林院校对于创新创业教育的认识深度和重视程度还不够，将其看作就业教育中的一个环节，简单地认为创新创业教育的目的在于拓宽学生的就业视野，往往忽视了创新创业教育过程中最为核心的思想，即促进学生创业价值观、创业意识、创业精神及创业能力素质的形成[9]。正因如此，目前部分农林院校面向林学专业本科生开设的创新创业课程比较少，一般只有 1~2 门必修课程，学时数量也比较少，偏少的课程和学时量难以真正满足学生对创新创业知识系统学习的需求。此外，部分学校把创新创业课程设置为通识类课程，多在大一、大二开设，但此时，学生尚未开始专业课程的学习，无法将创业知识、专业教育与职业规划紧密结合[10-11]。

2.2 专业教材缺乏，与专业融合度不高

农林领域与其他领域的特点不同，其创业的难度和要求也不同，但目前部分高等院校将大学生创新创业教育作为通识教育的一部分，因此，大多以认知拓展和思维意识训练为主，很少有根据不同专业特点、结合专业知识和职业规划开展相应教育，导致林学专业领域的创新创业指导教材尤其是新形态教材缺乏[12]，学生除了课堂听取任课教师讲解外没有其他合适的可供自学的教材和资料，且不同专业之间的教学内容大同小异，导致创新创业教育的专业针对性不强，与专业的融合度不高。相关研究统计：有 86.5% 的学生认为创新创业教育缺乏专业针对性，希望学校能够根据专业特点开展分类教育，针对不同专业的大学生群体和有着强烈创业愿望的学生系统开设不同的创新创业课程[9]。

2.3 师资力量不足，教学模式方法单一

创新创业学是一门理论性和专业性结合紧密的学科，这对创新创业课程教学师资提出了很高的要求。但目前大部分农林院校没有创新创业教育方面的专门师资队伍，大部分创新创业课程教学任务都是由辅导员或其他管理人员兼职完成，这些人员大多缺乏创新创业背景，没有接受过系统的创新创业教育，也普遍缺乏农林企业管理、市场营销、财务投资等方面的专业知识，部分人员毕业之后到高校工作，社会经验相对不足，对各类企业尤其是农林企业的实际运作过程和管理体系特点也不清楚，因而，在课堂教学中，教学模式和方法都比较单一[13]，大多以讲授一些通用的创业基本理论和相关注意事项为主，很少进行模拟实验教学，即便引用一些案例，也大多来源于网络，内容陈旧，缺乏新意，且与专业的相关性不高，对学生吸引力不强，教学效果不佳。

2.4 实践机会不多，考核评价体系欠缺

创新创业学是一门实践性很强的学科，实践教学应在整个教学活动中占据主导地位。但由于各种条件限制，目前，各类农林院校的创新创业课程教学与其他理论课程并没有太大区别，都侧重于理论知识讲解，学生参与创新创业实践和接触企业的机会

不多。虽然一些学校也会举办一些创新创业类的赛事，但活动一般比较单一，且覆盖面小，每次都只有部分学生参加[14]。根据相关研究统计：目前有 92% 的学生认为创新创业课程的实践性亟待提高[9]。另外，无论是对教师还是学生，都缺乏相应的实践教学效果考核体系，教师只要求完成教学任务，学生只要课程学分，导致创新创业教育理论与实践环节脱节，难以满足学生、用人单位和社会的需求。

3 林学专业本科生创新创业课程教学改革对策

3.1 增加课程数量，建立贯穿式课程体系

各农林院校要从学校层面做好顶层设计，根据林学专业特点和相关行业对创新创业人才培养需要，增加必修课与选修课的数量，满足不同阶段不同学生的个性化学习需求。同时，制定循序渐进、有机衔接、科学合理的培养方案和课程体系，使课程内容充分涵盖创新创业意识培养和思维训练、农林企业管理、市场营销、财务投资等方面，并合理安排各类课程的开设时间，建立价值塑造、知识传授、能力培养“三位一体”，贯穿学生整个大学阶段的课程教学体系，开展分段式培养[15]。其中，一年级以创新创业思维意识培养和价值塑造为主，二年级以创新创业专业理论知识讲授学习为主，三年级以创新创业大赛模拟实践体验教学为主，四年级以到孵化基地、企业参与企业管理，培养实践能力为主。

3.2 加强教材建设，充实专业性教辅资料

教材是教育教学中不可或缺的一环，它是学生学习的主要来源和重要依据，也是教师教学的基础和学校教学管理的重要依据，在教育教学中具有重要作用[16]。因此，各农林院校应重视教材的编写和使用，不断完善教材体系，提高教材的科学性、系统性和实用性，为学生的学习和教师的教学提供更好的保障。针对林学专业，应编写符合专业特色、与专业结合紧密、反映学科前沿热点的创新创业教材，为学生提供高质量的学习资源，帮助学生系统、全面、高效地掌握相关知识。同时，在教材建设过程中，积极加强与农林企业的沟通合作，全方位收集创新创业案例资源，共同编制与教材配套的创新创业案例资源库等教辅资料，为创新创业教学提供生动丰富的新形态教学教辅等资料。

3.3 加强师资建设，构建多元化教学模式

师资队伍是创新创业教育质量和水平的决定性因素，他们的知识结构、教学能力和专业素养直接关系到学生的学习效果。各农林院校应积极建设专业的创新创业师资队伍，一方面，可从现有教师队伍中选择既具有创新创业相关背景，又具有丰富专业知识的教师开展专题培训，提高其创新创业教学能力；另一方面也可从农林企业等单位聘请相关负责人、专家作为兼职教师或学生的创新创业校外实践导师，定期为学生开展创新创业主题学术报告、案例分析讲解、模拟体验、创业实训等指导，从而构建“案例—模拟—实训”多元化、多层次、递进式教学模式。同时，增强课堂的开放性，通过情景创设、讨论交流、氛围打造等增强课堂的趣味性和吸引力，充分调动学生的学习积极性。

3.4 强化实践引领，搭建产学研实训平台

以行动实践为导向，积极加强与农林企业的沟通与产学研合作，搭建创新创业实践训练平台，从而实施体验（Experience）、情境（Environment）、沉浸（Engaging）、延展（Expand）的“4E”教学育人机制[17]。充分利用校企双方各自的优点，实现资源和优势互补，联合开发与教材相配套的创新创业虚拟仿真实训系统，实现创新创业实训教学场景化、模块化、智能化，使学生能够深度体验创新创业各类情景。联合各类农林企业，加强创新创业赛事组织，增加赛事数量和扩大赛事覆盖面，从而达到以赛代练、以赛促学的目的。加强校企协同育人，通过与农林企业建设实训基地，使学生直接参与企业运营实践，解决企业面对的问题，并从中寻找创新创业灵感，全方位提升创新创业技能。

4 结束语

创新创业是民族复兴和国家繁荣昌盛的根本战略选择。高等院校作为培养高级人才的主阵地，更是创新创业教育实践的主战场。林业既是国家的公益性事业，也是我国的基础性产业，在国民经济和社会可持续发展中具有重要地位与作用，通过先进的科学技术和管理手段，促进林业的可持续发展，充分发挥森林的多种功能效益，对于促进人口、经济、社会、环境和资源协调发展具有重要意义。近年来，随着中央对生态文明建设的不断重视，国家从资金、政策等各方面都给予了林业较大的支持，林业迎来了快速发展的重要机遇期，其对创新创业高级专业人才的培养也提出了更高的要求。但目前，各农林院校在林业创新创业人才培养课程教学中还存在着课程学时较少，开设时间不合理，专业教材缺乏、与专业融合度不高，师资力量不足、教学模式方法单一，实践机会不多、考核评价体系欠缺等问题。为更好地满足经济社会发展对林学专业创新创业人才的需求，各农林院校在林学专业创新创业教学中应增加课程数量，建立价值塑造、知识传授、能力培养“三位一体”，贯穿学生整个大学阶段的课程教学体系；加强教材建设，不断建设科学性、系统性和实用性的教材体系；加强师资建设，构建“案例—模拟—实训”多元化、多层次、递

进式教学模式；强化实践引领，搭建有利于实施体验、情境、沉浸、延展的“4E”教学育人机制的产学研实践平台，从而为实现中华民族伟大复兴提供强有力的林业人才保障和智力支持。

参考文献

[1] 蒋向利 . 打造创新创业升级版 推动“双创”高质量发展 国务院发布《关于推动创新创业高质量发展 打造“双创”升级版的意见》[J]. 中国科技产业 ,2018(10):80.

[2] 张林平 , 吴斐 , 张扬 , 等 . “互联网 +”背景下林学专业大学生创新创业现状分析——以江西农业大学为例 [J]. 生物灾害科学 ,2022,45(2):236–240.

[3] 王玉涛 , 陆秀君 , 张晓林 , 等 . 基于创新创业能力培养的林木种苗学实践教学改革与探索 [J]. 黑龙江农业科学 , 2017(9):116–119.

[4] 刘平 , 王玉涛 , 刘青柏 , 等 . 基于创新创业能力培养的林学专业教学模式探索研究 [J]. 黑龙江农业科学 ,2016(6): 123–125.

[5] 王志维 , 王庆材 , 张晓文 , 等 . 林科类大学生创新创业教育的实践研究 [J]. 当代教育实践与教学研究 ,2020(3): 163–164.

[6] 宋华明 , 朱佳俊 . 江苏涉农高校创新创业课程学生满意度研究 [J]. 中国农业教育 ,2023,24(3):29–42.

[7] 金阳 . 创新创业型人才培养与高校思政课教学改革研究 [J]. 当代教研论丛 ,2023,9(8):114–117.

[8] 张倩 , 张志萍 , 吕宵宵 . 地方高校创新创业师资队伍建设路径探究 [J]. 科技风 ,2023(24):66–68.

[9] 齐宪磊 , 盛艳萍 , 王庆材 , 等 . 地方农林院校创新创业社团在大学生创业教育中的实践与探索——以山东农业大学林学院为例 [J]. 长春工程学院学报 (社会科学版), 2019,20(4):83–86.

[10] 李琪 , 李永峰 , 席恩华 , 等 . 双创背景下涉林学科大学生创新创业能力培养改革与实践 [J]. 教育教学论坛 ,2020(24): 191–192.

[11] 段祖安 , 蔷薇 , 丁修堂 , 等 . 对现代大学生创新创业教育实践的思考 [J]. 实验室科学 ,2019,22(2):238–240.

[12] 孙冰爽 . “互联网 +”背景下创新创业教育新形态教材建设的实践与思考 [J]. 电脑知识与技术 ,2021,17(28):281–282,284.

[13] 王雅莉 . 基于个性化发展的创新创业教育教学模式改革研究与实践 [J]. 教育视界 ,2023(32):53–57.

[14] 谭占明 , 施明登 , 程云霞 , 等 . 基于学科竞赛驱动的设施专业学生创新创业能力培养方案初探 [J]. 农业工程技术 , 2022,42(16):80–83.

[15] 李艳洁 , 王庆材 , 张晓文 , 等 . 大学生创新创业教育分段式培养体系研究与探索——以山东农业大学林学院为例 [J]. 传播力研究 ,2018,2(33):171.

[16] 周晶 , 吕晓芳 . 基于自我效能感提升的大学生创新创业教材建设 [J]. 黑河学院学报 ,2021,12(8):92–94.

[17] 陈永平 . 技术赋能的高职教学资源 : 多元化情境、沉浸式体验与多维度质量提升 [J]. 职教论坛 ,2022,38(8):58–66.

（上接第 27 页）

性思考 [J]. 中国电化教育 ,2022(8):24–31,74.

[9] 郭文革 . “互联网 + 教育”的三大发展趋势 [J]. 远程教育杂志 ,2022(4):91.

[10] 陆钧 . “互联网 + 教育”与“课内翻转”的联系 [J]. 考试周刊 ,2023(2):1–4.

[11] 张宪明 , 周知进 . 机械制造工艺学课程项目式教学实践探索 [J]. 中国机械 ,2023(3):89–92.

[12] 王雪晴 , 李阔湖 , 卫亚博 , 等 . 新工科背景下“互联网 + 教育”教学模式探索与评价方法研究——以《自动检测技术》混合式教学为例 [J]. 创新创业理论研究与实践 ,2021,4(9):126–127,130.

[13] 朱立达 , 巩亚东 , 于天彪 . 基于“互联网 +”与大数据的机械类专业教学改革探析 [J]. 中国现代教育装备 , 2022(11):14–16.

[14] 王寅昊 . 互联网时代高校体育教学模式浅析 [J]. 课程教育研究 ,2020(8):221–222.

《创新创业理论研究与实践》杂志维权声明

《创新创业理论研究与实践》杂志是经国家新闻出版署批准，黑龙江出版传媒股份有限公司主管、黑龙江格言杂志社有限公司主办的学术期刊，国内刊号CN 23-1604/G4,国际连续出版物号ISSN 2096-5206。

近期，接部分读者、作者反映和举报，有人以《创新创业理论研究与实践》杂志社名义或冒用《创新创业理论研究与实践》编辑部名义，通过网站、邮件、电话、书信等方式进行非法约稿、组稿，并收取版面费用，严重侵害我社和《创新创业理论研究与实践》杂志权益，严重损害了杂志社和刊物的声誉，造成恶劣影响。对此，我杂志社严正声明：

我杂志社编辑出版的《创新创业理论研究与实践》杂志选稿、用稿均由《创新创业理论研究与实践》编辑部在职在岗编辑和编委会专家执行“三审三校”制度，严格筛选、审查后择优录用，我社从未委托任何机构、任何网站或个人面向社会征稿。

《创新创业理论研究与实践》编辑部网站http://www.cxcybjb.com是唯一官网，cxcybjb@126.com是编辑部指定收稿邮箱。互联网上出现的其他任何关于《创新创业理论研究与实践》刊物的所谓网址、邮箱，任何冒用我社或编辑部名义的约稿、组稿以及收取版面费等信息均属伪造，且严重违反国家法律法规及国家新闻出版的有关规定，我杂志社将依法追究相关责任人的法律责任。

特此声明。

请相关单位及广大作者、读者提高警惕，谨防上当。

如有问题请拨打我社电话：0451-84611887

本社社址：哈尔滨市道里区田地街100号

《创新创业理论研究与实践》编辑部

2020-4-20

2.3 乡村振兴背景下加强林学专业人才培养的思考

◆ 学 前 教 育

◆ 教 育 广 角

乡村振兴背景下加强林学专业人才培养的思考

李青粉，邓　成*
（华南农业大学，广东　广州　510642）

摘　要　随着我国加快建设农业强国步伐实施乡村振兴战略以来，对农业实践应用型人才的需求也越来越多。林学专业是我国农科领域的传统专业之一，在乡村振兴的各个领域中发挥着极其重要的作用。推进林业发展必将为早日实现乡村振兴提供有力的支撑，而全面推进乡村振兴更是离不开林业的深度参与。现代化林业的发展也离不开人才，但目前大部分农林院校在林学专业人才培养方面尚未完全跟上乡村振兴战略的实施步伐。文章全面分析了乡村振兴战略背景下对林学专业人才的需求特点，并深入剖析了目前农林院校林学专业人才培养中存在的主要问题，最后针对性地提出了相关的对策与建议，以期为高等院校更好地开展乡村振兴战略所需的林学专业人才培养提供参考与借鉴。

关键词　乡村振兴；林学专业；实践能力；人才培养
中图分类号：C961　　**文献标识码**：A

林学专业是农科领域的传统专业之一，其高质量的人才培养对于保障乡村振兴战略的顺利实施具有重要意义[1]。随着时代的发展，社会对林学专业人才的具体需求也在不断变化，乡村振兴战略背景下，如何紧跟时代步伐，创新培养理念，培养出真正契合乡村发展需求的应用型人才成了各大农林院校需要思考和面对的问题[2-3]。为此，本文拟通过分析乡村振兴战略对林学专业人才的需求特点的同时，剖析农林院校林学专业人才培养存在的问题，进而找出加强林学专业人才培养的对策与建议，将为高等院校更好地开展乡村振兴战略所需的林学专业人才培养提供参考和借鉴。

1 乡村振兴对林学人才的需求特点

我国乡村土地辽阔，民俗文化多样，森林资源丰富，到处都是绿水青山，风景优美，但总体来说工业、商业、金融、文化、教育、卫生事业的发展水平较低，资源开发利用程度不高，无论是资源类型、资源特色还是发展环境等都与城市有着较大的差别，这也决定了其发展路径和方式与城市会有着很大的差异，对林学等人才的需求也有着其自身的特点。总的来说，乡村振兴所需要的林学人才应具有以下特点。

1.1 懂乡村懂农业，专业能力强

乡村的生活地理环境、农业生产特征和发展方式都有着其自身的特点，农村的制度等都有着其深厚的历史背景和时代特征，农村古老的传统节日习俗和民俗文化也与其发展紧密相关。要想治理和发展好乡村，就必须真正懂乡村懂农业，并具有较强的专业知识能力的专业性人才。首先，要真正懂乡村地理环境和人文特点，这样才能结合当地农业特色、农村现状、农民基础，彻底弄明白要“发展什么”“怎么发展”“发展成什么样”等现实问题[4]；其次，要懂乡村发展的各项农业政策，才能充分利用好各种农业政策调节农村生产关系、维护农民整体的长远利益、促进科技发展[5]；再次，要懂林业并具有较强的专业能力，才能将专业理论知识和具体生产实践紧密结合起来，利用专业知识开展现代化经营，促进农林业的可持续发展；最后，还要懂农民语言、农民心理，说农民群众听得懂的话，才能真正了解农民群众的需求，更好地推动乡村建设、乡村振兴。

1.2 爱农村爱农民，“三农”情怀深

在新时代的中国，实现乡村振兴和全面建设社会主义现代化国家的目标，离不开一支真正爱农村、爱农民的，具有深厚情怀的工作队伍[6]。由于发展速度较慢，目前大部分乡村的医疗、教育、卫生环境等方方面面都暂时落后于城市，生活条件较差，加之许多人因为没有经历过乡村的生活，对农村生活一知半解，导致其认知产生了偏差，从而对农村和农民会产生一定的偏见和误解。这导致很多专业人才虽然学习的是农林专业，但却不愿意到林业基层就业，即便到了基层，也对农林工作缺乏足够的热情，不愿意在农村扎根，甚至想方设法要“逃离”农村，致使各项工作成效都大打折扣。因此，加强乡村振兴，需要真正了解乡村，发自真心热爱农村，对“三农”工作热心，愿意倾心付出，能够经常走进群众家里、时刻走进群众心里，将心比心、以心换心，担着责任、带着感情，团结农民群众，当作自己的本分帮助群众解决实际问题，具有深厚“三农”情怀的人才。

1.3 懂规划懂实践，动手能力强

实施乡村振兴战略是一项长期的历史性任务，也是

一篇大文章，需要统筹谋划，必须注重规划先行、突出重点、分类实施、典型引路、科学推进的策略。推进乡村振兴具有前所未有的长远性和全局性，必须坚持规划先行，加快形成城乡融合、区域一体、多规合一的规划体系，强化乡村振兴战略的规划引领作用[7]。对于林业，也要始终坚持以各级相关文件精神为引领，根据农村实际，认真谋划相关领域的发展规划。同时，对于规划的各项目标和指标，还需要通过实践落实到具体领域，这也需要进一步深刻认识乡村各地林业发展的实际情况，通过找差距、勤改进、强突破、再创新，将各项工作内容细化落实，如进一步深化农村土地制度改革，完善承包地三权分置制度，发展多形式适度规模经营，加快推进林业现代化治理，实现小农户和现代林业发展有机衔接。可见，实施乡村振兴，急需全面掌握“3S”等现代林业技术，既懂规划又懂落实，具有较强动手能力的现代化专业人才。

1.4 懂经营懂策划，市场意识强

过去，在传统农林业经营理念和模式管理下的森林资源，其生态产品价值尚没有充分转化和变现，未能给当地居民带来较好的经济效益，致使当地居民守着丰富的“绿水青山”资源却无法转化为“金山银山”，只能过着较为清贫的日子。随着我国经济发展进入新常态，传统产业在国民经济中的比重逐渐下降，节能、循环、低碳的发展方式渐成主流，发展生态经济成为了从居山守贫走向生态富民的光明之路。党的十九大报告中明确了乡村振兴的总要求为“产业兴旺，生态宜居，乡风文明，治理有效，生活富裕”[8]。因此，大力加强森林经营和林下经济、森林康养、森林旅游等林业产业建设，着力增加生态产品供给，是让良好生态环境成为人民生活质量增长点，让人民群众幸福指数节节攀升，进而实现乡村繁荣振兴的重要途径。而林下经济、森林康养、森林旅游等产业的发展，急需既懂森林资源经营，又懂线上线下产品营销策划，具有敏锐市场意识的综合型农商人才。

2 农林院校林学专业人才培养存在的问题

2.1 了解乡村的渠道和窗口不够

全面了解乡村和林业的真实状况应是林学专业人才培养过程中的重要环节。但目前，大部分农林院校的林学专业人才培养都是以学校理论知识学习为主，除了少部分学生有机会参与假期学校组织的“三下乡”活动外，大部分学生接触、了解乡村的渠道和窗口有限，真正深入乡村的机会更是少之又少，无法与“三农”建立直接的链接，虽然有部分来自农村的学生对于农村环境状况和经济发展水平等有一定认知，但其也缺乏真正深入全面了解农村林业经济发展、产业运营管理、深层次运行逻辑和行业现代化治理的机会和途径。同时，相关专业的许多教师对于乡村的了解也不够深入，接触乡村的渠道和窗口有限，无法给予学生更多乡村实际情况的介绍。这就使得所培养的人才对于乡村缺乏真正深入全面的了解，从而造成“学农不知农、学农不懂农”的尴尬局面[9]。

2.2 “三农”情怀思政课程设置较少

相对于热闹繁华的都市，乡村生活相对要冷清无聊得多，而且农村如何建设、环境如何整治、村民如何团结、文化如何引导等也都是摆在乡村振兴面前的重要难题。乡村振兴建设者只有发自真心热爱农村，才愿意倾心付出，才会愿意扎根农村、建设农村，才能在农村广阔天地大施所学、大展才华。但目前，社会对于农村还存在一些偏见和误解，这也导致部分林学专业的学生与农村的“爱”意无法建立，觉得乡村工作不但苦和累，还没有前途，不愿意到乡村去工作。因此急需改变这种认知偏见，但目前大部分农林院校的思政课程都还是以传统爱国主义情怀教育为主，和专业有机结合，针对性的生态文明、“三农”情怀思政课程较少[10]，林学专业学生存在“轻农厌农”偏见的局面没有得到根本性转变。

2.3 实践教学效果重视程度不够

目前在农林院校懂农村、爱农业、爱农民“一懂两爱”人才培养方面，不少教育工作者都深入分析了其重要性和必要性[11-12]，也有学者从较为宏观视角对具体实施措施提出了很有针对性的建议[13]。但在具体教学模式和课程建设方面，仍然属于摸索阶段，尤其是实践教学方面，大部分农林院校还缺乏相应的效果评价体系，大多采用与理论教学相同的评价方法体系。对于学生，实践能力评价很少根据学生的实践操作情况和分析解决实际问题的能力等综合开展考评，多是“走流程”的形式完成，大多是交一份报告或填一些记录表格就行，对于教师，则更多的是考核和检查，没有激励机制，导致老师对相关工作的积极性不高。这些都使得学生的动手实践能力无法得到实质性提升[14]。

2.4 复合管理人才培养效果不佳

乡村振兴建设涉及的面比较广，尤其是对于新时代的林业建设，既涉及第一产业即森林种植业，同时也涉及第二产业如林产品加工业和第三产业如森林康养、森林旅游等，且其发展内涵和形式也发生了很大变化，其经营管理也面临着很多新的挑战。因此，对于林学专业人才的要求比以往要高得多，既需要懂森林资源经营培育，又要懂现代林业信息管理技术，还要懂市场经济和产品营销策

划。但目前大部分农林院校的林学专业人才培养还是按照以往以林学专业课程教育为主的模式进行,对于林业新政策、新技术、市场营销和经营管理类课程及相应的交叉学科课程设置较少,导致所培养的人才还是以专业型为主,缺乏既懂林业又懂市场,既懂技术又懂管理的高素质新型农商复合型人才[15]。

3 加强林学专业人才培养的对策建议

根据乡村振兴对林学专业人才的需求特点及农林院校林学专业人才培养中存在的问题,各大农林院校应针对性地改进人才培养模式,不断提高人才培养针对性和质量,积极破解乡村振兴中林业人才缺乏问题,为乡村振兴提供源源不断的智力支持。

3.1 拓展"知农"窗口,破解"学农不懂农"困境

创新人才培养的理念,改革教学模式,匹配大学生个性化发展需求。在相关课堂教学中积极加强案例教学和场景模拟教学,增加学生了解乡村的途径,使学生通过场景模拟和案例分析更多了解乡村的实际情况。加强实训,积极聘请业界实践者对学生进行生态产品新媒体营销专题培训,分层次多场景组织指导学生参与农林类相关企业的链接互动。将行业实践与校内教学有机结合,为身处象牙塔中的大学生打开真实触摸"三农"的窗口,拓宽其专业视野,加强其对农林行业的认识,使其逐步了解、领会农林产业发展和相关企业经营管理的现状和存在的问题,引导其应用所学的专业理论知识去分析和解决实际生产中存在的问题,从而打破"学农不懂农"的囧境。

3.2 塑造"爱农"情怀,摒弃"轻农厌农"偏见

不可否认,农村的条件暂时还不如城市,但另一方面,农村正因为没有都市的高楼大厦、宽阔道路,没有都市的车水马龙、霓虹满目,才多了那一份宁静祥和,给基层人才定下心神、努力工作、专心服务提供了良好机会,使其能够在沉淀中成长、成熟、成才。农林院校要在课程教学和实践过程中,加强对学生的价值塑造以及社会责任感和情怀教育,通过与农林业龙头企业合作讲企业故事,品乡村文化底蕴,让学生理解企业家精神与企业的使命与社会担当,引导培养学生对农村、农民、农林业的热爱,以及其对推动乡村特色文化传承和农林产业高质量发展的使命感,不断深化其"知农懂农"领域的认知,厚植"三农"情怀,夯实大学生服务乡村振兴的思想基础和情感认同。

3.3 强化"强农"实践,逾越"想-说-做"鸿沟

改革培养方案,完善实践教学体系,强化实践训练,树立"干中学"理念,通过以赛代练、以践促学等多种方式,加强学生实践应用能力的培养。积极组织和指导学生参与各类农林企业品牌创意、农产品文化创意、产品形象设计、包装创意设计以及文旅创意设计等赛事实践,锻炼大学生的调研营销与组织策划的能力;加强产学研交流合作,搭建学生与农林企业交流互动平台,给学生创造了解企业诉求、直接介入企业运营实践和分析并参与解决农林企业实际问题的机会,鼓励学生自主提供问题解决方案;加强大学生创新创业教育训练,积极为大学生提供创新创业的实践舞台,全方位培养大学生服务乡村振兴的实践动手能力,从而培养"敢想、能说、会做"的实干型专业人才。

3.4 提升"兴农"才干,培养"新型农商"人才

当前,我国农林业正处于从传统产业向现代产业过渡的转型期,其经营方式也在从粗放型向集约型转变,对从业者的要求也从单纯的生产技术型向市场意识引领下的生产+经营管理综合型转变,农林业产业发展对新型农商复合型人才的需求越来越多。各农林院校应在加强学生专业能力培养的基础上,进一步强化学生综合素质的培养。应积极改革课程设置方案,增加农林政策法规、森林资源管理、林下经济发展、森林康养、森林旅游等与乡村振兴紧密相关的专业类课程的比例,同时,加强"3S"技术等现代林业信息技术和规划设计类、文旅开发类、市场营销类、财务经济类、企业管理类课程和相关交叉学科设置,从而培养出既懂林业、又懂市场,既懂技术、又懂管理的复合性新型农商人才。

4 结语

随着我国乡村振兴战略的大力实施,农村经济社会发展需要大量人才,尤其需要真正懂农业,爱农村,爱农民的"一懂两爱"人才。林业作为农科领域的传统专业之一,其高质量的人才培养对于乡村振兴战略的实施具有重要意义。为满足乡村振兴人才培养的需求,各农林院校应进一步创新林业人才培养理念,改革教学模式,匹配大学生个性化发展需求,改革培养方案,不断完善实践教学体系,强化实践考核,改革课程设置,通过进一步完善视野拓展、价值塑造、能力培养和知识传授"四位一体"的育人过程,培养学生爱农情怀和责任担当,增强其服务现代林业发展的实践应用能力,为他们未来的职业发展奠定坚实的基础,使之成为"懂'三农'""有情怀""重实践""强能力"的新型复合型农商人才,为乡村振兴战略的顺利实施提供强有力的人才保障和智力支持。

*通讯作者:邓成

★基金项目:华南农业大学林学与风景园林学院2021年度教改项目—林学专业课程思政改革初探;华南

农业大学林学与风景园林学院2023年度教改项目—课程思政在《环境保护与可持续发展》教学中的探索与实践。

作者简介：李青粉（1987—），女，汉族，河南濮阳，博士，讲师，硕士生导师，研究方向为林学教学和相关科学研究；邓成（1981—），男，汉族，湖南邵阳，博士，讲师，硕士生导师，研究方向为林学教学和相关科学研究。

参考文献

[1] 袁杰,贾小明,李卫忠,等.“新农科”建设背景下林学专业本科人才培养方案的改革——以西北农林科技大学为例[J].中国林业教育,2023(3):19-23.
[2] 刘慧芳,王挺,许慧.乡村振兴背景下“三农”人才培养的困境与出路——对高职院校农林专业人才培养的思考[J].安徽农业科学,2023(15):269-272.
[3] 苏小菱,黄坚钦,林海萍.基于乡村振兴的卓越农林人才培养思考与实践——以浙江农林大学林学专业为例[J].福建茶叶,2019(9):51-52.
[4] 蒋晓蕾.乡村振兴中的人才问题研究[D].济南:山东大学,2019.
[5] 陈良正,陈蕊,王雪娇,等.云南省高原特色农业产业政策创新探析[J].江西农业学报,2019(11):138-145.
[6] 张俊.“一懂两爱”的“三农”人才队伍建设路径探究[J].南方农业,2021(18):161-162.
[7] 封丹.开好局、起好步乡村振兴第一个五年战略规划出台[J].科技智囊,2018(11):23-29.
[8] 吴海峰.乡村产业兴旺的基本特征与实现路径研究[J].中州学刊,2018(12):35-40.
[9] 代莉,冯建灿,胡选振,等.乡村振兴背景下的新农科建设[J].中国高等教育,2021(21):54-56.
[10] 黄庆丰,唐雪海,欧强新,等.林学专业森林经理学课程思政教学实践[J].安徽农业科学,2022(18):255-256.
[11] 安美忱.涉农高校思想政治教育融入知农爱农新型人才培养对策研究[J].思想政治教育研究,2022(2):136-141.
[12] 石定芳,吴能表.新农科建设驱动下农林人才培养模式的改革路向研究[J].中国农业教育,2021(1):44-50.
[13] 贺华良,薛进,邱林,等.“知农爱农”新型人才培养的困境、机遇与改革探索[J].高等农业教育,2021(5):22-27.
[14] 孟志兴,解晓悦.乡村振兴背景下农村区域发展专业实践教学体系建设研究——以山西农业大学为例[J].商业经济,2020(7):181-183.
[15] 董银英.乡村振兴战略实施对农业复合人才的专业英语要求[J].中国农业资源与区划,2022(1):9,17.

（上接第276页）少的教师和学生面对面接触，缺乏对于学生的情况的了解，只能通过技术分析手段能够更为精确地对学生在线学习情况进行评估，及时跟进相关的学习支持服务。

4 结语

通过对中国知网CNKI数据库2023年之前的在线学习行为分析模型研究文献进行分析，得出如下主要结论：第一，2016年以来，相关文献发表处于上升状态，源于在线教育发展得到了国家和社会的广泛重视；第二，相关研究的主要阵地和研究成果的主要来源都是高等师范院校，开放教育体系的机构也较为积极参与相关研究；第三，《中国电化教育》《电化教育研究》等前五的期刊发表的相关论文质量较高，其可以给研究者提供较高的研究价值；第四，目前主流的模型研究是基于概率分析和机器学习的分析方式，深度学习的应用还处于研究起步阶段，随着近年来数据挖掘技术的发展，在线学习行为分析的手段也更丰富更多样化；第五，相关研究面向大学生、慕课学习者、成人教育学生，研究的主要目的都是通过技术分析手段能够更为精确地对学生在线学习情况进行评估以及为后续学习支持服务提供依据。

★基金项目：广西高校中青年教师科研基础能力提升项目“基于大数据的远程教育学习支持服务精准度提升研究”（2021KY1923）。

作者简介：陶兴海，男，硕士，工程师。

参考文献

[1] 陈然.远程学习者在线学习危机预警及干预研究——英国开放大学OU Analyse项目剖析[J].成人教育,2021,41(11):12-17.
[2] 宗阳,孙洪涛,张亨国,等.MOOCs学习行为与学习效果的逻辑回归分析[J].中国远程教育,2016(5):14-22,79.
[3] 叶俊民,罗达雄,陈曙.基于短文本情感增强的在线学习者成绩预测方法[J].自动化学报,2020,46(9):1927-1940.

电子版
科教导刊

主管单位：长江出版传媒股份有限公司
主办单位：湖北长江报刊传媒（集团）有限公司
编辑出版：科教导刊（电子版）编辑部
编 辑 部：李政　朱敬　冯春娟　刘付豪
美术编辑：易晓敏

刊　　期：旬刊
地　　址：武汉市洪山区珞喻路78号长江传媒大厦2109室
电　　话：027-87508018　027-87508618
投稿邮箱：kjdkdzb@126.com
发　　行：武汉广渊广告有限公司

二、科研项目

1. 主持项目

表9 科研课题情况
李青粉 主持的课题

序号	项目名称	评审等级	项目来源	合同经费/实到经费	立项时间	结题时间	课题组总人数	本人排名	是否结题	备注
1	细胞外囊泡对川西云杉抵御性细胞系衣霉菌的作用机制	B	人事部	5.0	2017-11-17	2019-01-15	2	1	是	
2	贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制劳务采购项目		横向	15.0	2022-07-04	2022-12-30	5	1	是	
3	西藏自治区天然林资源保护工程二期2020年自治区级复查野外调查及成果编制劳务采购项目		横向	5.25	2022-07-04	2022-09-30	5	1	是	
4	湖北省国家级公园数据整合汇编		横向	10.0	2022-11-16	2023-06-30	7	1	是	
5	云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析	A	科技部	70.0	2023-12-13	2026-11-30	5	1	否	

科技处审核人及盖章：

年 月 日

1.1 云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析

子课题编号：2023YFD2200102-02　　密　级：公开

国家重点研发计划
子课题任务书

子课题名称：	云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析
所属课题：	体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析
所属项目：	林木体胚形成的分子调控机制研究
所属专项：	林业种质资源培育与质量提升
课题牵头承担单位：	南京大学
子课题承担单位：	华南农业大学
子课题负责人：	李青粉
执行期限：	2023 年 12 月 至 2028 年 11 月

南京大学 制
2023 年 12 月 09 日

填 写 说 明

一、任务书甲方即课题牵头承担单位，乙方即子课题承担单位。

二、任务书中的单位名称，请按规范全称填写，并与单位公章一致。

三、任务书中文字须用宋体小四号字填写。

四、凡不填写内容的栏目，请用“无”表示。

五、乙方完成任务书的填写，提交甲方审核确认后，用A4纸打印、装订、签章。一式六份报课题牵头承担单位和项目牵头单位，其中项目牵头单位二份，课题承担单位二份，子课题承担单位二份。

六、《项目申报书》、《项目任务书》和《课题任务书》是本任务书填报的重要依据，子课题任务书填报不得降低考核指标，不得自行对主要研究内容作大的调整。《项目申报书》、《项目任务书》、《课题任务书》和子课题任务书将共同作为子课题过程管理、综合绩效评价（验收）和监督评估的重要依据。

子课题基本信息表

<table>
<tr><td colspan="2">子课题名称</td><td colspan="6">云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析</td></tr>
<tr><td colspan="2">子课题编号</td><td colspan="6">2023YFD2200102-02</td></tr>
<tr><td colspan="2">所属课题</td><td colspan="6">体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析（2023YFD2200102）</td></tr>
<tr><td colspan="2">所属项目</td><td colspan="6">林木体胚形成的分子调控机制研究（2023YFD2200100）</td></tr>
<tr><td colspan="2">密级</td><td colspan="2">■公开 □秘密 □机密</td><td>单位总数</td><td colspan="3">1</td></tr>
<tr><td colspan="2">子课题类型</td><td colspan="6">■基础前沿 □重大共性关键技术 □应用示范研究 □其他</td></tr>
<tr><td colspan="2">课题活动类型</td><td colspan="6">■基础前沿 □应用研究 □试验发展</td></tr>
<tr><td colspan="2">经费预算</td><td colspan="6">总预算 70.00 万元，其中中央财政专项经费 70.00 万元</td></tr>
<tr><td colspan="2" rowspan="2">子课题周期节点</td><td>起始时间</td><td>2023 年 12 月</td><td>结束时间</td><td colspan="2">2028 年 11 月</td></tr>
<tr><td>实施周期</td><td>共 60 个月</td><td>预计中期时间点</td><td colspan="2">2026 年 05 月</td></tr>
<tr><td rowspan="6">子课题承担单位</td><td>单位名称</td><td colspan="2">华南农业大学</td><td>单位性质</td><td colspan="2">事业单位</td></tr>
<tr><td>单位所在地</td><td colspan="2">广东省广州市</td><td>组织机构代码</td><td colspan="2">124400004554165634</td></tr>
<tr><td>通信地址</td><td colspan="2">广东省广州市天河区五山路 483 号</td><td>邮政编码</td><td colspan="2">510642</td></tr>
<tr><td>银行账号</td><td colspan="2">3602002609000310520</td><td>法定代表人姓名</td><td colspan="2">薛红卫</td></tr>
<tr><td>单位开户名称</td><td colspan="5">华南农业大学</td></tr>
<tr><td>开户银行（全称）</td><td colspan="5">中国工商银行股份有限公司广州五山支行</td></tr>
<tr><td rowspan="6">子课题负责人</td><td>姓 名</td><td>李青粉</td><td>性 别</td><td>□男☑女</td><td>出生日期</td><td>1987-02-05</td></tr>
<tr><td>证件类型</td><td>身份证</td><td>证件号码</td><td colspan="3">410901198702052023</td></tr>
<tr><td>所在单位</td><td colspan="5">华南农业大学</td></tr>
<tr><td>最高学位</td><td colspan="5">☑博士 □硕士 □学士 □其他</td></tr>
<tr><td>职 称</td><td colspan="3">□正高级 □副高级 ☑中级 □初级 □其他</td><td>职务</td><td></td></tr>
</table>

	电子邮箱	li63757416@163.com	移动电话	18998825286
子课题参加人数	6 人。其中：	高级职称 2 人，中级职称 2 人，初级职称 ___ 人，其他 2 人；		
		博士学位 3 人，硕士学位 1 人，学士学位 2 人，其他 ___ 人。		
子课题简介（限 500 字以内）	针叶树种原胚团的分化能力随着增殖时间的延长往往会出现不同程度的降低甚至丧失的问题已日渐成为林木生产和科学研究中的瓶颈，原因与增殖阶段内源激素及多胺等含量的不断累积及相关基因的表达密切相关。因此，探索并阐明针叶树原胚团分化能力降低背后的生理和分子机制有着重要的科学意义和应用价值。本子课题拟利用不同增殖时间的培养和各项生理指标的测定揭示欧洲云杉胚性愈伤在增殖阶段累积的内源激素和多胺对体细胞胚形态建成影响的生理机制，并利用单细胞转录组测序、生物信息学分析、基因编辑等技术解析增殖培养过程中分化能力降低的分子机制，可以为欧洲云杉遗传转化和种质创新提供重要的基因资源，同时推动针叶树体胚发生技术在大规模应用过程中瓶颈问题的解决。			

一、目标及考核指标、评测方式/方法

1. 子课题目标

本子课题拟利用不同增殖时间的培养和各项生理指标的测定揭示欧洲云杉胚性愈伤在增殖阶段累积的内源激素和多胺对体细胞胚形态建成影响的生理机制，并利用单细胞转录组测序、生物信息学分析、基因编辑等技术解析增殖培养过程中分化能力降低的分子机制，可以为欧洲云杉遗传转化和种质创新提供重要的基因资源，同时推动针叶树体胚发生技术在大规模应用过程中瓶颈问题的解决。

2. 考核指标

（1）挖掘云杉体胚发生的关键调节因子 1 个以上；

（2）鉴定细胞分化标记基因 1 个以上；

（3）筛选细胞特异元件 1 个以上；

（4）申请国家发明专利 1 件以上，发表高水平研究论文 2 篇以上；

（6）培养青年学术骨干 2 名，培养硕士研究生 3 名。

3. 评测方式/方法

（1）档案查阅

包括查阅原始实验数据、测序数据、学位论文、学术论文、专利证书或受理申请材料、财务审计报告及凭证。

（2）过程管理

通过专家指导、专题研讨、年度总结、中期检查、结题验收等加强过程管理。

4. 拟形成的代表性成果

云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制。

本子课题拟通过筛选和鉴定关键基因在欧洲云杉愈伤组织长期增殖培养过程中发挥的功能，揭示欧洲云杉原胚团随着增殖时间的延长分化能力降低的分子机制，以期推动针叶树体细胞胚胎发生技术在大规模应用过程中瓶颈问题的解决。

请填写下表。

子课题目标、预期成果与考核指标表

课题目标[1]	预期成果				考核指标[2]				考核方式(方法)及评价手段[4]
	预期成果名称			预期成果类型	指标名称	立项时已有指标值/状态	中期指标值/状态[3]	完成时指标值/状态	
本子课题拟利用不同增殖时间的培养和各项生理指标的测定揭示欧洲云杉胚性愈伤在增殖阶段累积的内源激素和多胺对体细胞胚形态建成影响的生理机制，并利用单细胞转录组测序、生物信息学分析、基因编辑等技术解析增殖培养过程中分化能力降低的分子机制，可以为欧洲云杉遗传转化和种质创新提供重要的基因资源，同时推动针叶树体胚发生技术在大规模应用过程中瓶颈问题的解决。	**主要成果**	1	云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制	□新理论 ☑**新原理** □新产品 □新技术 □新方法 □关键部件 □数据库 □软件 □应用解决方案 □实验装置/系统 □临床指南/规范 □工程工艺 □标准 ☑**论文** ☑**发明专利** □其他	指标 2.1 重要调控因子	/	挖掘胚性干细胞调控云杉体胚发生的重要调控因子 1 个。	挖掘云杉体胚形成过程中胚性干细胞启动、愈伤细胞干性维持以及分化形成体胚过程中的调控机理，鉴定重要调控因子 1 个以上。	查阅原始数据、学术论文、毕业论文以及专利技术文件等。
					指标 2.2 细胞分化标记基因	/	鉴定细胞分化标记基因 1 个。	鉴定细胞分化标记基因 1 个以上。	
					指标 2.3 细胞特异元件	/	筛选细胞特异元件 1 个。	筛选细胞特异元件 1 个以上。	
					指标 2.4 专利和论文	/	申请国家发明专利 1 件；发表学术论文 1 篇。	授权国家发明专利 1 件以上；发表学术论文 2 篇以上。	

科技报告考核指标	序号	报告类型[5]	数量	提交时间	公开类别及时限[6]
	1	子课题年度技术进展报告	4	2024 年 11 月、2025 年 11 月 2026 年 11 月、2027 年 11 月	延期公开（2 年）
	2	子课题中期技术进展报告	1	2026 年 05 月	延期公开（2 年）
	3	子课题最终科技报告	1	2029 年 01 月	延期公开（2 年）

其他目标与考核指标：

培养青年学术骨干 2 名，培养硕士研究生 3 名，其中项目结题验收时毕业研究生 2 名以上。

备注：

1. **“课题目标”**，应从以下方面明确描述：（1）研发主要针对什么问题和需求；（2）将要解决哪些科学问题、突破哪些核心/共性/关键技术；（3）预期成果；（4）成果将以何种方式应用在哪些领域/行业/重大工程等，并拟在科技、经济、社会、环境或国防安全等方面发挥何种的作用和影响。（5）所列主要成果原则上不超过5项，如有其他重要成果放在“其他”成果中表述。
2. **“考核指标”**，指相应成果的数量指标、技术指标、质量指标、应用指标和产业化指标等，其中，数量指标可以为专利、产品等的数量，论文代表作应注重质量，不以数量作为评价标准；技术指标可以为关键技术、产品的性能参数等；质量指标可以为产品的耐震动、高低温、无故障运行时间等；应用指标可以为成果应用的对象、范围和效果等；产业化指标可以为成果产业化的数量、经济效益等。同时，对各项考核指标需填写立项时已有的指标值/状态以及课题完成时要到达的指标值/状态。同时，考核指标也应包括支撑和服务其他重大科研、经济、社会发展、生态环境、科学普及需求等方面的直接和间接效益。如对国家重大工程、社会民生发展等提供了关键技术支撑，成果转让并带动了环境改善、实现了销售收入等。若某项成果属于开创性的成果，立项时已有指标值/状态可填写“无”，若某项成果在立项时已有指标值/状态难以界定，则可填写“/”。
3. **“中期指标”**，各专项根据管理特点，确定是否填写，鼓励阶段目标明确的项目课题填写中期指标。
4. **“考核方式方法”**，应提出符合相关研究成果与指标的具体考核技术方法、测算方法等。
5. **“科技报告类型”**，包括项目综合绩效评价（验收）前撰写的全面描述研究过程和技术内容的最终科技报告、项目年度或中期检查时撰写的描述本年度研究过程和进展的年度技术进展报告以及在项目实施过程中撰写的包含科研活动细节及基础数据的专题科技报告（如实验报告、试验报告、调研报告、技术考察报告、设计报告、测试报告等）。其中，每个项目在综合绩效评价（验收）前应撰写一份最终科技报告；研究期限超过2年（含2年）的项目，应根据管理要求，每年撰写一份年度技术进展报告；每个项目可根据研究内容、期限和经费强度，撰写数量不等的专题科技报告。科技报告应按国家标准规定的格式撰写。
6. **“公开类别及时限”**，公开项目科技报告分为公开或延期公开，内容需要发表论文、申请专利、出版专著或涉及技术诀窍的，可标注为“延期公开”。需要发表论文的，延期公开时限原则上在2年（含2年）以内；需要申请专利、出版专著的，延期公开时限原则上在3年（含3年）以内；涉及技术诀窍的，延期公开时限原则上在5年（含5年）以内。涉密项目科技报告按照有关规定管理。

二、子课题研究内容、研究方法及技术路线

（一）子课题的主要研究内容

1. 拟解决的重大科学问题或关键技术问题

阐明欧洲云杉原胚团分化能力的降低的分子机制

本研究拟通过测定不同处理和不同增殖时间的愈伤组织产生的不同发育阶段的体细胞胚中内源激素和多胺的含量，阐明原胚团增殖阶段激素和多胺的累积与体细胞胚形态建成间的内在关系。然后拟采用单细胞转录组测序以及亚细胞定位和基因编辑等技术筛选并鉴定关键基因在欧洲云杉愈伤组织长期增殖培养过程中发挥的功能，揭示欧洲云杉原胚团随着增殖时间的延长分化能力降低的分子机制。

2. 主要研究内容

（1）明确欧洲云杉原胚团分化能力的降低与增殖阶段内源激素和多胺累积间的内在关系

本研究拟通过测定不同增殖时间的愈伤组织产生的不同发育阶段的体细胞胚中内源激素和多胺的含量，阐明原胚团增殖阶段激素和多胺的累积与体细胞胚形态建成间的内在关系。

（2）阐明欧洲云杉原胚团胚性维持的分子机制

本研究拟采用单细胞转录组测序以及亚细胞定位和基因编辑等技术筛选并鉴定关键基因在欧洲云杉愈伤组织长期增殖培养过程中发挥的功能，揭示欧洲云杉原胚团随着增殖时间的延长分化能力降低的分子机制。

（二）子课题采取的研究方法

1. 欧洲云杉原胚团的增殖、分化和萌发实验

将欧洲云杉原胚团在添加细胞分裂素（拮抗物）的培养基上进行增殖培养，然后分别在不同增殖时间取样进行体细胞胚的诱导和萌发实验，并对不同增殖时间的愈伤组织及其分化的不同阶段的体细胞胚进行组织细胞学观察和内源激素、多胺的含量以及抗氧化酶活性的测定。最后，采用 R 软件对上述实验数据进行方差分析和多重比较，揭示欧洲云杉原胚团增殖过程中累积的内源激素和多胺影响体细胞胚发育的生理机制。

2. 单细胞转录组测序及生物信息学分析

对1中不同增殖培养时间的愈伤组织及其分化得到的体细胞胚进行单细胞转录组测序。分析欧洲云杉不同增殖时间的愈伤组织间及其分化的球形胚间在细胞水平上的差异，绘制愈伤组织及体细胞胚的细胞图谱、细胞分布图谱和基因分布图谱；构建愈伤组织到体细胞胚发育的轨迹（拟时分析）和欧洲云杉胚性维持分子调控网络，探究愈伤组织向体细胞胚转化的机制。最后，筛选出参与体细胞胚形态建成的关键基因，并采用实时荧光定量PCR（qRT-PCR）技术对关键基因的表达量进行验证，解析欧洲云杉原胚团增殖培养对体细胞胚形态建成影响的分子机制。

3. 关键基因在欧洲云杉原胚团胚性维持中的功能鉴定

根据2中生物信息学分析结果筛选出1~2个在欧洲云杉原胚团增殖过程中起关键作用的基因进行结构分析，并进行亚细胞定位；然后构建CRISPR/Cas9植物表达载体和超表达载体并进行遗传转化；最后，通过激光共聚焦显微镜检测、阳性转基因体细胞胚表型观察、qRT-PCR检测以及靶位点测序分析等手段，鉴定该基因在欧洲云杉原胚团增殖过程中的功能并阐明其调控机制。

（三）子课题的技术路线

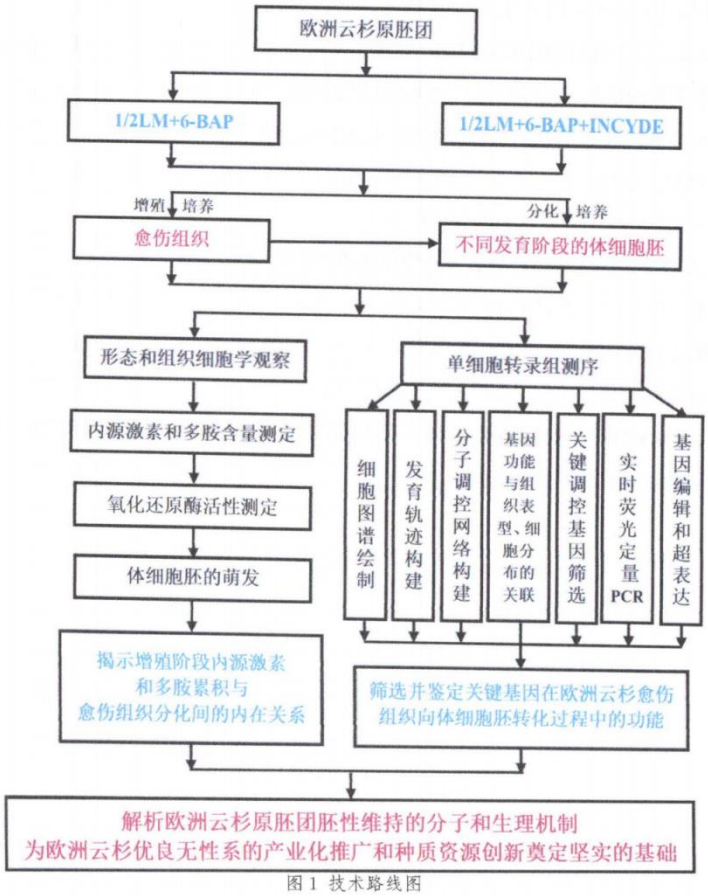


图 1 技术路线图

三、子课题年度计划

按年度制定子课题执行的计划进度，应将子课题的考核指标分解落实到年度计划中。

年度	任务	考核指标	成果形式
2023 年 12 月 | 2024 年 05 月	1.不同处理下愈伤组织的增殖培养实验； 2.不同处理不同增殖时间愈伤组织的生理指标测定。	1. 生理指标数据的获取。	1. 技术报告。
2024 年 06 月 | 2024 年 11 月	1. 欧洲云杉原胚团的分化； 2.全面分析总结项目已取得的成果，撰写并提交子课题年度技术进展报告。	1. 完成子课题年度技术进展报告 1 份。	1. 年度技术进展报告。
2024 年 12 月 | 2025 年 05 月	1. 欧洲云杉体细胞胚的萌发实验； 2. 体细胞胚不同部位的生理指标测定； 3. 专利申请书的撰写与提交。	1. 申请国家发明专利 1 件。	1. 专利申请书。
2025 年 06 月 | 2025 年 11 月	1. 体细胞胚的单细胞转录组测序； 2. 分析总结项目已取得的成果，完成相关学术论文的数据整理、撰写与发表； 3. 全面分析总结项目已取得的成果，撰写并提交子课题年度技术进展报告。	1. 绘制云杉体胚单细胞转录图谱。 2. 撰写学术论文 1 篇； 3. 完成子课题年度技术进展报告 1 份。	1. 测序数据； 2. 学术论文初稿； 3. 年度技术进展报告。
2025 年 12 月 | 2026 年 05 月	1. 图谱绘制以及关键基因的筛选等生物信息学分析； 2. 欧洲云杉原胚团胚性维持分子调控网络的构建。	1. 挖掘云杉体胚发生的关键调节因子 1 个。 **中期考核：** 1. 投稿学术论文 1 篇； 2. 申请国家发明专利 1 件； 3. 构建分子调控网络 1 个。	1. 关键调节因子； 2. 中期技术进展报告。
2026 年 06 月 | 2026 年 11 月	1. 关键基因的表达量验证实验（qRT-PCR）； 2. 分析总结项目已取得的成果，完成相关学术论文的数据整理、撰写与发表； 3. 全面分析总结项目已取得的成	1. 发表学术论文 1 篇； 2. 培养硕士研究生 1 名； 3. 完成子课题年度技术进展报告 1 份。	1. 学术论文； 2. 硕士研究生学位论文； 3. 年度技术进展报告。

	果，撰写并提交子课题年度技术进展报告。		
2026年12月 | 2027年05月	1. 亚细胞定位载体的构建和遗传转化以及激光共聚焦显微镜检测。	1. 鉴定细胞分化标记基因1个。	1. 细胞分化标记基因。
2027年06月 | 2027年11月	1. 基因编辑载体构建和遗传转化； 2. 撰写并提交年度技术进展报告。	1. 参加与研究主题相关的国内外学术会议并做学术报告1次； 2. 完成子课题年度技术进展报告1份。	1. 学术报告； 2. 年度技术进展报告
2027年12月 | 2028年05月	1. 超表达载体构建和遗传转化； 2. 分析总结项目已取得的成果，完成相关学术论文的数据整理、撰写与发表。	1. 筛选细胞特异元件1个； 2. 发表学术论文1篇。	1. 细胞特异元件； 2. 学术论文。
2028年06月 | 2028年11月	1. 全面分析总结项目取得的研究成果，撰写并提交子课题最终科技报告。	1. 培养硕士研究生1名； 2. 完成子课题最终科技报告1份。	1. 硕士研究生学位论文； 2. 最终科技报告。

四、需要约定的其他内容

1. 子课题承担单位将按照科技计划项目科学数据汇交的有关要求，制定科技资源汇交方案，将科学数据汇交到课题组和有关方面认可的科学数据中心，并出具汇交凭证。

2. 子课题承担单位将按照国家重点研发计划项目安全管理的有关要求，切实履行项目安全管理职责，加强人员培训教育，强化科研过程安全管理。

3. 子课题负责人严格按照时间节点向项目组和课题组提交研究报告，并接受项目组和课题组统一管理。

4. 子课题经费使用严格按照《国家重点研发计划资金管理办法》（财教〔2021〕178号）和任务经费预算合理使用。

5. 所有考核指标要求提交的研究成果为第一标注：国家重点研发计划（课题编号：2023YFD2200102）。

五、子课题参加人员基本情况表

填表说明： 1.专业技术职称：A、正高级 B、副高级 C、中级 D、初级 E、其他；
2.投入本课题的全时工作时间（人月）是指在课题实施期间该人总共为课题工作的满月度工作量；累计是指课题组所有人员投入人月之和；
3.课题固定研究人员需填写人员明细；
4.是否有工资性收入：Y、是 N、否；
5.人员分类代码：B、课题负责人 C、项目/课题骨干 D、其他研究人员；
6.工作单位：填写单位全称，其中高校要具体填写到所在院系。

序号	姓名	性别	出生日期	证件类型	证件号码	专业技术职称	职务	最高学位	专业	投入本课题的全时工作时间（人月）	人员分类代码	在课题中分担的任务	是否有工资性收入	工作单位
1	李青粉	女	1987-2-5	身份证	410901198702052023	C	无	博士	林木遗传育种	30	B	项目统筹设计实施	Y	华南农业大学林学与风景园林学院
2	毛娟	女	1984-9-18	身份证	21052219840918322X	B	无	博士	林木遗传育种	30	C	云杉转化体系构建	Y	华南农业大学林学与风景园林学院
3	邓成	男	1981-9-20	身份证	430522198109206371	C	无	博士	森林培育	30	C	生物信息学分析	Y	华南农业大学林学与风景园林学院
4	林元震	男	1979-5-24	身份证	350322197905242554	B	无	博士	林木遗传育种	30	C	云杉体胚体系优化	Y	华南农业大学林学与风景园林学院
5	黄小玲	女	1985-10-15	身份证	422801198510151041	C	无	硕士	林木遗传育种	30	C	云杉体胚体系构建	Y	华南农业大学林学与风景园林学院
6	周艳萍	女	1998-8-28	身份证	362201199808282429	E	无	学士	林业	30	D	云杉体胚生理实验	N	华南农业大学林学与风景园林学院
固定研究人员合计										180	/	/	/	/

流动人员或临时聘用人员合计	120	/	/	/	/
累计	300	/	/	/	/

六、经费预算

子课题（2023YFD2200102-02）承担单位基本情况表

<table>
<tr><td colspan="8">填表说明：1. 组织机构代码指企事业单位国家标准代码，单位若已三证合一请填写单位社会信用代码，无组织机构代码的单位填写“000000000”；
2. 单位公章名称必须与单位名称一致。</td></tr>
<tr><td colspan="2">子课题编号</td><td colspan="3">2023YFD2200102-02</td><td>执行周期（月）</td><td colspan="2">60</td></tr>
<tr><td colspan="2">子课题名称</td><td colspan="6">云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析</td></tr>
<tr><td rowspan="9">子课题承担单位</td><td>单位名称</td><td colspan="6">华南农业大学</td></tr>
<tr><td>单位性质</td><td colspan="6">事业单位</td></tr>
<tr><td>单位主管部门</td><td colspan="3">广东省人民政府</td><td>隶属关系</td><td colspan="2">直属</td></tr>
<tr><td>单位组织机构代码</td><td colspan="6">124400004554165634</td></tr>
<tr><td>单位法定代表人姓名</td><td colspan="6">薛红卫</td></tr>
<tr><td>单位所属地区</td><td>广东省</td><td colspan="2">地市
（市、自治州、盟）</td><td colspan="3">广州市</td></tr>
<tr><td>电子邮箱</td><td colspan="6">li63757416@163.com</td></tr>
<tr><td>通信地址</td><td colspan="6">广东省广州市天河区五山路 483 号</td></tr>
<tr><td>邮政编码</td><td colspan="6">510642</td></tr>
<tr><td rowspan="10">相关责任人</td><td rowspan="5">子课题负责人</td><td>姓名</td><td colspan="5">李青粉</td></tr>
<tr><td>身份证号码</td><td colspan="5">410901198702052023</td></tr>
<tr><td>工作单位</td><td colspan="5">华南农业大学</td></tr>
<tr><td>电话号码</td><td>020-85280256</td><td>手机号码</td><td colspan="3">18998825286</td></tr>
<tr><td>电子邮箱</td><td>li63757416@163.com</td><td>邮政编码</td><td colspan="3">510642</td></tr>
<tr><td>通信地址</td><td colspan="5">广东省广州市天河区五山路 483 号林学与风景园林学院 616 室</td></tr>
<tr><td rowspan="4">财务部门负责人</td><td>姓名</td><td colspan="5">朱俏萍</td></tr>
<tr><td>电话号码</td><td>020-85280087</td><td>手机号码</td><td colspan="3">13828428501</td></tr>
<tr><td>传真号码</td><td colspan="5">020-85280087</td></tr>
<tr><td>电子邮箱</td><td colspan="5">104725665@qq.com</td></tr>
</table>

子课题预算表

子课题编号：2023YFD2200102-02　子课题名称：云杉体胚发生过程中胚性干细胞启动、维持与转变的分子机制解析　金额单位：万元

预算科目名称	金额
一、中央财政专项资金	**70.00**
（一）直接费用	**58.33**
1. 设备费	0.00
1.1 购置设备费	0.00
1.2 试制设备费	0.00
1.3 设备改造与租赁费	0.00
2. 业务费	46.155
2.1 材料费	18.685
2.2 测试化验加工费	13.50
2.3 燃料动力费	1.56
2.4 出版/文献/信息传播/知识产权	4.80
2.5 会议/差旅/国际合作交流费	7.26
2.5.1 会议费	1.10
2.5.2 差旅费	6.16
2.5.3 国际合作交流费	0.00
2.6 其他支出	0.35
2.6.1 土地租凭费	0.00
2.6.2 财务验收审计费	0.35
3. 劳务费	12.175
3.1 人员劳务费	8.80
3.2 专家咨询费	3.375
（二）间接费用	11.67
二、其他来源资金	0.00
三、合计	70.00

预算说明

一、中央财政资金

预算的编制要坚持任务相关性、政策相符性和经济合理性，实事求是编制提出课题预算。填报时，直接费用应按设备费 、业务费 、劳务费三个类别填报，每个类别结合科研任务按支出用途进行说明。除 50 万元以上的设备外，其他费用只提供基本测算说明，不需要提供明细。

1.设备费（是指项目实施过程中购置或试制专用仪器设备，对现有仪器设备进行升级改造，以及租赁外单位仪器设备而发生的费用等。计算类仪器设备和软件工具可在设备费科目编列。填报时，50 万元以上的设备详细说明，50 万元以下的设备费用分类说明）

无。

2.业务费（是指在项目实施过程中消耗的各种材料、低值易耗品等、发生的测试化验加工、燃料动力、出版文献、信息传播、知识产权事务、会议、差旅、国际合作与交流以及其他与项目实施直接相关的各项费用。编报时，对单笔大额支出、对外委托支出重点说明）

业务费共 46.155 万元，其中包括材料费 18.685 万元，测试化验加工费 13.500 万元，燃料动力费 1.560 万元，出版/文献/信息传播/知识产权事务费 4.800 万元，会议/差旅/国际合作交流费 7.260 万元，其他支出 0.350 万元。全部为中央财政资金。

2.1 材料费 18.685 万元

本子课题主要开展云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究，涉及到组培培养、载体构建、遗传转化和基因编辑等研究，需要购买相关的试剂、耗材等。

（1）植物组织培养常用试剂和耗材费，9.755 万元。

主要用途：用于云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中，胚性愈伤诱导、愈伤继代增殖、体胚诱导等，植物组织培养常用试剂主要用于培养基制备、转化植株的筛选与培养所涉及到的试剂和材料。

测算依据：预算中的数量是根据课题研究任务的需要进行测算，报价为参考目

前市场上的平均报价。具体如下：

MS 培养基，用于云杉愈伤组织原胚团继代、分化、再生及遗传转化的必需培养基配制，预计需要购置 10 瓶，单价 400 元/瓶，共 400 元/瓶×10 瓶=**0.400 万元**。

1/2MS 培养基，用于云杉成熟子叶胚材料生根培养基的配制，每瓶可配 50 L，预计需购置 10 瓶，单价 350 元/瓶，共 350 元/瓶×10 瓶=**0.350 万元**。

植物激素等添加剂，用于云杉愈伤组织原胚团继代、分化和再生培养基的配制，预计需要购置 2，4-二氯苯氧乙酸 2,4-D 一瓶，6-苄氨基嘌呤 6-BA，苯基噻二唑脲 TDZ，脱落酸，水解酪蛋白，肌醇，活性炭各一瓶，单价分别为 100 元/瓶，550 元/瓶，1100 元/瓶，500 元/瓶，1000 元/瓶，250 元/瓶和 1200 元/瓶，（100+550+1100+500+1000+250+1200）元/瓶×1 瓶=**0.470 万元**；植物凝胶 3 瓶，单价 1800 元/瓶，共 1800 元/瓶×3 瓶=**0.540 万元**；蔗糖和琼脂粉各 40 瓶，单价分别为 60 元/瓶和 150 元/瓶，共（60+150）元/瓶×50 瓶=**1.050 万元**；二水合氯化钙，氯化钾，硼酸，硝酸锌六水合物，硫酸镁 七水合物，硫酸锰一水合物，硫酸铜 五水合物，硫酸锌七水合物，硫酸亚铁七水合物，碘化钾，磷酸钾一元，钼酸钠二水合物，烟酸，乙二胺四乙酸二钠盐二水合物，吡哆醇盐酸盐，硫胺盐酸盐和硫酸铁(II) 七水合物各 2 瓶，单价分别为 500 元/瓶，660 元/瓶，830 元/瓶，1450 元/瓶，1000 元/瓶，1050 元/瓶，730 元/瓶，570 元/瓶，210 元/瓶，1050 元/瓶，1450 元/瓶，1030 元/瓶，470 元/瓶，950 元/瓶，650 元/瓶，810 元/瓶，1000 元/瓶，共（500+660+830+1450+1000+1050+730+570+210+1050+1450+1030+470+950+650+810+1000）元/瓶×1 瓶=**1.440 万元**。

植物组织培养常用耗材，5.655 万元。

一次性培养皿和组培瓶，用于云杉愈伤组织原胚团继代和分化，萌发和植株再生，预计需要购置各 50 箱，单价均为 375 元/ 箱，共 375 元/箱×2×50 箱=**3.755 万元**。

营养土，蛭石，珍珠岩，营养盆，用于云杉植株移栽使用，预计需要购置各 10 袋，单价分别为 150 元/袋，50 元/袋，100 元/袋和 300 元/袋，共（150+50+100+300）元/袋×20 袋=**1.200 万元**。

无粉乳胶手套，用于云杉组织培养实验操作时使用，预计需要购置 5 箱，单价为 700 元/箱，共 700 元/箱×10 箱=**0.700 万元**。

（2）植物遗传转化试剂费和常用耗材，8.780 万元。

主要用途：用于云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中，关键调控基因克隆和载体构建和遗传转化株系的分子水平鉴定。

测算依据：预算中的数量是根据课题研究任务的需要进行测算，报价为参考目前市场上的平均报价。具体如下：

质粒提取试剂盒，RACE 试剂盒和 LR 克隆酶 Gateway 系统，用于云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中关键调控基因的克隆和连接，预计各需要购置 3 盒，3 盒和 1 盒，单价分别为 1500 元/盒，8600 元/盒和 7500 元/盒，共（8600+1500）元/盒×3 盒+7500 元/盒×1 盒=**3.780 万元**；此外，还需要购置大肠杆菌 DH5α 感受态和农杆菌感受态 EHA105 感受态各 6 包，单价分别为 250 元/包和 900 元/包，共（250+900）元/包×6 包=**0.690 万元**。

验证关键基因的表达量需要荧光实时定量 PCR 实验：预计需要 RNA 提取试剂盒和反转录试剂盒各 5 盒，价格分别为 3200 元/盒和 1300 元/盒，此外，需要荧光定量分析试剂 1 包，价格为 1800 元/包，共（3200+1300）元/盒×5 盒+1800 元/包×1 盒=**2.430 万元**。

植物遗传转化常用耗材，用于云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中分子实验，预计需要 0.2 mL 离心管，1.5 mL 离心管，2 mL 离心管，10 ml 离心管，50 mL 离心管，10 μL 移液器吸头，200 μL 移液器吸头，1000 μL 移液器吸头，5000 μL 移液器吸头各 5 箱，单价分别为：1000 元/箱，800 元/箱，800 元/箱，1200 元/箱，700 元/箱，1000 元/箱，800 元/箱，600 元/箱和 900 元/箱，共（1000+800+800+1200+700+ 1000+800+600+900）×2 箱=**1.560 万元**；此外，还需要定量 96 孔板和定量 96 孔板密封膜各 2 盒，定价分别为 200 元/盒和 1400 元/盒，共（200+1400）元/盒×2 盒=**0.320 万元。**

2.2 测试化验加工费 13.500 万元

子课题主要开展云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究，涉及到组培培养、载体构建、遗传转化和基因编辑等研究内容，其中目标基因的筛选与克隆、载体构建和转化株系的分子鉴定需要大量合成引物、DNA 片段测序，转化株系的表型鉴定，需要进行生理指标测试等试验。

（1）愈伤组织的单细胞测序，7.200 万元。

主要用途：根据研究目标和任务，子课题在云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中，为了筛选出调控云杉胚性维持的重要基因，需要对不同时间处理的原胚团进行单细胞转录组测序。

测算依据：需对 2 个处理的样品（每个样品 2 次生物学重复）进行单细胞转录组的测序，根据广州基迪奥生物科技有限公司报价，单细胞测序价格为 19000 元/个样品（120 GB 数据），共需 18000 元/个样品×4 个样品=7.200 万元。

（2）引物合成，2.500 万元。

主要用途：根据研究目标和任务，子课题在云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中，进行大量基因克隆、克隆载体构建、遗传转化载体构建和转基因植株的分子检测，需要进行引物合成和目标序列测定。

测算依据：需合成原胚团组织特异性启动子克隆引物 100 条、重要功能相关基因克隆 500 条、目标基因的克隆载体构建引物 100 条、遗传转化载体构建引物 100 条、转化植株中载体序列的鉴定引物 100 条和目标基因插入情况的鉴定引物 100 条，共 1000 条。根据广州基迪奥生物科技有限公司报价，引物合成价格为 1 元/碱基，每条引物平均 25 个碱基，每条引物合成需 25 元，共 25 元/条引物×1000 条**=2.500 万元**。

（3）目标基因克隆序列测定，2.000 万元。

主要用途：在关键基因的遗传转化研究中，目标基因 PCR 片段、重组载体和遗传转化植株目标片段，需要进行特定目标序列的 Sanger 测序，以判断序列是否为目标序列。

测算依据：目标基因的克隆与验证，需测定 200 个克隆片段；遗传转化载体的构建与验证，测定 100 个克隆片段，每个片段正反各测一个反应，预计需测序 600 个反应。获得遗传转化阳性植株后，需进行载体序列和目标基因的整合情况的分子鉴定，预计需测序 400 个反应。共计需要进行 Sanger 测序 1000 个反应。根据广州基迪奥生物科技有限公司报价，每个反应 20 元，共需要 20 元/个反应×1000 个**=2.000 万元**。

（4）生理指标的测定，1.800 万元。

主要用途：根据研究目标和任务，子课题在云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析的研究中，为了研究不同时长增殖培养过程中原胚团生理水平上的变化，需要对其内源激素的水平进行测定。

测算依据：根据实验需要一共设置三个处理，每个处理 3 个生物学重复，需要测定生长素，细胞分裂素，赤霉素，脱落酸和油菜素内酯，根据诺敏科达生物科技有限公司的报价，生长素，细胞分裂素，赤霉和脱落酸四种激素的测定为 1000 元/样品，油菜素内酯测定为 1000 元/样品，因此，激素的测定共需（1000+1000）元/样品×9 样品=1.800 万元。

2.3 燃料动力费，1.560 万元。

主要用途：用于培养云杉愈伤、体胚和幼苗的人工培养箱、组织培养间、人工气候室及配套设备的水电消耗等燃料动力费。

测算依据：子课题进展过程中，由于保存植物材料的需要，需使用全自动温室、光照培养箱、空调、超低温冰箱、-20 度和 4 度冰箱、恒温摇床等大型仪器，平均每月耗电约 200 度，按工业用电平均价格 1.02 元/度，共需电费 1.02 元/度×200 度/月×50 个月=1.020 万元；子课题实施过程中，温室和苗圃中繁殖试验植物材料需要消耗自来水，平均每月耗水约 20 吨，按水费 5.40 元/吨，共需水费 5.40 元/吨×20 吨/月×50 个月=0.540 万元。合计 1.560 万元。

2.4 出版/文献/信息传播/知识产权事务费，4.800 万元。

主要用途：用于本子课题考核指标所规定的文献检索、查新、论文发表、专利申请等费用，还包括研究过程中相关材料打印、复印、装订及邮寄等所产生的费用。

测算依据：预计课题研究成果在国内外重要刊物发表高质量学术论文 2 篇，平均每篇论文的版面费 10000 元，计 10000 元/篇×2 篇=2.000 万元；因研究需要进行文献信息检索、文献资料、查新等费用，按每年 2000 元，计 2000 元/年×5 年=1.000 万元；子课题预计申请发明专利 1 件，相关费用按每件 8000 元，计 8000 元/件×1 件=0.800 万元；课题总结报告打印、复印、装订费，各子任务成果宣传及展示材料制作等费用，按每年 1000 元，计 1000 元×5 年=0.500 万元；课题和子任务研究相关技术和财务等材料、资料邮寄费，按每年 1000 元，计 1000 元×5 年=0.500 万元。合计 4.800 万元。

2.5 会议/差旅/国际合作交流费，7.260 万元。

（1）会议费，1.100 万元。

主要用途：用于支付子课题召开会议所需相关费用。

测算依据：子课题需要召开子课题启动、子课题年度研讨、子课题中期评估、子课题总结等协作会议等 5 次，会期每次 2 天，平均每次 2 人参加。按参会 550 元/人天，每次会议需 550 元/人天×2 人×2 天=0.220 万元，5 次会议共需 0.220 万元/次×5 次=1.100 万元。

（2）差旅费，6.160 万元

主要用途：主要用于子课题开展过程中，在研人员、相关研究生野外采集研究材料，参加与课题研究相关会议、课题交流外埠出差以及因试验、协作、材料等课题相关事宜用于市内交通所需要的旅差费。

测算依据：开展云杉体胚形成过程中胚性干细胞的启动、干性维持以及命运转变的分子机制解析研究，由子课题承担单位所在地广州去往甘肃、湖北、云南等地进行实验材料的采集工作，平均每年 1 人次，平均每人次出差 5 天，住宿 4 天，平均往返路费 1500 元/人次，按照按照《中央和国家机关差旅费管理办法》（财行[2013]531 号）和《关于调整中央和国家机关差旅住宿费标准等有关问题的通知》（财行[2015]497 号）标准，一般地区补助费 180 元/人天，住宿费平均 300 元/人天，每年需外埠差旅费（180 元/人天×5 天+300 元/人天×4 天+1500 元/人次）×1 人次=3600 元，5 年共需 3600 元/年×5 年=1.800 万元；子课题研究人员和研究生赴外地参加中国林业学术大会、中国林业青年学术年会等课题相关学术会议和交流，平均每年 1 人次，平均每人次差旅 5 天，住宿 4 天，平均往返路费 1500 元/人次，会议注册费每人次 1500 元，根据相关政策和财务标准规定，补助费 180 元/人天，住宿费平均 300 元/人天，每年需外埠差旅费（180 元/人天×5 天+300 元/人天×4 天+1500 元/人次+1500 元/人次）×1 人次= 5100 元，5 年共需 5100 元/年×5 年=2.550 万元；子课题开展过程中，因市内采样、仪器维修、实验交流、汇报学习等相关事宜用于市内交通费，每年需 3620 元，5 年共需 3620 元/年×5 年=1.810 万元。合计 **6.160 万元**。

（3）国际合作交流费，0 万元。

无。

2.6 其他支出，0.350 万元。

财务验收审计费：0.350 万元

3.劳务费 12.175 元。

主要用途：在子课题执行过程中，除子课题组固定成员外，还需要研究生、杂交制种、野外采种和组培间管护的临时劳务人员进行研究和辅助性劳务工作，需支付子课题执行过程中培养的研究生和雇佣的临时劳务人员的劳务费。

测算依据：硕士研究生科研补贴按人均 800 元/人·月，临时用工人员按聘用人员的实际情况测算，各类人员在项目中的投入时间按照完成任务的预计时长测算。

3.1 硕士研究生劳务费 4.800 万元

参与本子课题研究的硕士研究生 2 名，主要负责云杉体胚体系建立与优化、关键调控因子鉴定、基因功能验证等实验以及相关数据分析整理等工作。硕士平均每人攻读硕士时间为 3 年，平均每人每月支付补助费 800 元，每年按工作 10 月计，共需硕士生劳务费 2 人×10 月/年×800 元/人月×3 年＝4.800 万元。合计 **4.800 万元**。

3.2 临时聘用人员劳务费 4.000 万元

由于子课题研究中采集试验材料、组培继代、温室及大田试验、抗性调查、生长物候调查等工作量大、持续时间长，需要雇用大量临时工人。5 年至少需要 200 个人工，按平均每人工支付劳务费 200 元算，临时工劳务费需 200 元/人工×200 人工=4.000 万元，其中：

试验材料采集：采集云杉未成熟种子用于愈伤诱导，每年需 10 个人工，5 年需 10 个人工/年×5 年=50 个人工；

组培管理：遗传转化技术研究，需要大量的组培操作，组培苗继代、洗刷组培瓶等工作，每年需 20 个人工，5 年共需 20 个人工/年×5 年=100 个人工；

温室管理：遗传转化植株在温室进行移栽、育苗等需雇工对温室的植株进行移栽、除草、除虫、施肥、浇水、管护，每年 16 个人工，5 年共需 10 个人工/年×5 年=50 个人工；

3.3 专家咨询费：3.375 万元

主要用途：用于支付课题执行过程中各类会议、现场查定、通讯等聘请、咨询专家的费用。

测算依据：子课题总计召开会议 5 次，平均每次聘请 2 人，平均每次 1 天，

根据《中央财政科研项目专家咨询费管理办法》（财科教[2017]128 号）文件规定，专家费按每人每天 2800 元（税前）计，5 年 5 次计：2800 元/人天×1 天×2 人×5 次=2.800 万元；本子课题与其他子课题之间以其他通讯形式临时咨询每年平均 5 人次，按 230 元/人次计算，5 年共需要 230 元/人次×5 人次/年×5 年=0.575 万元。合计 **3.375 万元**。

二、其他来源资金

对其他来源资金主要用途、支出预算做简要说明。

无。

七、相关附件

1. 乙方与参加单位有关协议（须加盖乙方与参加单位公章、法人签字签章；协议文件须扫描上传。如无参加单位，则不填）；

任务书签署

甲乙双方根据《国务院印发关于深化中央财政科技计划（专项、基金）管理改革方案的通知》（国发〔2014〕64号）、《国务院关于优化科研管理提升科研绩效若干措施的通知》（国发〔2018〕25号）、《国务院办公厅关于改革完善中央财政科研经费管理的若干意见》（国办发〔2021〕32号）、《科技部 财政部关于印发<国家重点研发计划管理暂行办法>的通知》（国科发资〔2017〕152号）、《财政部 科技部关于印发<国家重点研发计划资金管理办法>的通知》（财科教〔2016〕113号）、《科技部财政部关于印发<中央财政科技计划（专项、基金等）监督工作暂行规定>的通知》（国科发政〔2015〕471号）等有关文件规定，以及有关法律、政策和管理要求，依据项目立项通知，签署本任务书。

同时，本单位和课题负责人**郑重承诺**：对本课题所有成果产出（包括但不限于新产品、新技术、标准、论文、专利等）的真实性、与项目（课题）的关联性等负责，将按要求落实科研作风学风和科研诚信主体责任；课题经费全部用于与本课题研究工作相关的支出，不截留、挪用、侵占，不用于与科学研究无关的支出；接受并积极配合相关部门的监督检查。如有违反，本单位和课题负责人以及相关成果产出者愿接受项目管理专业机构和相关部门做出的各项处理决定，包括但不限于终止课题执行、追回课题经费，取消一定期限国家科技计划项目（课题）申报资格，记入科研诚信严重失信行为数据库以及主要负责人接受相应党纪政纪处理等。

课题牵头承担单位（甲方）：

法定代表人签字（签章）：

（公章）

2023年12月13日

课题负责人签字（签章）：

2023年12月13日

子课题承担单位（乙方）：华南农业大学

法定代表人签字（签章）：

（公章）

2023年12月13日

子课题负责人签字（签章）：

2023年12月13日

1.2 细胞分裂素对川西云杉胚性细胞系长期增殖的作用机制

中国博士后科学基金资助证书

Certificate of China Postdoctoral Science Foundation Grant

华南农业大学 李青粉 博士后研究人员，

经专家评审，获得第 62 批中国博士后科学基金面上资助一等资助。特颁此证。

This is to certify that has received the –class General Financial Grant from the China Postdoctoral Science Foundation.

博士后编号Postdoctor No.: 173001

资助编号Grant No.: 2017M622717

2017 年 11 月 13 日

1.3 贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制劳务采购项目

HXKJHT20221781

技术服务合同

项目名称： 贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制劳务采购项目

接受方（甲方）： 国家林业和草原局中南调查规划院

提供方（乙方）： 华南农业大学

签订时间： 2022年 7月 4日

签订地点： 湖南长沙

有效期限： 合同签订之日至2022年12月30日

1

接受方（甲方）：国家林业和草原局中南调查规划院

住　所　地：湖南省长沙市香樟东路 143 号

法定代表人：周学武

项目联系人：丁山

联系方式：176173146605

通讯地址：湖南省长沙市香樟东路 143 号

电　话：0731-85679737　传　真：0731-85679825

电子信箱：

提供方（乙方）：华南农业大学

住　所　地：广东省广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：邓成

联系方式：18825090920

通讯地址：广东省广州市天河区五山路 483 号

电　话：18825090920　传　真：

电子信箱：154083733@qq.com

根据《中华人民共和国民法典》的有关规定，甲、乙双方经过平等协商，在真实、充分地表述各自意愿的基础上，自愿签订本劳务合同，以兹双方共同遵守：

第一条　甲方因开展 贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制 项目需要，接受乙方提供 野外调查及成果编制 方面的劳务。

第二条　甲方要求乙方完成的工作目标、工作内容和工作方式如下：

1、工作目标：贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制项目工作。

2、工作内容：协助甲方完成：（1）收集并核实与贡觉县天保工程建设相关的资金分解、任务完成、组织管理、责任落实、资源保护等资料；（2）对各项资金使用、森林管护、责任落实、公益林建设、森林抚育等情况进行现地核实查验；（3）调查、评估工程实施的生态效益、社会效益、经济效益；（4）完成贡觉县林地小班数据更新及补充调查工作，检查并修正逻辑、拓扑关系，制作调查数据库；（5）汇总调研与调查材料，综合分析工程建设任务完成情况、各项政策的落实情况和执行情况、工程管理情况、工程实施的建设成效和目标实现情况等，构建科学合理的评估指标体系，客观评价工程实施成效；（6）相关成果汇编、验收、评审事宜。

3、工作方式：协作。

第三条　乙方应按下列要求提供劳务：

1、工作地点：湖南省长沙市、西藏自治区昌都市、广东省广州市。

2、工作进度：按照甲方所确定的工作进度计划执行。

3、工作成果：（1）外业调查成果材料；（2）相关成果编制材料（含报告、附表、附图等）。

4、质量要求：按照甲方要求进行。

5、其他要求：在整个项目工作期间，工作认真负责，遵守项目所在地的政策法规和风俗习惯，处理好与地方干部、当地群众和有关人员的关系，自觉注意自己的言行举止，不得参与和从事违法违纪的活动，自觉维护甲方形象。乙方人员必须严格遵守甲方安全生产和保密管理的各项规章制度，确保安全生产，违反甲方安全生产和保密管理规章制度而造成的一切后果，甲方概不负责。严格保守在合同期内可能知晓的甲方的商业和技术秘密，在合同期内取得的全部资料的所有权归甲方，未经甲方同意不得擅自提供给第三方。对所提供的成果的真实性和可靠性负责，并负责成果释疑和纠错，同时应按甲方的要求对成果进行完善和修改。外业工作期间，乙方应为专业技术人员购买相关的野外保险，以及劳保用品、外业工作药品等，乙方专业技术人员如果发生人身安全事故、因病住院等，均由乙方负责妥善处理，并承担由此所发生的相关费用。

第四条　为保证乙方有效开展工作，甲方应当向乙方提供下列技术资料、工作条件和协作事项：

1、提供技术资料：

（1）相关标准、规程规范及有关文件；

（2）有关基础资料。

2、提供工作条件和协作事项：

（1）指导和协助乙方完成相关技术工作；

（2）组织乙方与有关部门进行协调和沟通等。

3、甲方提供上述技术资料、工作条件和协作事项的时间及方式：自合同签订之日起至项目完成时止。

第五条　合同期限

1、本合同期限：合同签订之日至 2022 年 12 月 30 日；

2、乙方应在合同期限内完成本合同第二条、第三条所规定的全部工作内容，并满足质量要求。非乙方原因导致乙方在合同期限内未能完成本合同第二条、第三条所规定的全部工作内容时，甲、乙双方可协商终止合同或变更合同。

第六条　乙方在承担项目工作任务期间，应遵守国家法律、法规和甲方的项目管理要求，服从甲方安排，按贡觉县天然林资源保护工程建设成效监测（2017-2020）工作野外调查及成果编制工作方案和有关要求开展工作，自觉维护甲方形象。

第七条　根据项目工作需要甲方可对乙方相关人员进行相应的技术培训，乙方应认真完成培训。如果在培训后乙方相关人员不能适应所从事的工作，甲方有权解除本合同。

第八条　服务费用及支付方式：

1、劳务费用总额为：人民币壹拾伍万元整（¥150000）。

2、劳务费用由甲方 在工作结束后一次性 支付乙方。

乙方开户银行名称、地址和帐号为：

开户银行：广州工行五山支行

地址：广州市天河区五山路 483 号

银行帐号：3602002609000310520

税务登记号：124400004554165634

3、乙方在劳务费用的使用上要符合国家有关财务规定，不得有损甲方声誉，甲方有权对乙方劳务费用的使用情况进行检查，但不得妨碍乙方的正常工作。

4、本条约定之费用已包括全部成本费用及报酬，除此之外，甲方无需再向乙方支付其他任何费用。

5、在甲方支付劳务费用前，乙方应提供等额增值税专用发票给甲方。否则，甲方有权迟延付款直至乙方提供发票为止。

第九条　乙方履行本合同过程中应遵守的保密义务如下：

1、保密内容（包括技术信息和经营信息）：项目涉及的数据、资料、阶段性成果、最终成果等，按《保密法》要求执行；

2、涉密人员范围：项目参与人员及接触人员；

3、保密期限：按国家有关规定执行；

4、涉密责任：按国家有关规定执行。

第十条　双方确定，按下列标准和方式对乙方提供的劳务工作成果进行验收：

1、工作成果形式：按甲方要求提供；

2、验收标准：由甲方按验收标准验收；

3、验收的时间和地点：按甲方要求的时间和地点。

第十一条　双方确定，在本合同有效期内，甲方指定丁山为甲方项目联系人，乙方指定邓成为乙方项目联系人。项目联系人承担以下责任：

1、负责双方意见的交流和传递；

2、通报工作进展；

3、协调双方合作事项。

一方变更项目联系人的，应当及时以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十二条　合同解除

1、乙方提供劳务不能满足甲方项目进度需要的，经过甲方书面催告仍然无法满足甲方项目进度需要，则甲方有权解除本合同，但应按乙方实际工作天数或工作成果支付相应的劳务费用；

2、乙方提供劳务期间出现违法违规行为，严重影响甲方项目权益或甲方声誉的，甲方有权解除本合同，并追究乙方相关责任；

3、乙方提供劳务期间未对乙方技术人员进行相应安全教育、采取安全防范措施的，与提供劳务工作安全保障的，甲方有权解除本合同，但应按乙方实际劳务工作天数或劳务工作成果支付相应的劳务费用；

4、乙方在合同期限内要求提前解除合同的，应提前5日通知甲方并征得甲方同意后方可解除本合同，甲方按乙方实际劳务工作天数

或劳务工作成果支付相应的劳务费用。乙方如单方擅自解除合同，甲方有权拒付乙方的劳务费用，并要求乙方赔偿甲方由此产生的全部经济损失；

5、双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

（1）、自然灾害等不可抗力；

（2）、甲方与业主单位因故解除合同。

第十三条　特别约定

1、乙方应在工作结束申请支付劳务费用前，或解除、终止合同前将所承担的有关工作向甲方进行移交，需要说明的应附书面说明，否则甲方有权拒付乙方的劳务费用。

2、乙方应根据甲方的项目工作内容与要求，在正式提供劳务前，应自行为乙方人员投保有关险种。如乙方人员在甲方项目工作期间发生人身意外伤害事故等，乙方所受到的全部损失应由保险公司进行赔偿，甲方不承担责任。乙方因个人身体健康状况出现问题引起的受伤和意外身亡等情况，甲方不承担责任。

3、乙方（含乙方人员）违反法律法规所引起的一切法律责任均由乙方自行承担。乙方因违反甲方项目管理要求或由于乙方自身的过错行为致使乙方人员受到伤害或给他人造成损失的，责任由乙方承担。

4、乙方在项目劳务服务期间完成的与甲方工作有关的相关智力成果的专利申请权、著作权等相关知识产权均归甲方所有。

5、乙方在项目劳务期间应注意遵守工作纪律和项目管理规定，

保守因劳务原因获知的他人成果和知识产权，严格保守国家秘密。

第十四条　双方确定：

1、在本合同有效期内，甲方利用乙方提供的劳务工作成果所完成新的技术成果，归___甲___方所有；

2、在本合同有效期内，乙方利用甲方提供的技术资料和工作条件完成的新的技术成果，归___甲___方所有。

第十五条　违约条款

1、乙方未经甲方同意，提前解除合同，甲方不应向其支付劳务报酬，乙方为此给甲方造成的一切经济损失及维权损失（包括但不限于律师费、诉讼费等费用）由乙方承担；

2、因乙方原因，乙方逾期完成合同工作内容，甲方为此产生的一切经济损失及维权损失（包括但不限于律师费、诉讼费等费用）由乙方承担；

3、乙方在劳务期间内，因主观过错或过失给第三方造成损失的，由乙方承担赔偿责任。如因此牵连甲方，甲方为此产生的一切经济损失及维权损失（包括但不限于律师费、诉讼费等费用）由乙方承担；

4、甲方在乙方劳务成果验收确认后，未按照合同约定付款的，应依照未付劳务费用每日万分之一的标准，向乙方支付逾期付款违约金；

5、__甲__方违反本合同第四 条规定，应当 承担违约责任，乙方有权要求顺延因此受到影响的服务成果提交时间，如果甲方技术资料或者工作条件提供时间严重耽误乙方人员工作，乙方有权要求甲方对

耽误时间进行适当补偿；

6、乙方违反本合同第二、第三、第六、第十条规定，应当承担违约责任，甲方有权要求乙方对推迟提交成果时间引起的损失进行赔偿，如果乙方提供的服务成果不合格，甲方有权要求乙方进行返工，直到服务成果符合要关规定。如果这种不合格的状况在乙方采取补救措施后仍然存在的，甲方有权单方面解除本合同并就本方受到的损失进行索赔。

第十六条　本合同的变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在 5 日内予以答复；逾期未予答复的，视为同意：

1、甲方要求乙方提前完成服务并提前提交服务成果；

2、乙方因甲方未按照合同约定及时提供技术资料或者工作条件、协作事项而推迟提供服务成果时间。

第十七条　双方约定本合同其他相关事项为：无。

第十八条　双方因履行本合同而发生的争议，应当友好协商解决，协商不成，任何一方均可向甲方住所地人民法院起诉。

第十九条　本合同一式 捌 份，甲方执 肆 份、乙方执 肆 份，具有同等法律效力。

第二十条　本合同经双方签字盖章后生效。

甲方：国家林业和草原局中南调查规划院（盖章）

法人代表（签字）：_____

委托代理人（签字）：_____

2022 年 7 月 4 日

乙方：华南农业大学（盖章）

法人代表（签字）：_____

委托代理人（签字）：_____

2022年 7 月 4 日

11

1.4 湖北省国家湿地公园数据整合汇编

合同编号：

技术服务合同

项目名称：湖北省国家湿地公园数据整合汇编

委 托 方：中国林业科学研究院资源信息研究所

（甲 方）

受 托 方：华南农业大学

（乙 方）

签订时间：2022 年 11 月 16 日

签订地点：北京

有效期限：2022 年 12 月-2023 年 6 月

中华人民共和国科学技术部印制

技术服务合同

委托方（甲方）：中国林业科学研究院资源信息研究所

住　所　地：北京市海淀区东小府 2 号

法定代表人：王宏

项目联系人：凌成星

联系方式 ：15001231520

通讯地址：北京市海淀区东小府 2 号中国林业科学研究院资源信息研究所

电　　话：01062889954　　传真：01062888500

电子信箱：Lingcx@ifrit.ac.cn

受托方（乙方）：华南农业大学

住　所　地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：邓成

联系方式 ：18825090920

通讯地址：广州市天河区五山路 483 号

电　　话：02087575942　　传真：

电子信箱：154083733@qq.com

本合同甲方委托乙方就湖北省江夏藏龙岛等 30 个国家湿地公园数据整合汇编项目进行的专项技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根

标准。

2．提供工作条件：

（1）为乙方在北京开展相关工作提供必要的办公场所

（2）对项目过程中乙方遇到的疑异问题进行必要解释

3．其他：无

4．甲方提供上述工作条件和协作事项的时间及方式：甲方根据乙方要求向乙方提供所需的相关技术资料或由双方协商按项目进度提供。

第四条：甲方向乙方支付技术服务报酬及支付方式为：

1．技术服务费总额为：100000.00 元（人民币：拾万元整）

2．技术服务费由甲方分两次（一次或分期）支付乙方。

具体支付方式和时间如下：

（1）签订合同后 7 个工作日内，进行第一笔拨款，拨款额度为总金额的 70%，计 70000.00 元（人民币：柒万元整）

（2）成果数据在规定时间内提交，并经过甲方验收合格后 7 个工作日内，进行第二笔拨款，拨款额度为总金额的 30%，计 30000.00 元（人民币：叁万元整）

乙方开户银行名称、地址和帐号为：

开户银行：广州工行五山支行

账户名称：华南农业大学

地址：广州市天河区五山路 483 号

条件所完成的新的技术成果，归___甲_____方所有。

第八条：双方确定，按以下约定承担各自的违约责任：

1．___甲____方违反本合同第___五__条约定，应当__以项目进度计划约定最后一月的最后一日为准，每逾期 1 日，支付本合同总价款的 0.1%的违约金，最高违约金不高于本合同总价款的 10 %_____。（支付违约金或损失赔偿额的计算方法）。

2．___乙___方违反本合同第___五、六、七__条约定，应当__以项目进度计划约定最后一月的最后一日为准，每逾期 1 日，支付本合同总价款的 0.1%的违约金，最高违约金不高于本合同总价款的 10 %。（支付违约金或损失赔偿额的计算方法）。

第九条：双方确定，在本合同有效期内，甲方指定__凌成星__为甲方项目联系人，乙方指定__邓成_____为乙方项目联系人。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1．发生不可抗力；

2．_无_____

第十一条：双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第___1___种方式处理：

1．提交_____北京市_____仲裁委员会仲裁；

甲方：中国林业科学研究院资源信息研究所（盖章）

法定代表人 / 委托代理人：（签名）

2022年 11月 16日

乙方：华南农业大学（盖章）

法定代表人 / 委托代理人：（签名）

2022 年 11 月 16 日

1.5 西藏自治区天然林资源保护工程二期 2020 年自治区级复查野外调查及成果编制劳务采购项目

HXNCJU[2022]782

技术服务合同

项目名称：西藏自治区天然林资源保护工程二期 2020 年自治区级复查野外调查及成果编制劳务采购项目

接受方（甲方）：国家林业和草原局中南调查规划院

提供方（乙方）：华南农业大学

签订时间：2022 年 7 月 4 日

签订地点：湖南长沙

有效期限：合同签订之日至 2022 年 9 月 30 日

1

接受方（甲方）：国家林业和草原局中南调查规划院

住　所　地：湖南省长沙市香樟东路 143 号

法定代表人：周学武

项目联系人：丁山

联系方式：17673146605

通讯地址：湖南省长沙市香樟东路 143 号

电　话：0731-85679737　传　　真：0731-85679825

电子信箱：

提供方（乙方）：华南农业大学

住　所　地：广东省广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：邓成

联系方式：18825090920

通讯地址：广东省广州市天河区五山路 483 号

电　　话：18825090920　传　真：

电子信箱：154083733@qq.com

根据《中华人民共和国民法典》的有关规定，甲、乙双方经过平等协商，在真实、充分地表述各自意愿的基础上，自愿签订本劳务合同，以兹双方共同遵守：

第一条　甲方因开展 西藏自治区天然林资源保护工程二期2020年自治区级复查野外调查及成果编制 项目需要，接受乙方提供野外调查及成果编制 方面的劳务。

第二条　甲方要求乙方完成的工作目标、工作内容和工作方式如下：

1、工作目标：西藏自治区天然林资源保护工程二期2020年自治区级复查野外调查及成果编制项目工作。

2、工作内容：协助甲方完成：（1）至昌都市天保工程建设县收集并核实2020年度的工程建设任务完成、组织管理、责任落实、资源保护等资料；（2）对工程建设各县2020年度财务档案资料进行查验，核实工程建设年度各项资金的下达与使用情况；（3）组织和抽样样本，对工程建设各县2020年度森林管护、责任落实、公益林建设、森林抚育等情况进行现地核实查验；（4）汇总调研与调查材料，综合分析工程建设年度任务完成情况、资金到位与使用情况、各项政策的落实和执行情况、工程组织管理情况等，总结工程实施经验做法和存在的问题与不足；（5）协助甲方完成年度复查报告等成果编制及其他相关事宜。

3、工作方式：协作。

第三条　乙方应按下列要求提供劳务：

1、工作地点：湖南省长沙市、西藏自治区昌都市、广东省广州市。

2、工作进度：按照甲方所确定的工作进度计划执行。

3、工作成果：（1）外业调查成果材料；（2）相关成果编制材料（含报告、附表、附图等）。

4、质量要求：按照甲方要求进行。

5、其他要求：在整个项目工作期间，工作认真负责，遵守项目所在地的政策法规和风俗习惯，处理好与地方干部、当地群众和有关人员的关系，自觉注意自己的言行举止，不得参与和从事违法违纪的活动，自觉维护甲方形象。乙方人员必须严格遵守甲方安全生产和保密管理的各项规章制度，确保安全生产，违反甲方安全生产和保密管理规章制度而造成的一切后果，甲方概不负责。严格保守在合同期内可能知晓的甲方的商业和技术秘密，在合同期内取得的全部资料的所有权归甲方，未经甲方同意不得擅自提供给第三方。对所提供的成果的真实性和可靠性负责，并负责成果释疑和纠错，同时应按甲方的要求对成果进行完善和修改。外业工作期间，乙方应为专业技术人员购买相关的野外保险，以及劳保用品、外业工作药品等，乙方专业技术人员如果发生人身安全事故、因病住院等，均由乙方负责妥善处理，并承担由此所发生的相关费用。

第四条　为保证乙方有效开展工作，甲方应当向乙方提供下列技术资料、工作条件和协作事项：

1、提供技术资料：

（1）相关标准、规程规范及有关文件；

（2）有关基础资料。

2、提供工作条件和协作事项：

（1）指导和协助乙方完成相关技术工作；

（2）组织乙方与有关部门进行协调和沟通等。

3、甲方提供上述技术资料、工作条件和协作事项的时间及方式：自合同签订之日起至项目完成时止。

第五条　合同期限

1、本合同期限：合同签订之日至 2022 年 9 月 30 日；

2、乙方应在合同期限内完成本合同第二条、第三条所规定的全部工作内容，并满足质量要求。非乙方原因导致乙方在合同期限内未能完成本合同第二条、第三条所规定的全部工作内容时，甲、乙双方可协商终止合同或变更合同。

第六条　乙方在承担项目工作任务期间，应遵守国家法律、法规和甲方的项目管理要求，服从甲方安排，按西藏自治区天然林资源保护工程二期 2020 年自治区级复查野外调查及成果编制工作方案和有关要求开展工作，自觉维护甲方形象。

第七条　根据项目工作需要甲方可对乙方相关人员进行相应的技术培训，乙方应认真完成培训。如果在培训后乙方相关人员不能适应所从事的工作，甲方有权解除本合同。

第八条　服务费用及支付方式：

1、劳务费用总额为：人民币伍万贰仟伍佰元整（¥52500）。

2、劳务费用由甲方 在工作结束后一次性 支付乙方。

乙方开户银行名称、地址和帐号为：

开户银行： 广州工行五山支行

地址： 广州市天河区五山路 483 号

银行帐号： 3602002609000310520

税务登记号： 124400004554165634

3、乙方在劳务费用的使用上要符合国家有关财务规定，不得有损甲方声誉，甲方有权对乙方劳务费用的使用情况进行检查，但不得妨碍乙方的正常工作。

4、本条约定之费用已包括全部成本费用及报酬，除此之外，甲方无需再向乙方支付其他任何费用。

5、在甲方支付劳务费用前，乙方应提供等额增值税专用发票给甲方。否则，甲方有权迟延付款直至乙方提供发票为止。

第九条　乙方履行本合同过程中应遵守的保密义务如下：

1、保密内容（包括技术信息和经营信息）： 项目涉及的数据、资料、阶段性成果、最终成果等，按《保密法》要求执行；

2、涉密人员范围： 项目参与人员及接触人员；

3、保密期限： 按国家有关规定执行；

4、涉密责任： 按国家有关规定执行。

第十条　双方确定，按下列标准和方式对乙方提供的劳务工作成果进行验收：

1、工作成果形式： 按甲方要求提供；

2、验收标准：由甲方按验收标准验收；

3、验收的时间和地点：按甲方要求的时间和地点。

第十一条　双方确定，在本合同有效期内，甲方指定 丁山 为甲方项目联系人，乙方指定 邓成 为乙方项目联系人。项目联系人承担以下责任：

1、负责双方意见的交流和传递；

2、通报工作进展；

3、协调双方合作事项。

一方变更项目联系人的，应当及时以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十二条　合同解除

1、乙方提供劳务不能满足甲方项目进度需要的，经过甲方书面催告仍然无法满足甲方项目进度需要，则甲方有权解除本合同，但应按乙方实际工作天数或工作成果支付相应的劳务费用；

2、乙方提供劳务期间出现违法违规行为，严重影响甲方项目权益或甲方声誉的，甲方有权解除本合同，并追究乙方相关责任；

3、乙方提供劳务期间未对乙方技术人员进行相应安全教育、采取安全防范措施的，与提供劳务工作安全保障的，甲方有权解除本合同，但应按乙方实际劳务工作天数或劳务工作成果支付相应的劳务费用；

4、乙方在合同期限内要求提前解除合同的，应提前 5 日通知甲方并征得甲方同意后方可解除本合同，甲方按乙方实际劳务工作天数

或劳务工作成果支付相应的劳务费用。乙方如单方擅自解除合同，甲方有权拒付乙方的劳务费用，并要求乙方赔偿甲方由此产生的全部经济损失；

5、双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

（1）、自然灾害等不可抗力；

（2）、甲方与业主单位因故解除合同。

第十三条　特别约定

1、乙方应在工作结束申请支付劳务费用前，或解除、终止合同前将所承担的有关工作向甲方进行移交，需要说明的应附书面说明，否则甲方有权拒付乙方的劳务费用。

2、乙方应根据甲方的项目工作内容与要求，在正式提供劳务前，应自行为乙方人员投保有关险种。如乙方人员在甲方项目工作期间发生人身意外伤害事故等，乙方所受到的全部损失应由保险公司进行赔偿，甲方不承担责任。乙方因个人身体健康状况出现问题引起的受伤和意外身亡等情况，甲方不承担责任。

3、乙方（含乙方人员）违反法律法规所引起的一切法律责任均由乙方自行承担。乙方因违反甲方项目管理要求或由于乙方自身的过错行为致使乙方人员受到伤害或给他人造成损失的，责任由乙方承担。

4、乙方在项目劳务服务期间完成的与甲方工作有关的相关智力成果的专利申请权、著作权等相关知识产权均归甲方所有。

5、乙方在项目劳务期间应注意遵守工作纪律和项目管理规定，

保守因劳务原因获知的他人成果和知识产权，严格保守国家秘密。

第十四条　双方确定：

1、在本合同有效期内，甲方利用乙方提供的劳务工作成果所完成新的技术成果，归___甲___方所有；

2、在本合同有效期内，乙方利用甲方提供的技术资料和工作条件完成的新的技术成果，归___甲___方所有。

第十五条　违约条款

1、乙方未经甲方同意，提前解除合同，甲方不应向其支付劳务报酬，乙方为此给甲方造成的一切经济损失及维权损失（包括但不限于律师费、诉讼费等费用）由乙方承担；

2、因乙方原因，乙方逾期完成合同工作内容，甲方为此产生的一切经济损失及维权损失（包括但不限于律师费、诉讼费等费用）由乙方承担；

3、乙方在劳务期间内，因主观过错或过失给第三方造成损失的，由乙方承担赔偿责任。如因此牵连甲方，甲方为此产生的一切经济损失及维权损失（包括但不限于律师费、诉讼费等费用）由乙方承担；

4、甲方在乙方劳务成果验收确认后，未按照合同约定付款的，应依照未付劳务费用每日万分之一的标准，向乙方支付逾期付款违约金；

5、__甲__方违反本合同第四 条规定，应当 承担违约责任，乙方有权要求顺延因此受到影响的服务成果提交时间，如果甲方技术资料或者工作条件提供时间严重耽误乙方人员工作，乙方有权要求甲方对

耽误时间进行适当补偿；

6、乙方违反本合同第二、第三、第六、第十条规定，应当承担违约责任，甲方有权要求乙方对推迟提交成果时间引起的损失进行赔偿，如果乙方提供的服务成果不合格，甲方有权要求乙方进行返工，直到服务成果符合要关规定。如果这种不合格的状况在乙方采取补救措施后仍然存在的，甲方有权单方面解除本合同并就本方受到的损失进行索赔。

第十六条　本合同的变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在5日内予以答复；逾期未予答复的，视为同意：

1、甲方要求乙方提前完成服务并提前提交服务成果；

2、乙方因甲方未按照合同约定及时提供技术资料或者工作条件、协作事项而推迟提供服务成果时间。

第十七条　双方约定本合同其他相关事项为：无。

第十八条　双方因履行本合同而发生的争议，应当友好协商解决，协商不成，任何一方均可向甲方住所地人民法院起诉。

第十九条　本合同一式捌份，甲方执肆份、乙方执肆份，具有同等法律效力。

第二十条　本合同经双方签字盖章后生效。

甲方：国家林业和草原局中南调查规划院　　（盖章）

法人代表（签字）：

委托代理人（签字）：

2022 年 7 月 4 日

乙方：华南农业大学　　（盖章）

法人代表（签字）：

委托代理人（签字）：李秀彩

2022年 7 月 4日

11

2. 主要参与项目

表9 科研课题情况
李青粉 参与的课题

序号	项目名称	评审等级	项目来源	合同经费/实到经费	立项时间	开始时间	结题时间	负责人	课题组总人数	本人排名	是否结题
1	植物ABCB1的质量控制及其协同油菜素内酯信号和生长素运输的分子机制	A	国家自然科学基金委员会	58.0	2019-08-16	2020-01-01	2023-12-31	刘林川	7	2	是
2	杉木解析木及生物量采样	按照个人到位经费定级	横向	9.994	2021-07-25	2021-07-25	2021-12-31	邓成	5	2	是
3	受乙烯诱导的ERF第七亚家族转录因子调控木质素合成的分子机理	A	国家自然科学基金委员会	72.0	2018-08-16	2019-01-01	2022-12-31	吴蔼民	8	3	是
4	红椿新品种选育	A	科技部	130.0	2021-12-30	2021-12-30	2026-11-30	李培	11	4	否

科技处审核人及盖章：

年 月 日

第1页

2.1 植物 ABCB1 的质量控制及其协同油菜素内酯信号和生长素运输的分子机制

项目批准号	31870653
申请代码	C161002
归口管理部门	
依托单位代码	51064208A0499-0932

31870653 1004943

国家自然科学基金委员会

资助项目计划书

资助类别：面上项目

亚类说明：

附注说明：

项目名称：受乙烯诱导的ERF第七亚家族转录因子调控木质素合成的分子机理

直接费用：60万元　　执行年限：2019.01-2022.12

负 责 人：吴蔼民

通讯地址：广州市天河区五山路483号

邮政编码：510642　　电　　话：020-85280259

电子邮件：wuaimin@scau.edu.cn

依托单位：华南农业大学

联 系 人：唐家林　　电　　话：020-85280070

填表日期：2018年08月17日

国家自然科学基金委员会制

Version：1.004.943

国家自然科学基金委员会资助项目计划书填报说明

一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办法及《国家自然科学基金资助项目资金管理办法》（请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。

二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行和检查、验收的依据。

三、《计划书》各部分填写要求如下：

（一）简表：由系统自动生成。

（二）摘要及关键词：各类获资助项目都必须填写中、英文摘要及关键词。

（三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果批准通知中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。

（四）资金预算表：根据批准资助的直接费用，按照《国家自然科学基金项目预算表编制说明》填报资金预算表和预算说明书。国家重大科研仪器研制项目、重大项目还应按照预算评审后批复的直接费用各科目金额填报资金预算表、预算说明书及相应的预算明细表。

（五）正文：

1. 面上项目、青年科学基金项目、地区科学基金项目：如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目明确要求调整研究期限和研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。

2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标以及验收技术指标）或缩减研究（研制）内容。此外，还要突出以下几点：

（1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；

（2）项目主要参与者分工，合作研究单位之间的关系与分工，重大项目还需说明课题之间的关联；

（3）详细的年度研究（研制）计划。

3. 国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
（1）研究方向；
（2）结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
（3）研究内容、研究方案及预期目标（限两个页面）；
（4）年度研究计划；
（5）研究队伍的组成情况。
4. 国家自然科学基金基础科学中心项目：须选择“根据研究方案修改意见更改”，应当根据评审委员会和现场考察专家组的意见和建议，进一步完善并细化研究计划，作为评估和验收的依据。按下列提纲撰写：
（1）五年拟开展的研究工作（包括主要研究方向、关键科学问题与研究内容）；
（2）研究方案（包括骨干成员之间的分工及合作方式、学科交叉融合研究计划等）；
（3）年度研究计划；
（4）五年预期目标和可能取得的重大突破等；
（5）研究队伍的组成情况。
5. 对于其他类型项目，参照面上项目的方式进行选择和填写。

简表

申请者信息	姓　　名	吴蔼民	性 别	男	出生年月	1969年02月	民 族	汉族
	学　　位	博士			职称	教授		
	是否在站博士后	否		电子邮件		wuaimin@scau.edu.cn		
	电　　话	020-85280259		个人网页				
	工 作 单 位	华南农业大学						
	所 在 院 系 所	林学与风景园林学院						
依托单位信息	名　　称	华南农业大学					代码	51064208A0499
	联 系 人	唐家林		电子邮件		kycjhk@scau.edu.cn		
	电　　话	020-85280070		网站地址		http://web.scau.edu.cn/kjc/		
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	受乙烯诱导的ERF第七亚家族转录因子调控木质素合成的分子机理						
	资 助 类 别	面上项目			亚 类 说 明			
	附 注 说 明							
	申 请 代 码	C161002:林木遗传改良						
	基 地 类 别							
	执 行 年 限	2019.01-2022.12						
	直 接 费 用	60万元						

项目摘要

中文摘要：

木质素在林木生长发育中起到不可或缺的作用，但同时也给制浆造纸、生物质能源利用等带来不少负面影响。因此，合理调节植物体内木质素的生物合成是目前的研究热点。木质素合成关键酶基因的转录调控研究主要集中在MYB家族转录因子，而其它类转录因子报道很少。乙烯能够促进木材的形成，但乙烯信号转导怎样调控木质素的合成还没有报道。本项目将分析受乙烯诱导的ERF第七亚家族转录因子，通过生物信息学分析、组织器官表达、胁迫表达、基因定位等研究ERF-VII成员的生物学特性，再通过过量表达和转录因子抑制型技术对ERF-VII基因的进行转基因研究，通过FT-IR、GC-MS、二维核磁等分析转基因杨树木质素等细胞壁成分的变化，同时通过转录组测序、反式激活和凝胶滞留分析ERF-VII对木质素合成关键酶的调控。这些研究不仅可以丰富木质素合成的调控途径，还可以为我们从上游进行基因工程改良木质素材料提供理论支持。

Abstract:

Lignin, one of the most abundant terrestrial biopolymers, is indispensable for plant structure and defense. However, it also has a negative effect on pulp and paper industry, biomass utilization. Therefore, the suitable modifying lignin biosynthesis is one of the hotest topic in plant. At present, The regulation of lignin biosynthesis mainly focused on MYB family of transcription factors, and less reported on other transcription factors. Ethylene can stimulate wood formation by cambium cells, but how ethylene signal transduction regulate lignin

synthesis has not yet been reported. This project will focus on seventh Ethylene Response Factors (ERF-VII) family which induced by ethylene treatment in poplar.

The members of ERF-VII family from poplar will be studied by bioimformatic analysis, expression levels in poplar, localization, and expression in stress condition. then we will overexpress the target genes or/and inhabit by Chimeric expressor Gene-Silencing technology to analyze lignin component changes in transgenic poplar plants by FT-IR, GC-MS, and two-dimensional NMR. Furthermore, how ERF-VII regulating lignin biosynthesis will be analyzed through RNA-Seq, trans-activation analysis and Electrophoretic Mobile Shift Assay (EMSA). These studies will not only rich the regulatory of lignin biosynthesis pathways, but also provide the genetically engineering method to modify lignin material from upstream regulatory genes

关键词(用分号分开)：次生代谢；代谢调控；ERF 转录因子；木质素；乙烯诱导

Keywords(用分号分开)： secondary metabolism; the regulation of metabolism; ethylene response transcription factors; lignin; ethylene induced

项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间（月）
1	吴蔼民	1969.02	男	教授	博士	华南农业大学	020-85280259	320113196902234973	项目负责人	5
2	龙健梅	1989.08	女	讲师	博士	华南农业大学	020-85280259	452123198908283146	转录调控网络研究	8
3	李青粉	1987.02	女	博士后	博士	华南农业大学	18922210205	410901198702052023	基因表达、定位及调控研究	8
4	王凯利	1990.12	女	博士生	硕士	华南农业大学	13610203509	370983199012053244	基因表达及异质性分析	10
5	李倩	1993.01	女	硕士生	学士	华南农业大学	18320728073	370782199301293060	基因、蛋白表达分析	10
6	尚娜	1991.06	女	硕士生	学士	华南农业大学	18320728039	410221199106240222	关键基因调控研究	10
7	陈辰	1995.11	男	硕士生	学士	华南农业大学	14755598988	342502199511072016	合成代谢网络影响研究	10
8	朱忆魁	1994.11	男	硕士生	学士	华南农业大学	15862146801	320311199411267633	成分分析	10

总人数	高级	中级	初级	博士后	博士生	硕士生
8	1	1	0	1	1	4

国家自然科学基金项目直接费用预算表 （定额补助）

项目批准号：31870653　　项目负责人：吴蔼民　　金额单位：万元

序号	科目名称	金额
1	项目直接费用合计	60.0000
2	1、 设备费	0.0000
3	(1)设备购置费	0.00
4	(2)设备试制费	0.00
5	(3)设备升级改造与租赁费	0.00
6	2、 材料费	24.0000
7	3、 测试化验加工费	14.5000
8	4、 燃料动力费	0.00
9	5、 差旅/会议/国际合作与交流费	6.0000
10	6、 出版/文献/信息传播/知识产权事务费	4.5000
11	7、 劳务费	11.0000
12	8、 专家咨询费	0.00
13	9、 其他支出	0.00

预算说明书（定额补助）

（请按照《国家自然科学基金项目预算表编制说明》的有关要求，对各项支出的主要用途和测算理由，以及合作研究外拨资金、单价≥10万元的设备费等内容进行必要说明。）

1、 设备费：无

2、 材料费 24.0 万：

（1）原材料、试剂、药品等消耗品购置费（22.0 万元）

引物合成 2.00 万元，PCR 相关试剂 3.00 万，工具酶类 3.00 万元，感受态细胞、质粒提取试剂盒和胶回收试剂盒 1.50 万元，RNA 提取试剂盒、DNA 提取试剂盒和 cDNA 反转录试剂盒 2.00 万元，蛋白纯化试剂 1.50 万元，抗体 1.00 万元，普通生化试剂 5.00 万元，其它常规耗材 3.00 万元，

（2）其他 （2.0 万元）

主要用于种植实验材料，共计 2.00 万元。

3、 测试化验加工费 14.5 万：

转录组测序 7.00 万元，扫描电镜 1.00 万元、透射电镜 1.00 万元；CHIP sequence 2.00 万元；细胞壁成分分析所要的 PROLYSIS 测定 1.00 万元、GC-MS 1.00 万元、FT-IR 0.50 万元、二维核磁 1.00 万元等仪器使用分析费。

4、 燃料动力费：无

5、 差旅/会议/国际合作与交流费：6.0 万：

市内交通费（广州化学所实验中心、中山大学测试中心、热林所）0.60 万元

参加国内相关学术会议及开展相关学术研究交流（ 0.30 万元/次×8 人次=2.40 万元）。

项目组成员赴国外交流交通及住宿费：2020 年参加世界林木生物技术大会或植物细胞壁大会 2 人 / 次。拟 2018 年赴瑞典农业大学与 Bjorn Sundberg 教授或他们组的 Totte Niittylä 副教授交流杨树细胞壁测定及木质素下降对纤维素丝的角度的影响，交流 1 人次，合计：3.00 万元。

6、 出版/文献/信息传播/知识产权事务费：4.5 万元

按照平均每个专利申报费为 0.3 万元，本项目申请专利 2 个，共计支出 0.60 万元；国内核心期刊论文的发表版面费大约为 0.25 万元/篇×2 篇=0.50 万元，国际论文的发表版面费大约 0.80 万元/篇×3 篇=2.40 万元；入网、信息查询、邮寄等支出 1.00 万元。合计 4.50 万元。

7、 劳务费 11.0 万：

主要用于支付博士、硕士研究生劳务费。平均每年支付 5 名研究生费用，每人每年 10 个月参与工作，4 年×5 人×10 月×550 元/月=11.0 万元。

8、 专家咨询费：无

9、 其他支出：无

项目负责人签字： 科研部门公章： 财务部门公章：

报告正文

研究内容和研究目标按照申请书执行。

国家自然科学基金资助项目签批审核表

<table>
<tr>
<td colspan="2">我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：31870653），严格遵守国家自然科学基金委员会关于资助项目管理、财务等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。

项目负责人（签章）：
年　月　日</td>
<td colspan="2">我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、财务等各项规定，并督促实施。

依托单位（公章）
年　月　日</td>
</tr>
<tr>
<td rowspan="3">本栏目由基金委填写</td>
<td colspan="3">科学处审查意见：

建议年度拨款计划（本栏目为自动生成，单位：万元）：
<table>
<tr><td>年度</td><td>总额</td><td>第一年</td><td>第二年</td><td>第三年</td><td>第四年</td><td>第五年</td></tr>
<tr><td>金额</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
负责人（签章）：
年　月　日</td>
</tr>
<tr>
<td colspan="3">科学部审查意见：

负责人（签章）：
年　月　日</td>
</tr>
<tr>
<td colspan="3">相关局室审核意见：

负责人（签章）：
年　月　日</td>
</tr>
<tr>
<td>本栏目主要用于重大项目等</td>
<td colspan="3">委领导审批意见：

委领导（签章）：
年　月　日</td>
</tr>
</table>

2.2 受乙烯诱导的 ERF 第七亚家族转录因子调控木质素合成的分子机理

项目批准号	31970187
申请代码	C020101
归口管理部门	
依托单位代码	51064208A0499-0932

3197018710038 19

国家自然科学基金委员会

资助项目计划书

资助类别：面上项目

亚类说明：

附注说明：

项目名称：植物ABCB1的质量控制及其协同油菜素内酯信号和生长素运输的分子机制

直接费用：58万元　　执行年限：2020.01-2023.12

负 责 人：刘林川

通讯地址：广东省广州市天河区五山路483号华南农业大学林学与风景园林学院617房间

邮政编码：201602　　电　　话：020-85280256

电子邮件：liulinchuan@163.com

依托单位：华南农业大学

联 系 人：倪慧群　　电　　话：020-85280070

填表日期：2019年08月23日

国家自然科学基金委员会制

Version：1.003.819

国家自然科学基金委员会资助项目计划书填报说明

一、项目负责人收到《关于国家自然科学基金资助项目批准及有关事项的通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办法及《国家自然科学基金资助项目资金管理办法》（请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金委员会资助项目计划书》（以下简称《计划书》）。

二、填写《计划书》时要求科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行和检查、验收的依据。

三、《计划书》各部分填写要求如下：

（一）简表：由系统自动生成。

（二）摘要及关键词：各类获资助项目都必须填写中、英文摘要及关键词。

（三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果批准通知中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。

（四）资金预算表：根据批准资助的直接费用，按照《国家自然科学基金项目预算表编制说明》填报资金预算表和预算说明书。国家重大科研仪器研制项目、重大项目还应按照预算评审后批复的直接费用各科目金额填报资金预算表、预算说明书及相应的预算明细表。

（五）正文：

1. 面上项目、青年科学基金项目、地区科学基金项目：如果《批准通知》中没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中“项目评审意见及修改意见表”中“对研究方案的修改意见”栏目明确要求调整研究期限和研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。

2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标以及验收技术指标）或缩减研究（研制）内容。此外，还要突出以下几点：

（1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；

（2）项目主要参与者分工，合作研究单位之间的关系与分工，重大项目还需说明课题之间的关联；

（3）详细的年度研究（研制）计划。

3. 国家杰出青年科学基金、优秀青年科学基金和海外及港澳学者合作研究基金项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
（1）研究方向；
（2）结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
（3）研究内容、研究方案及预期目标（限两个页面）；
（4）年度研究计划；
（5）研究队伍的组成情况。
4. 国家自然科学基金基础科学中心项目：须选择“根据研究方案修改意见更改”，应当根据评审委员会和现场考察专家组的意见和建议，进一步完善并细化研究计划，作为评估和验收的依据。按下列提纲撰写：
（1）五年拟开展的研究工作（包括主要研究方向、关键科学问题与研究内容）；
（2）研究方案（包括骨干成员之间的分工及合作方式、学科交叉融合研究计划等）；
（3）年度研究计划；
（4）五年预期目标和可能取得的重大突破等；
（5）研究队伍的组成情况。
5. 对于其他类型项目，参照面上项目的方式进行选择和填写。

简表

申请者信息	姓　　名	刘林川	性 别	男	出生年月	1983年01月	民 族	汉族
	学　　位	博士			职称	教授		
	是否在站博士后	否		电子邮件	liulinchuan@163.com			
	电　　话	020-85280256		个人网页				
	工 作 单 位	华南农业大学						
	所 在 院 系 所	林学与风景园林学院						
依托单位信息	名　　称	华南农业大学				代码	51064208A0499	
	联 系 人	倪慧群		电子邮件	kycjhk@scau.edu.cn			
	电　　话	020-85280070		网站地址	http://kjc.scau.edu.cn/			
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	植物ABCB1的质量控制及其协同油菜素内酯信号和生长素运输的分子机制						
	资 助 类 别	面上项目			亚 类 说 明			
	附 注 说 明							
	申 请 代 码	C020101:植物结构与功能			C020407:植物激素与生长调节物质			
	基 地 类 别							
	执 行 年 限	2020.01-2023.12						
	直 接 费 用	58万元						

项目摘要

中文摘要：

植物ABC转运蛋白利用ATP水解提供能量将底物进行逆浓度梯度转运，在植物的生长和发育过程中起着非常重要的作用。生长素在特定的细胞合成后需要转运至不同的组织来行使生理功能，ABCB1作为一种重要的ABC转运蛋白在细胞膜上参与了对生长素的转运。然而，我们发现ABCB1在拟南芥twd1-6突变体背景下被滞留在内质网中并且被快速降解，特别是twd1-6和ABCB1蛋白能够共同定位于内质网。尽管我们目前对动物、酵母和植物细胞中糖蛋白在内质网中的质量控制和降解机制有了一定的了解，但是对转运蛋白的内质网滞留和降解途径认识还十分有限。在本项目申请中，我们以遗传学为基础，结合生物化学和细胞生物学研究手段，研究ABCB1在内质网中的质量控制机制。同时，我们还探索植物生长过程中ABCB1是如何来协同油菜素内酯信号和生长素的运输。

Abstract:

ABC transporters are essential for plant growth and development, which can transport complex organic materials against concentration gradients energized by ATP. Auxin are synthesized in specialized cells and are transported to target tissues. ABCB1, an ABC transporter, has been shown to be involved in auxin transport in plasma membrane. However, we found a single missense Arabidopsis twd1(twisted dwarf1) mutant, twd1-6, in which ABCB1 was retained in the ER and rapidly degraded. Especially, twd1-6 is co-localized with ABCB1 in the ER. Despite primary understanding of ERQC/ERAD of glycosylated proteins has been ascertained through investigating yeast, mammalian and plant cells, the functional studies of the ERQC/ERAD processes on membrane transporter proteins remained rather limited. In this study, we will focus on the folding, maturation, and degradation of ABCB1 to screen and identify the components involved in these processes by using genetics methods combined with biochemical and cell biology approaches. In addition, we are going to explore the critical roles of ABCB1 in the interactions between BR signaling and auxin transport during plant growth.

关键词(用分号分开)：运输途径；蛋白质分选；蛋白质降解；油菜素内酯信号；生长素运输

Keywords(用分号分开): transport pathway; protein sorting; protein degradation; BR signaling; auxin transport

项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间（月）
1	刘林川	1983.01	男	教授	博士	华南农业大学	020-85280256	21100219830126013X	项目负责人	10
2	李青粉	1987.02	女	工程师	博士	华南农业大学	18998825286	410901198702052023	遗传材料构建与分析	10
3	胡莉	1994.05	女	博士生	硕士	华南农业大学	18320726497	430621199405101041	蛋白质磷酸化修饰分析	10
4	韦健娘	1993.09	男	博士生	硕士	华南农业大学	13570444591	440681199309212639	细胞生物学分析	10
5	段志豪	1996.03	男	硕士生	学士	华南农业大学	18825195959	361127199603044213	蛋白质相互作用分析	10
6	周淑瑶	1997.02	女	硕士生	学士	华南农业大学	13422003209	440681199702234746	细胞生物学分析	10
7	伍慧祥	1997.12	男	硕士生	学士	华南农业大学	13632190418	360782199712094419	蛋白质相互作用分析	10

总人数	高级	中级	初级	博士后	博士生	硕士生
7	1	1	0	0	2	3

国家自然科学基金项目直接费用预算表 （定额补助）

项目批准号：31970187　　项目负责人：刘林川　　金额单位：万元

序号	科目名称	金额
1	项目直接费用合计	58.0000
2	1、 设备费	0.0000
3	(1)设备购置费	0.0000
4	(2)设备试制费	0.0000
5	(3)设备升级改造与租赁费	0.0000
6	2、 材料费	22.0000
7	3、 测试化验加工费	11.0000
8	4、 燃料动力费	0.0000
9	5、 差旅/会议/国际合作与交流费	5.8000
10	6、 出版/文献/信息传播/知识产权事务费	3.2000
11	7、 劳务费	16.0000
12	8、 专家咨询费	0.0000
13	9、 其他支出	0.0000

预算说明书（定额补助）

（请按照《国家自然科学基金项目预算表编制说明》等的有关要求，对各项支出的主要用途和测算理由，以及合作研究外拨资金、单价≥10万元的设备费等内容进行必要说明。）

1.设备费

无

目前，本团队和所在科研平台已具备开展本项目所需的实验条件，如各种仪器设备等，因此本项目无仪器设备购置的预算。

2.材料费

预算经费：22.00 万元

(1) 基因克隆和载体构建，预算经费 8.60 万元。

Taq 酶（1000U/支，Takara），每支 200 元，购置 50 支，合计 1.00 万元；

高保真 Taq 酶（1000U，Vazyme），每支 800 元，购置 20 支，合计 1.60 万元；

购买电泳普通琼脂糖每瓶 300 元，购置 50 瓶，合计 1.50 万元；

DNA 内切酶、连接酶、反转录酶和重组酶等酶类，合计 2.50 万元；

植物 DNA 提取试剂盒、RNA 提取试剂盒、琼脂糖凝胶 DNA 回收试剂盒等，合计 2.00 万元。

(2) 基因表达分析，预算经费 1.12 万元。

定量 PCR 试剂盒（Bio-Rad，100 次，iScript cDNA Synthesis Kit），每个 1600 元，购置 7 盒，合计 1.12 万元；

(3) 蛋白表达纯化及功能检测，预算经费 6.20 万元。

原核表达蛋白 His 标签、GST 标签、MBP 标签纯化用介质，合计 0.90 万元；

BCA 蛋白质定量试剂盒，合计 0.30 万元；

蛋白浓缩用超滤管，每个 50 元，购置 100 个，合计 0.50 万元；

购买标签抗体、磁珠，用于免疫共沉淀和蛋白质相互作用检测，合计 4.50 万元。

(4) 植物组织培养试剂，预算经费 2.70 万元。

植物组培用琼脂粉、培养基、糖类等培养基，合计 1.20 万元。

转基因筛选用抗生素，潮霉素、头孢霉素、利福平、卡那霉素等，合计 1.50 万元；

(5) 常用低值易耗品，预算经费 3.38 万元。

一次性培养皿，每箱 160 元，购置 30 箱，合计 0.48 万元；

一次性移液吸头和离心管，合计 2.00 万元；

一次性 96 孔 PCR 板（Axygen），每包 30 元，购置 200 包，合计 0.60 万元；

一次性乳胶手套、口罩等，合计 0.30 万元；

3.测试化验加工费

预算经费：11.00 万元

(1) 引物合成，用于基因的图位克隆，基因表达的鉴定，载体构建等实验，合计 1.80 万元；

(2) DNA 测序，用于基因克隆、载体构建，定点突变的鉴定等，合计 1.50 万；

(3) 蛋白质质谱分析，用于蛋白质定性和定量分析，磷酸化位点分析等，合计 2.50 万元。

(4) 抗体制备，用于蛋白质稳定性检测和其它生化实验。每个 8000 元，制备 4 个，

合计 3.20 万元；
进行转录组测序分析，每个样本 2000 元，预计 10 个样本，合计 2.00 万元。

4.燃料动力费
无

5.差旅/会议/国际合作与交流费
预算经费：5.80 万元
用于参加国内、国际学术会议注册费用和住宿、交通费用。
课题人员参加国内学术会议交流 6 人次，会议注册费、交通费、住宿费合计 3.00 万元。参加国际学术会议 2 人次，会议注册费、交通费、住宿费合计 2.80 万元。

6.出版/文献/信息传播/知识产权事务费
预算经费：3.20 万元
项目研究期间发表高水平 SCI 国际论文 2 篇，论文版面费以每篇 1.50 万元计；资料查阅、文献检索费等 0.20 万元；合计 3.20 万元。

7.劳务费
预算经费：16.00 万元
参加课题研究工作的博士研究生 2 人，按照华南农业大学的博士研究生待遇标准课题组每人每月平均补助 800 元，2 名博士研究生共计 80 人月，合计 6.40 万元；
参加课题研究工作的硕士研究生 3 人，按照华南农业大学硕士研究生待遇标准每人每月平均补助 800 元，3 名硕士研究生共计 120 人月，合计 9.60 万元。

8.专家咨询费
无

9.其他支出
无

项目负责人签字：　　科研部门公章：　　财务部门公章：

报告正文

研究内容和研究目标按照申请书执行。

国家自然科学基金资助项目签批审核表

<table>
<tr>
<td colspan="2">我接受国家自然科学基金的资助，将按照申请书、项目批准意见和计划书负责实施本项目（批准号：31970187），严格遵守国家自然科学基金委员会关于资助项目管理、财务等各项规定，切实保证研究工作时间，认真开展研究工作，按时报送有关材料，及时报告重大情况变动，对资助项目发表的论著和取得的研究成果按规定进行标注。

项目负责人（签章）：
年　月　日</td>
<td>我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、财务等各项规定，并督促实施。

依托单位（公章）
年　月　日</td>
</tr>
<tr>
<td rowspan="3">本栏目由基金委填写</td>
<td colspan="2">科学处审查意见：

建议年度拨款计划（本栏目为自动生成，单位：万元）：
<table>
<tr><td>年度</td><td>总额</td><td>第一年</td><td>第二年</td><td>第三年</td><td>第四年</td><td>第五年</td></tr>
<tr><td>金额</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
负责人（签章）：
年　月　日</td>
</tr>
<tr>
<td colspan="2">科学部审查意见：

负责人（签章）：
年　月　日</td>
</tr>
<tr>
<td colspan="2">相关局室审核意见：

负责人（签章）：
年　月　日</td>
</tr>
<tr>
<td>本栏目主要用于重大项目等</td>
<td colspan="2">委领导审批意见：

委领导（签章）：
年　月　日</td>
</tr>
</table>

2.3 红椿新品种选育

子课题编号：2021YFD2200305-2　　密　级：

国家重点研发计划
子课题任务书

子课题名称：	红椿新品种选育
所属课题：	椿树新品种选育
所属项目：	北方珍贵林木新品种选育
子课题承担单位：	华南农业大学
课题承担单位：	中国林业科学研究院亚热带林业研究所
子课题负责人：	李培
执行期限：	2021.12　至　2026.11

中华人民共和国科学技术部制

2021 年 12 月 30 日

填 写 说 明

一、任务书甲方即项目牵头承担单位，乙方即课题承担单位。

二、任务书通过“国家科技计划管理信息系统公共服务平台”，按照系统提示在线填写。

三、任务书中的单位名称，请按规范全称填写，并与单位公章一致。

四、任务书要求提供乙方与所有参加单位的合作协议，需对原件进行扫描后在线提交。

五、任务书中文字须用宋体小四号字填写。

六、凡不填写内容的栏目，请用“无”表示。

七、乙方完成任务书的在线填写，提交甲方审核确认后，用A4纸在线打印、装订、签章。一式八份报项目牵头承担单位签章，其中课题承担单位一份，课题负责人一份，作为项目任务书附件六份。

八、如项目下仅设一个课题，课题任务书只需填报课题预算部分。

九、涉密课题请在“国家科技计划管理信息系统公共服务平台”下载任务书的电子版模板，按保密要求离线填写、报送。

十、《项目申报书》和《项目任务书》是本任务书填报的重要依据，任务书填报不得降低考核指标，不得自行对主要研究内容作大的调整。《项目申报书》、《项目任务书》和本任务书将共同作为课题过程管理、综合绩效评价（验收）和监督评估的重要依据。

子课题基本信息表

<table>
<tr><td colspan="2">子课题名称</td><td colspan="6">红椿新品种选育</td></tr>
<tr><td colspan="2">子课题编号</td><td colspan="6">2021YFD2200305-2</td></tr>
<tr><td colspan="2">所属课题</td><td colspan="6">椿树新品种选育</td></tr>
<tr><td colspan="2">所属项目</td><td colspan="6">北方珍贵林木新品种选育</td></tr>
<tr><td colspan="2">子课题类型</td><td colspan="6">☐基础前沿 ☑ 重大共性关键技术 ☐应用示范研究 ☐其他</td></tr>
<tr><td colspan="2">经费预算</td><td colspan="6">130 万元，其中中央财政专项资金 130 万元</td></tr>
<tr><td colspan="2" rowspan="2">子课题周期节点</td><td>起始时间</td><td colspan="2">2021 年 12 月</td><td>结束时间</td><td>2026 年 11 月</td></tr>
<tr><td>实施周期</td><td colspan="2">共 60 个月</td><td>预计中期时间点</td><td>年 月</td></tr>
<tr><td rowspan="6">子课题承担单位</td><td>单位名称</td><td colspan="3">华南农业大学</td><td>单位法定代表人姓名</td><td>刘雅红</td></tr>
<tr><td>单位性质</td><td colspan="3">事业单位</td><td>组织机构代码</td><td>12440000455416563 4</td></tr>
<tr><td>单位所属地区</td><td colspan="3">广东省</td><td>地市（市、自治州、盟）</td><td>广州市</td></tr>
<tr><td>通信地址</td><td colspan="3">广东省广州市天河区五山路 483 号</td><td>邮政编码</td><td>510642</td></tr>
<tr><td>单位开户名称</td><td colspan="2">华南农业大学</td><td>开户银行（全称）</td><td colspan="2">中国工商银行股份有限公司广州五山支行</td></tr>
<tr><td>银行账号</td><td colspan="3">3602002609000310520</td><td>银行机构代码</td><td>102581000546</td></tr>
<tr><td rowspan="6">子课题负责人</td><td>姓 名</td><td>李培</td><td>性 别</td><td>☐男 ☑ 女</td><td>出生日期</td><td>1985.03</td></tr>
<tr><td>证件类型</td><td>身份证</td><td>证件号码</td><td colspan="3">220103198503080420</td></tr>
<tr><td>所在单位</td><td colspan="5">华南农业大学</td></tr>
<tr><td>最高学位</td><td colspan="5">☑ 博士 ☐硕士 ☐学士 ☐其他</td></tr>
<tr><td>职 称</td><td colspan="3">☐正高级 ☐副高级 ☑ 中级 ☐初级 ☐其他</td><td>职务</td><td></td></tr>
<tr><td>电子邮箱</td><td colspan="2">lipei-meinv@163.com</td><td>移动电话</td><td colspan="2">13925087374</td></tr>
<tr><td rowspan="2">子课题参加人数</td><td rowspan="2">9 人。
其中： 人</td><td colspan="5">高级职称 1 人，中级职称 6 人，初级职称 0 人，其他 2 人；</td></tr>
<tr><td colspan="5">博士学位 7 人，硕士学位 0 人，学士学位 2 人，其他 0 人。</td></tr>
</table>

子课题简介（限500字以内）	红椿（*Toona ciliata*）隶属楝科（Meliaceae）香椿属（*Toona*），素有“中国桃花心木”之称，是优等家具用材，为国家Ⅱ级重点保护野生濒危植物，也是我国优先重点发展的乡土珍贵树种之一。针对红椿材质优良和资源高效良种缺乏的问题，以前期所选择的优良种源幼苗为材料，开展红椿生长、材质、耐寒等重要性状遗传变异规律，选育资源高效型、优质、抗逆专适品种；基于红椿全基因组，挖掘椿树重要目标性状相关的候选基因，建立其遗传转化及基因编辑技术体系，创制抗旱、耐寒新种质。

一、目标及考核指标、考核方式/方法

（一）、子课题目标

针对红椿育种周期长、新种质创制技术未突破，缺乏材质优良、抗逆抗逆专适良种等问题，利用前期所选择的优良种源幼苗为材料，开展非生物胁迫下椿树重要目标性状遗传变异规律研究、椿树群体种质基因型鉴定与关联遗传分析、育种群体构建、资源高效和抗逆专适品种选育等方面研究内容，通过进行低温及持续干旱胁迫处理，测定相对电导率、植物体内活性氧、净光合速率等目标性状，获得其变异规律红椿生长、材质、耐寒等重要性状遗传变异规律；以红椿种源试验林为材料选育资源高效型、优质、抗逆专适品种；基于红椿全基因组，挖掘椿树重要目标性状相关的候选基因，建立其遗传转化及基因编辑技术体系。

（二）、考核指标

1、制定遗传测定和优良品种评价等选育体系，审（认）定速生或抗寒良种 1 个，遗传增益提高 15%以上；

2、创制转基因耐寒新种质 2 份；

3、申请发明专利 2 件；

4、繁育苗木 10 万株；

5、营建试验示范林 200 亩；

6、发表 SCI 论文 1 篇（本项目为第一标注、SCI 论文为二区及以上期刊、中文论文为 CSCD(C)库收录期刊）。

（三）、测评方式/方法

1、 制定遗传测定和优良品种评价等选育体系通过试验示范林现场查定、原始资料核查和技术报告予以核实；

2、试验示范林的面积测评，结合现场查定和课题验收等过程管理环节，邀请本领域专家进行现场测定、审查。

3、繁育苗木数量以苗木繁育过程和现场影像资料予以核实；

4、专利以授权或申请受理文件测评；

5、学术论文通过核查出版物原件（复印件）或录用证明作为测评依据；

6、审（认）定速生或抗寒良种以国家或省级良种证书或申请受理通知书予以核实；

7、创制的转基因耐寒新种质测评，邀请本领域专家组成专家组，对种质创制过程中所

			基地	□数据库 □软件 □应用解决方案 □实验装置/系统 □临床指南/规范 □工程工艺 □标准 □论文 □发明专利 ☑ 其他 繁育苗木、试验示范林	指标 3.2 繁育苗木	0	2 万株	10 万株	苗木繁育过程和现场影像资料
		…							
	其他成果				指标				
				同上					

	序号	报告类型[5]	数量	提交时间	公开类别及时限[6]
科技报告考核指标	1	年度进展报告	4	2022 年 11 月、2023 年 11 月 2024 年 11 月、2025 年 11 月	延期公开 3 年(含 3 年)以内
	2	中期进展技术进展报告	1	2024 年 5 月	延期公开 3 年(含 3 年)以内
	3	最终技术进展报告	1	2026 年 12 月	延期公开 3 年(含 3 年)以内
其他目标与考核指标					

备注：

1. **“课题目标”**，应从以下方面明确描述：（1）研发主要针对什么问题和需求；（2）将要解决哪些科学问题、突破哪些核心/共性/关键技术；（3）预期成果；（4）成果将以何种方式应用在哪些领域/行业/重大工程等，并拟在科技、经济、社会、环境或国防安全等方面发挥何种的作用和影响。（5）所列主要成果原则上不超过5项，如有其他重要成果放在“其他”成果中表述。
2. **“考核指标”**，指相应成果的数量指标、技术指标、质量指标、应用指标和产业化指标等，其中，数量指标可以为专利、产品等的数量，论文代表作应注重质量，不以数量作为评价标准；技术指标可以为关键技术、产品的性能参数等；质量指标可以为产品的耐震动、高低温、无故障运行时间等；应用指标可以为成果应用的对象、范围和效果等；产业化指标可以为成果产业化的数量、经济效益等。同时，对各项考核指标需填写立项时已有的指标值/状态以及课题完成时要到达的指标值/状态。同时，考核指标也应包括支撑和服务其他重大科研、经济、社会发展、生态环境、科学普及需求等方面的直接和间接效益。如对国家重大工程、社会民生发展等提供了关键技术支撑，成果转让并带动了环境改善、实现了销售收入等。若某项成果属于开创性的成果，立项时已有指标值/状态可填写“无”，若某项成果在立项时已有指标值/状态难以界定，则可填写“/”。
3. **“中期指标”**，各专项根据管理特点，确定是否填写，鼓励阶段目标明确的项目课题填写中期指标。
4. **“考核方式方法”**，应提出符合相关研究成果与指标的具体考核技术方法、测算方法等。
5. **“科技报告类型”**，包括项目综合绩效评价（验收）前撰写的全面描述研究过程和技术内容的最终科技报告、项目年度或中期检查时撰写的描述本年度研究过程和进展的年度技术进展报告以及在项目实施过程中撰写的包含科研活动细节及基础数据的专题科技报告（如实验报告、试验报告、调研报告、技术考察报告、设计报告、测试报告等）。其中，每个项目在综合绩效评价（验收）前应撰写一份最终科技报告；研究期限超过2年（含2年）的项目，应根据管理要求，每年撰写一份年度技术进展报告；每个项目可根据研究内容、期限和经费强度，撰写数量不等的专题科技报告。科技报告应按国家标准规定的格式撰写。
6. **“公开类别及时限”**，公开项目科技报告分为公开或延期公开，内容需要发表论文、申请专利、出版专著或涉及技术诀窍的，可标注为“延期公开”。需要发表论文的，延期公开时限原则上在2年（含2年）以内；需要申请专利、出版专著的，延期公开时限原则上在3年（含3年）以内；涉及技术诀窍的，延期公开时限原则上在5年（含5年）以内。涉密项目科技报告按照有关规定管理。

二、子课题研究内容、研究方法

（一）子课题的主要研究内容

拟解决的关键科学问题、关键技术问题，针对这些问题拟开展的主要研究内容，限 1000 字以内。

（1）非生物胁迫下椿树重要目标性状遗传变异规律研究

以前期所选择的优良种源幼苗为材料，进行枝条低温胁迫处理，通过测定相对电导率、植物体内活性氧等目标性状，对不同遗传背景材料的抗寒性进行评价，获得其变异规律；材料同低温胁迫实验，进行持续干旱胁迫实验，测定净光合速率等指标，获得红椿不同幼苗中各种物质的变化规律。

（2）群体种质基因型鉴定与关联遗传分析

基于红椿全基因组，以胸径、树高、木材密度、导管长、心材色泽等目标性状作为全基因组关联分析的生长性状和木材性状，筛选出大于 100 份的红椿种质资源进行目标性状测定及重测序，利用混合模型进行全基因组关联分析，精确鉴定出与表型变异密切相关的 SNP 位点，获得与目标性状相关联的候选基因或基因区域。

（3）育种群体构建

构建椿树遗传转化及 CRISPR/Cas9 基因编辑技术体系，创制抗旱、耐寒新种质。

（4）资源高效和抗逆专适品种选育

以红椿种源试验林为材料，分析不同种源的树高、胸径、枯稍率等重要性状差异，选育速生、耐寒优良种源。

（二）子课题采取的研究方法

针对子课题研究拟解决的问题，拟采用的方法、原理、机理、算法、模型等

限 1000 字以内。

（1）非生物胁迫下椿树重要目标性状遗传变异规律研究

① 优良种源选择

利用 SAS 软件进行方差分析。

种源试验地单点随机区组的方差分析线性模型为：

$$Y_{ij} = \mu + B_i + P_j + E_{ij}$$

式中，Y_{ij} 为第 i 个种源第 j 个区组数值，μ 为群体平均值，B_i 为第 i 个区组的效应值，P_j 为第 j 个种源的效应值，E_{ij} 为剩余项。

遗传参数估算为提供育种选择提供依据。

种源遗传力(H^2)：$H^2=\frac{\sigma_P^2}{\sigma_e^2/B+\sigma_P^2}$

单株遗传力（H_i^2）：$H_i^2=4\sigma_f^2/(\sigma_e^2+\sigma_f^2)$

其中， B 为重复数（区组数），σ_e^2为环境方差，σ_f^2为家系方差，σ_P^2为种源方差。

利用 ASReml-R 进行育种值估算。

BLUP 预测性状育种值的公式为：

$$\hat{a}_i=h_i^2(y_i-\hat{\mu})$$

其中，$\hat{a}_i$为预测的育种值；h_i^2为第 i 个个体的遗传力，y_i表示第 i 个个体的观测值，$y_i-\hat{\mu}$根据固定效应进行表型值的矫正。

② 室内低温胁迫、干旱及测定

计算低温胁迫处理后的相对电导率（REC）。计算公式如下：

$Y=(E_1-E_0)/(E_2-E_0)\times100\%$

式中，Y 为相对电导率，E_1为样品低温处理后的电导率，E_2样品沸水浴后的电导率，E_0为无离子水电导率。

用硫代巴比妥酸（TBA）显色法测定丙二醛（MDA）。

采用磺基水杨酸法测定游离脯氨酸（fPro）。

采用蒽酮比色法测定可溶性糖含量。

采用考马斯亮蓝 G-250 染色法测定可溶性蛋白质含量。

A. 半致死温度计算

建立相对电导率拟合 Logistic 回归方程，计算半致死温度（LT_{50}）。Logistic 回归方程为

$y=K/(1+ae^{-bt})$

式中 t 为回归曲线的拐点，即低温胁迫的半致死温度（LT_{50}），t_1为组织细胞膜受伤的起始温度，t_2为组织细胞膜接近全透性时的温度。

用 SPSS 19.0 软件进行估算方程参数，拟合方程，然后求出 LT_{50}、t_1和 t_2值。

干旱胁迫采用光合-光响应、光合-日进程及光合色素含量测定。

（2）红椿 CRISPR/Cas9 基因编辑技术体系构建

目标基因的靶点设计，利用在线软件工具包 CRISPR-GE 进行设计筛选，主要筛选原则包括：

A. 目的基因的正链和负链都可以设计靶点，靶点序列结构为 5'-N20NGG-3' (20N 为靶点序列，NGG 为 PAM 区)；

B. 靶点序列的 GC 含量对其打靶效率有影响，靶点 GC%应不低于 40%，GC% 高时有较高的打靶效率，可选择范围 50%~70%；

C. 应对靶点进行特异性分析，以减少非特异脱靶突变，尽量选择脱靶估值小于 0.6 的靶点；

D. 靶点内尽量不要有连续 4 个以上的碱基 T；

E. 避免选择使用与 sgRNA 序列连续配对 8 个碱基以上的靶点，否则会抑制靶 点与染色体 DNA 的结合；

F. 如果用一个靶点序列敲除两个或者两个以上的同源基因时，应该选用同源 基因之间碱基完全相同的区域作为目标靶位点。从候目标基因的候选靶点中选择了一个靶点，根据引物结构和注意事项（标准的靶点格式为 N20NGG，如果位于互补链，则需要转换成标准格式）设计靶点引物。

三、主要创新点

围绕基础前沿、共性关键技术或应用示范等层面，简述子课题的主要创新点。具体内容应包括该项创新的基本形态及其前沿性、时效性等，并说明是否具备方法、理论和知识产权特征。每项创新点的描述限 500 字以内。

1、创新点 1：构建适合红椿的 CRISPR/Cas9 基因编辑体系

利用常规育中筛选优良种源及单株，对红椿抗逆性强的优良种源或单株进行高效无性繁殖；充分利用红椿全基因组信息，结合转录组测序，获得个体或种源进行差异性分析，深度挖掘及鉴定低温及干旱胁迫下及优良与非优良种源的差异表达基因，构建适合红椿的 CRISPR/Cas9 基因编辑载体，利用红椿遗传转化体系，创制新种质。该项创新具备理论创新和集成创新特征，其中部分单项技术可以申请发明专利。红椿作为珍贵速生用材树种，生长及成材周期长，常规育种与分子育种相结合，进行早期选择，可缩短育种周期，并随着深入研究不断完善。

四、预期经济社会效益

子课题的科学、技术、产业预期指标及科学价值、社会、经济、生态效益。限 500 字以

内。

红椿是优质速生阔叶树种，其前期生长十分迅速，且红椿材质良好，木材鲜红，花纹美丽，纹理通直，是上等的家具用材，被誉为“中国桃花心木”。但是，红椿育种周期长、新种质创制技术未突破，缺乏材质优良、抗逆抗逆专适良种。通过本课题的实施，重点突破红椿抗逆良种选育技术，营建试验示范林 200 亩以上，培育良种苗木 10 万株，申请国家专利 2 项，重点突破红椿抗寒、抗旱、速生、优质的红椿良种选育，间接经济和社会效益明显；并利用基因编辑技术创制新种质，遗传增益提高 15%，显著提升珍贵用材林的育种水平，有力地推动红椿造林推广和产业化发展，并能有效地保护红椿的种质资源。

五、子课题年度计划

按每6个月制定形成子课题的计划进度，应将子课题的考核指标分解落实到年度计划中。

1、年度：2021 年 12 月—2022 年 5 月

任务：签订子课题任务书，制定详细研究计划及实施方案，在现有的红椿试验林的基础上，选择优良种源、家系和单株；开展无性系扩繁。

考核指标：选择优良种源 3-5 个、优良家系 10-15 个、优良单株 15-20 个。

成果形式：实施方案等

2、年度：2022 年 6 月—2022 年 11 月

任务：落实试验示范林基地的选址；以前期所选择的优良种源幼苗为材料，开展枝条低温胁迫及持续干旱处理。

考核指标：选定 200 亩营建示范林基地；

成果形式：年度进展报告、与试验基地签订的单位的合作协议等

3、年度：2022 年 12 月—2023 年 5 月

任务：继续开展低温胁迫及持续干旱实验，测定相对电导率、植物体内活性氧等目标性状；开展红椿不同种源材性差异分析，获得转录组数据；开展红椿遗传转化体系优化实验；

考核指标：转录组差异基因及材性成分分析，初步鉴定与材性相关的关键功能基因 5-10 个；申请发明专利 1 项

成果形式：专利申请受理书等；

4、年度：2023 年 6 月—2023 年 11 月

任务：对不同遗传背景材料的抗寒性进行评价，获得其变异规律；测定净光合速率等指标，获得红椿不同幼苗中各种物质的变化规律；继续开展材性转录组分析，并测定非生物胁迫处理的红椿转录组；试验示范林抚育整地

考核指标：完成 2-3 个关键基因功能解析

成果形式：年度进展报告、示范林现场实物展示

5、年度：2023 年 12 月—2024 年 5 月

任务：分析非生物胁迫处理的转录组数据，深度挖掘差异表达基因，获取红椿速生、抗寒及抗旱的功能基因；分析不同种源的树高、胸径、枯稍率等重要性状差异，选育速生、耐寒优良种源；开展 CRISPR/Cas9 基因编辑技术体系构建；营建试验示范林

考核指标：获取抗性功能基因 2-3 个，营建试验示范林 100 亩；

成果形式：示范林现场实物展示

6、年度：2024 年 6 月—2024 年 11 月

任务：继续进行功能基因分析；基于红椿全基因组，以胸径、树高、木材密度、导管长、心材色泽等目标性状鉴定出与表型变异密切相关的 SNP 位点，获得与目标性状相关联的候选基因或基因区域；继续开展开展 CRISPR/Cas9 基因编辑技术体系构建。

考核指标：SNP 位点分析

成果形式：年度进展报告等

7、年度：2024 年 12 月—2025 年 5 月

任务：继续开展 SNP 位点鉴定；结合红椿遗传转化体系及 CRISPR/Cas9 基因编辑技术体系，利用筛选获得的非生物胁迫基因，创制抗旱、耐寒新种质；继续营建试验示范林，培育苗木

考核指标：培育新品质苗木 5 万株；营建试验示范林 50 亩；

成果形式：示范林、良种苗木为现场实物展示

8、年度：2025 年 6 月—2025 年 11 月

任务：继续创制红椿抗旱、耐寒新种质；获得转基因幼苗

考核指标：研究论文 1 篇；审（认）定速生、抗寒良种 1 个

成果形式：年度进展报告、公开发表刊物等

9、年度：2025 年 12 月—2026 年 5 月

任务：建立红椿遗传转化体系及 CRISPR/Cas9 基因编辑技术体系；继续营建试验示范林

考核指标：培育新品质苗木 5 万株，申请发明专利 1 项，营建试验示范林 50 亩；

成果形式：专利申请受理书等；示范林、良种苗木为现场实物展示

10、年度：2026 年 6 月—2026 年 11 月

任务：完成任务书规定的各项考核指标；提交结合报告，进行课题项目总结验收。

考核指标：审（认）定速生、抗寒良种 1 个，遗传增益提高 15%以上

成果形式：审定良种，总结报告等

六、子课题组织实施机制及保障措施

1、子课题的内部组织管理方式、协调机制等，限 500 字以内。

本子课题负责人对子课题的研究工作及经费负责，严格遵守课题及项目组要求，负责撰写报告及上报。在财务管理方面，依据国家、省市相关法律法规，学校建立了完整的项目及资金管理办法，项目严格实行专项经费管理试行办法，执行财务会计制度和内部控制制度，对课题经费支出实行详细预算控制和辅助账管理，确保科技经费专款专用，财务信息真实有效。

本子课题研究团队是一支实力雄厚、梯队合理的创新团队，申报人一直从事红椿选育及遗传多样性等工作，项目参与人为多年从事林木遗传育种的专业技术人才，并熟练掌握转录组测序技术原理，在承担任务领域具有熟练的实验操作技能，具有大数据分析的丰富经验，完全具备完成本项课题的人员条件，为红椿新品种选育等研究提供了良好的研究平台。团队成员明确主要分工，确保具有充足的时间和经历参与课题研究。

强化本子课题执行管理，及时整理相关实施方案、计划等，按部就班进行科研工作，并按时完成上级课题要求的实施进展或中期检查等。

2、子课题实施的相关政策，已有的组织、技术基础，支撑保障条件，限 500 字以内。

近年来，课题组在国家林业局林业公益性行业科研专项资金及“十三五”国家重点研发计划的资助下，以红椿为材料，开展了红椿地理变异及遗传多样性、种源及家系试验和红椿高效组培快繁及遗传转化体系研究，取得了阶段性的成果：

1)在我国红椿的全分布区共收集红椿种源 30 个，国外种源 11 个；546 份单株种子；

2)建立红椿采穗圃和基因库；

3)已在广东建立了种源和定向培育试验林；

4)初步建立了组织快繁及遗传转化体系；

5)制定了育苗技术规程 2 项；申请红椿相关专利 6 项；在中文核心期刊公开发表论文 11 篇，SCI 论文 6 篇；

6)开展虫害胁迫及自身虫害防御转录组分析等相关基因分析和鉴定。

华南农业大学拥有国家重点实验室、国家工程技术研究中心和国家工程技术实验室六个国家级科技平台，并有教育部、农业部、广东省重点实验室（工程研究中心）43 个省部级重点科技平台。本课题实施依托亚热带农业生物资源保护与利用国家重点实验室及广东省森林植物种质创新与利用重点实验室，拥有国内一流的试验条件和实验设备，以上实验条件和技术平台完全可以保证本项目研究的顺利开展。

3、对实现课题总目标的支撑作用，及与项目、课题内其他课题的协同机制，限 500 字以内。

本子课题是课题“椿树新品种选育”内设的子课题之一，主要针对非生物胁迫下椿树重要目标性状遗传变异规律研究、群体种质基因型鉴定与关联遗传分析、遗传转化体系及基因编辑技术等技术瓶颈为主要突破点，可为项目、课题拟解决的关键科学技术问题起重要支撑作用。本课题的顺利实施，研究目标的实现，可影响课题目标的完成。

本子课题以红椿为研究对象，其主要分布于西南、华南、华东等地，因无法克服寒害等问题，无法进行大范围推广，制约了可持续发展。至此，须加强多研究机构和多方面合作，充分与其他课题进行广泛交流研究，如开展资源调查、获取优质种源等。子课题团队人员与其它课题通过多年的长期合作，已经建立了良好的学术联系和学术氛围，可开展课题间学术交流等活动，互通有无，相互借鉴，及时调整研究思路。子课题承担单位和其它参加单位均拥有多个科研平台，课题间优势互补，相互借鉴各自的技术优势，促进各课题协同创新。

七、知识产权对策、成果管理及合作权益分配

限 500 字以内。

本子课题将按照国家科技成果的相关规定，严格执行《科技成果登记办法》，实行国家科技计划重大成果报告制度，实施过程中取得重大成果时，及时向科技部的计划管理机构报告，并根据科技成果特点，按照法律法规的规定适时通过专利申请登记等方式予以保护。

本课题属于重大共性关键技术研究，其成果主要以学术论文、发明专利、繁育基地和试验示范林、良种等形式呈现。成果如涉及国家机密，应严格遵照《中国人民共和国保守国家秘密法》和《科学技术保密规定》及相关规定实施管理。论文应标注项目类别、任务（课题）名称或编号，论文版权归出版单位所有；发明专利归专利权人所有；子课题实施过程中建立的技术平台、繁育基地以及试验示范林的产权由承担单位和参与建设单位按相应的责任和权益共同分享。

八、需要约定的其他内容

限 500 字以内。

无

九、子课题参加人员基本情况表

填表说明： 1. 专业技术职称：A、正高级　B、副高级　C、中级　D、初级　E、其他；
2. 投入本课题的全时工作时间（人月）是指在课题实施期间该人总共为课题工作的满月度工作量；累计是指课题组所有人员投入人月之和；
3. 课题固定研究人员需填写人员明细；
4. 是否有工资性收入：Y、是　N、否；
5. 人员分类代码：B、课题负责人　C、项目/课题骨干　D、其他研究人员；
6. 工作单位：填写单位全称，其中高校要具体填写到所在院系。

序号	姓名	性别	出生日期	证件类型	证件号码	专业技术职称	职务	最高学位	专业	投入本课题的全时工作时间（人月）	人员分类代码	在课题中分担的任务	是否有工资性收入	工作单位
1	李培	女	1985-03-08	身份证号	220103198503080420	C	无	博士	林木遗传育种	40	B	项目实施统筹及设计实施	Y	华南农业大学林学与风景园林学院
2	欧阳昆唏	男	1983-02-25	身份证号	330327198302255375	B	无	博士	林木遗传育种	24	C	红椿全基因组分析挖掘抗性基因	Y	华南农业大学林学与风景园林学院
3	周玮	女	1986-08-07	身份证号	420582198608070020	C	无	博士	林木遗传育种	24	C	红椿材性分析	Y	华南农业大学林学与风景园林学
4	李青粉	女	1987-02-05	身份证号	4109011	C	无	博士	林木遗传	24	C	干旱胁迫目	Y	华南农业大学林学与风景园林学

					9870205 2023				育种			标性状遗传变异规律研究		
5	张俊杰	女	1989-09-22	身份证号	4206831 9890922 3168	C	无	博士	林木遗传育种	24	C	全基因组关联分析，筛选 SNP 位点	Y	华南农业大学林学与风景园林学院
6	骈瑞琪	男	1981-04-22	身份证号	1404021 9810422 2014	C	无	博士	林木遗传育种	24	C	低温胁迫目标性状遗传变异规律研究	Y	华南农业大学林学与风景园林学院
7	阙青敏	男	1990-08-27	身份证号	4228011 9900827 3670	C	无	硕士	林木遗传育种	32	C	优良种源、家系及单株选择	Y	华南农业大学林学与风景园林学院
8	宋慧云	女	1996-04-05	身份证号	4102251 9960405 5826	E	无	学士	林木遗传育种	40	D	红椿 CRISPR/Cas 9 基因编辑技术体系	N	华南农业大学林学与风景园林学院

9	李悦	女	1998-05-12	身份证号	610115199805123521	E	无	学士	林木遗传育种	40	D	红椿遗传转化体系优化	N	华南农业大学林学与风景园林学院
10	周祥斌	男	1989-08-04	身份证号	500228198908045471	C	无	硕士	林木遗传育种	15	D	试验林地建设及管理	Y	广东省连山林场（广东鹰扬关森林公园管理处）
11	陈志	男	1965-07-02	身份证号	440230196507022419	B	无	无	行政管理	15	D	试验林地建设及管理	Y	广东省连山林场（广东鹰扬关森林公园管理处）
固定研究人员合计										302	/	/	/	/
流动人员或临时聘用人员合计										300	/	/	/	/
累计										602	/	/	/	/

十、经费预算

子课题预算表

表 B1　　子课题编号：　　子课题名称：　　金额单位：万元

序号	预算科目名称	金额
	（1）	（2）
1	一、中央财政专项资金	130
2	（一）直接费用	103
3	1. 设备费	3.8
4	其中：购置设备费	3.8
5	2. 业务费	77.6
6	3. 劳务费	21.6
7	（二）间接费用（自动计算）	27
8	二、其他来源资金	0
9	三、合计	130

注：1. 间接费用无需编制预算说明；2. 绩效支出在间接费用中无比例限制。承担单位在统筹安排间接费用时，要处理好合理分摊间接成本和对科研人员激励的关系，绩效支出安排与科研人员在课题工作中的实际贡献挂钩。

预算说明

一、中央财政资金

预算的编制要坚持任务相关性、政策相符性和经济合理性，实事求是编制提出课题预算。填报时，直接费用应按设备费、业务费 、劳务费三个类别填报，每个类别结合科研任务按支出用途进行说明。除 50 万元以上的设备外，其他费用只提供基本测算说明，不需要提供明细。

1.设备费（是指项目实施过程中购置或试制专用仪器设备，对现有仪器设备进行升级改造，以及租赁外单位仪器设备而发生的费用等。计算类仪器设备和软件工具可在设备费科目编列。填报时，50 万元以上的设备详细说明，50 万元以下的设备费用分类说明）

（1）购置设备费 3.8 万元

① PYLODYN 皮罗钉木材检测仪 1 台

主要用途：用于椿树种质资源高通量评价、野外采集样品保存、木材生长无损测定、室内实验等工作。

与课题的相关性、必要性：本单位现有 PYLODYN 皮罗钉木材检测仪购置于 2018 年，且该设备受限于冲击针使用次数的磨损程度，目前已经达到最大化，无法完成后续实验。本课题在种质资源高通量评价中需要完成椿树木材无损测定，故需要更新 PYLODYN 皮罗钉木材检测仪 1 台。

购置 1 台，单价为 3.8 万元，小计 3.8 万元。

设备名称	型号	功能、性能	报价（万元）	经销商电话	备注
PILODYN 皮罗钉木材检测仪	FBK	皮罗钉木材检测仪是用来测量活树及木质结构有效工具	3.8	点将（上海）科技股份有限公司 刘 斌 13524489082	

（2）试制设备费

无。

（3）设备改造费

无。

（4）设备租赁费

无

2.业务费（是指在项目实施过程中消耗的各种材料、低值易耗品等、发生的测试化验加工、燃料动力、出版文献、信息传播、知识产权事务、会议、差旅、国际合作与交流以及其他与项目实施直接相关的各项费用。编报时，对单笔大额支出、对外委托支出重点说明）

（1）材料费 31.9 万元

本课题共有 5 项研究内容，分别为种质资源高通量评价、非生物胁迫下重要目标性状变异规律解析、育种群体构建、新种质创制、资源高效和抗逆专适良种选育。以下按 5 项内容分别测算：

① 种质资源高通量评价材料费 15.9 万元

主要用途：该任务中的种质资源胸径、树高、心材色泽等等指标的动态监测，发掘重要性状高度关联的 SNPs，获得与目标性状相关联的候选基因或基因区域等内容，需要购买试验示范林管护的化肥、农药、铝合金树体标识牌、塑料标牌等，以及实验室内开展木材密度、导管长等指标测定所需要的试剂耗材。

与课题的相关性、必要性：依据种质资源高通量动态监测的需要，以广东广州、湖南汨罗等地的种源及家系试验林约 100 亩为样地，测定红椿相关指标；同时需要在实验室内开展 100 份以上种质资源的木材密度、导管长度等指标测定。依据发掘重要性状高度关联的 SNPs 的需要，采集 100 份以上资源的叶片、幼龄材等植物样本，需购买 FAA 固定液、干燥剂、液氮、干冰等试剂耗材。

测算依据：数量依据是根据该项研究任务的需要，报价依据是参考目前市场上的平均报价。

A、化肥购置费 8.00 万元。培育种质苗木 2 万株，需要育苗地 20 亩，施氮磷钾复合肥 1.00 万元；连续 4 年对 100 亩种源、家系试验林进行施肥，每亩地 200 元/年，计 8.00 万元。

B、铝合金树体标识牌购置费 1.2 万元。购置铝合金标识 1000 套，包括铝合金弹簧绳和标识牌，单价 12 元。

C、其他农资购置费 3.20 万元。主要包括修枝剪、高枝剪、测高杆、塑料绳、铁锹、塑料标签等农资产品一批。

D、实验室内实验试剂耗材购置费 3.50 万元。开展木材密度、导管长度等指标测定所需要的酒精、硝酸溶液、氯酸钾、番红溶液等试剂耗材一批。

② 非生物胁迫下重要目标性状变异规律解析实验材料费 7.00 万元

主要用途：该任务中进行低温及持续干旱胁迫处理，通过测定相对电导率、植物体内活性氧、净光合速率等目标性状，对不同遗传背景材料的抗寒性及抗旱性进行评价，获得其变异规律，需要购置实验室内开展非生物胁迫下指标测定所需要的试剂耗材。

与课题的相关性、必要性：依据非生物胁迫重要目标性状变异规律解析的需要，以红椿优良种源及家系幼苗为样本，在实验室内开展非生物胁迫处理，并进行 500 份以上样品的功能叶片、带芽枝条等植物样本相对电导率度、净光合速率等指标测定，需要购买液氮、硫代巴比妥酸（TBA）、蔗糖、PEG-6000 等试剂耗材。

测算依据：数量依据是根据该项研究任务的需要，报价依据是参考目前市场上的平均报价。

实验室内实验试剂耗材购置费 7.00 万元。开展非生物胁迫处理等所需要的等硫代巴比妥酸（TBA）、蔗糖、PEG-6000 等试剂耗材一批。

③ 新种质创制实验材料费 9.0 万元

主要用途：该任务中构建遗传转化体系，并构建适合红椿的 CRISPR/Cas9 基因编辑载体，需要购置实验室内开展分子生物学实验的试剂耗材。

与课题的相关性、必要性：依据新种质创新任务需要，课题需利用红椿幼苗叶片为受体系统，构建高效的遗传转化体系，并且构建红椿基因编辑体系，需要载体、抗生素、培养基等一系列分子生物学实验耗材，实现种质创新。

测算依据：数量依据是根据该项研究任务的需要，报价依据是参考目前市场上的平均报价。

用于购买荧光定量、RNA 提取试剂盒、胶回收试剂盒等共计 3.50 万元。

用于购买 Taq 酶、dNTP、高保真 Taq 酶（TaKaRa）、大肠杆菌感受态细胞（天根）、DNA/RNA/蛋白质提取、细菌培养、组织培养及遗传转化等药品常用化学试剂共计 2.50 万元。

用于组织培养的玻璃器皿、常规分子实验的塑料制品 3.00 万元。

（2）测试化验加工费 22.9 万元

主要用于以下 4 个方面的测试，测算单价依据近 2 年市场价格，测试数量依据研究任务的需要确定。

① 幼龄材木材材性测定费 8.10 万元：

主要用途：主要用于支付红椿样本材性测定所需费用。

与课题的相关性、必要性：为了顺利开展优质速生新品种选育研究，需要对红椿不同种源的材性进行测定分析，并以此为选择指标进行新品种选育。

以红椿种源试验林为采集地，每个地点每个种源测定 3 个分生株，共测定样品量为 180 个（30 个种源×3 株×2 个地点），测定指标为基本密度、硬度、管胞长度、径腔比、管孔率，每个样品测试费为 450 元，180 个样品共计 8.10 万元。

② 100 份以上红椿重测序测试费 7.20 万元。

主要用途：主要用于支付红椿样本重测序所需的费用。

与课题的相关性、必要性：为了精确鉴定出与表型性状、木材密度及心材色泽变异密切相关的 SNP 位点，进行 100 份以上的红椿样本重测序。

预计共有 120 份样本，全基因组选择 10*深度测序价格为 600 元/样（3G 数据量），120 个样品共计 7.20 万。

③ 样品转录组及代谢产物含量测定费 5.34 万元

主要用途：主要用于支付红椿木材代谢产物含量测定所需费用。

与课题的相关性、必要性：为了明确红椿木材色泽产生来源，为顺利开展材性测定分析，需要对红椿不同种源木材进行转录组及代谢物含量测定，进行关联分析，确保为新品种筛选及种质创新提供依据。

选定红椿木材心材色泽表现差异显著的 3 个种源作为采集地。转录组测序共计 30 个样品（心材：3 个种源×5 株；边材：3 个种源×5 株），每个样品测试费 700 元，30 个样品共计 2.1 万元；代谢组测序共计 54 个样品（心材：3 个种源×6 株；边材：3 个种源×6 株；木材浸出液：3 个种源×6 株），测定其代谢产物含量，每个样品测试费为 600 元，共计 3.24 万元；转录组及代谢组测试费共计 5.34 万元。

④ 其他相关测试 2.26 万元

主要用途：主要包括植物切片制作费 1.26 万元、引物合成、抗体制备、Sanger

测序、激光共聚焦显微镜观察 1.00 万元。

（3）燃料动力费 1.00 万元

主要用途：用于支付本课题承担单位实验所需电费等支出

（4）出版/文献/信息传播/知识产权事务费 4.6 万元

主要用途：用于本课题考核指标所规定的专利申请、良种审（认）定、发表论文等方面的费用和研究过程中所需要的打印、复印、装订、文献检索、科技查新等费用。

与项目的相关性、必要性：依据项目专业管理机构要求，课题中期评估会议 1 次，故需要复印、打印、装订课题执行进展、成果凝练相关材料；按照考核指标要求，需要支付专利申请、良种审（认）定、发表论文等方面的申请费、专利费、版面费等。

测算依据：根据考核指标申请发明专利 2 件，申请费每件 8000 元，小计 1.60 万元；发表论文 1 篇，每篇版面费平均 15000 元，小计 1.50 万元；打印、复印、装订、文献检索、科技查新、科技摄影、展板制作等费用每年约 0.30 万元，5 年小计 1.50 万元。共计 4.6 万元

（4）会议/差旅/国际合作交流费共计 10.3 万元

用于支出本课题研究过程中参加国内学术交流活动的会议费用，包含注册费、往返长途交通费、公杂费、住宿和市内交通费用。

（6）专家咨询费：4.9 万元

用于支付课题执行过程中会议等聘请咨询专家的费用。

依据专业管理机构管理规范及课题研究任务开展的需要，课题执行期召开启动会议 2 次，中期总结发展会议 1 次，需聘请相关领域专家。

测算依据：依据“《中央财政科研项目专家咨询费管理办法》（财科教[2017]128 号）文件”文件精神，会议,按高级职称 1500-2400 元（含税）/人·天测算。

① 子课题启动会议：召开子课题启动会议 1 次，聘请 5 位专家进行现场指导，会期为 2 天，专家咨询费 1600 元/人，共计 0.80 万元。

② 子课题年度总结会议：召开子课题年度会议，共 4 次，每次聘请 5 为专家进行现场指导，专家咨询费 1600 元/人，共计 3.2 万元；

③ 子课题中期总结发展会议：召开中期总结发展会议 1 次，每次聘请 5 位专家进行现场指导，会期为 2 天，专家咨询费 1600 元/人，共计 0.80 万元。

④ 专家咨询其他费用 0.1 万元

（7）其他支出 2 万元

土地租赁费 2 万元

主要用途：用于支付苗木繁育、试验示范林营建所需土地的租金。

3. 劳务费（是指在项目实施过程中支付给参与项目的研究生、博士后、访问学者以及项目聘用的研究人员、科研辅助人员、科研（财务）助理等的劳务性费用；支付给临时聘请的咨询专家的费用等。项目聘用人员由单位缴纳的社会保险补助、住房公积金等可纳入劳务费列支。）

（1）劳务费：21.6 万元

支付课题执行过程中育苗基地建设、苗木繁育、试验示范林硬件和管护等方面的临时性用工劳务费。

课题组成员有 7 名固定成员，固定成员分别按照任务需要承担实验设计、数据分析、种质创新等相关工作，但固定人员无法进行体系优化、林分管护等工作，无法满足完成课题任务的需要。故还需要研究生参与课题方面的研究任务，需要聘请试验示范林管护、苗木繁育的临时工等。

测算依据：硕士研究生科研补贴按人均 1500 元/人·月，博士研究生科研补贴按人均 2000 元/人·月，财务助理按 7000 元/月（含五险一金），临时用工人员按聘用人员的实际情况测算，各类人员在项目中的投入时间按照完成任务的预计时长测算。

① 研究生科研补助：课题执行过程中，有 3 名研究生参与课题研究，其中 2 名硕士研究生，1 名博士研究生，硕士研究生科研补助为 600 元/人·月，博士研究生科研补助为 1200 元/人·月，每名研究生每年发放补助 10 个月，连续 3 年，共计 7.20 万元。

② 临时聘用人员劳务费：① 优质苗木繁育基地长期聘用 1 名临时工参与苗木繁育和管护等工作，每年发放劳务 10 个月/人，3000 元/人·月，连续聘请 3 年，共计 9.00 万元；② 营造试验示范林 200 亩，需要用工 50 个，单价 200 元，合计 1.00 万元；③ 新造试验示范林管护用工费：连续 3 年对试验示范林进行常规管护，

200 亩每年需要用工 50 个，3 年合计 150 个，单价 200 元，合计 3.00 万元；野外种质资源收集过程中需要雇佣当地群众做向导和采集种条，合计 1.4 万元。共计 14.4 万元

二、其他来源资金

对其他来源资金主要用途、支出预算做简要说明。

无

任务书签署

双方根据《国务院印发关于深化中央财政科技计划（专项、基金）管理改革方案的通知》（国发〔2014〕64号）、《国务院关于优化科研管理提升科研绩效若干措施的通知》（国发〔2018〕25号）、《国务院办公厅关于改革完善中央财政科研经费管理的若干意见》（国办发〔2021〕32号）、《科技部 财政部关于印发<国家重点研发计划管理暂行办法>的通知》（国科发资〔2017〕152号）、《财政部 科技部关于印发<国家重点研发计划资金管理办法>的通知》（财科教〔2016〕113号）、《科技部财政部关于印发<中央财政科技计划（专项、基金等）监督工作暂行规定>的通知》（国科发政〔2015〕471号）等有关文件规定，以及有关法律、政策和管理要求，依据项目立项通知，签署本任务书。

同时，本单位和子课题负责人**郑重承诺：**对本子课题所有成果产出（包括但不限于新产品、新技术、标准、论文、专利等）的真实性、与项目（课题）的关联性等负责，将按要求落实科研作风学风和科研诚信主体责任；项目经费全部用于与本项目研究工作相关的支出，不截留、挪用、侵占，不用于与科学研究无关的支出；接受并积极配合相关部门的监督检查。如有违反，本单位和子课题负责人以及相关成果产出者愿接受项目管理专业机构和相关部门做出的各项处理决定，包括但不限于终止子课题执行、追回子课题经费，取消一定期限国家科技计划项目（课题）申报资格，记入科研诚信严重失信行为数据库以及主要负责人接受相应党纪政纪处理等。

课题承担单位（甲方）：

法定代表人签字（签章）：王浩杰

（公章）

年 月 日

课题负责人签字（签章）：刘平

2021 年 12 月 30 日

子课题承担单位（乙方）：

法定代表人签字（签章）：

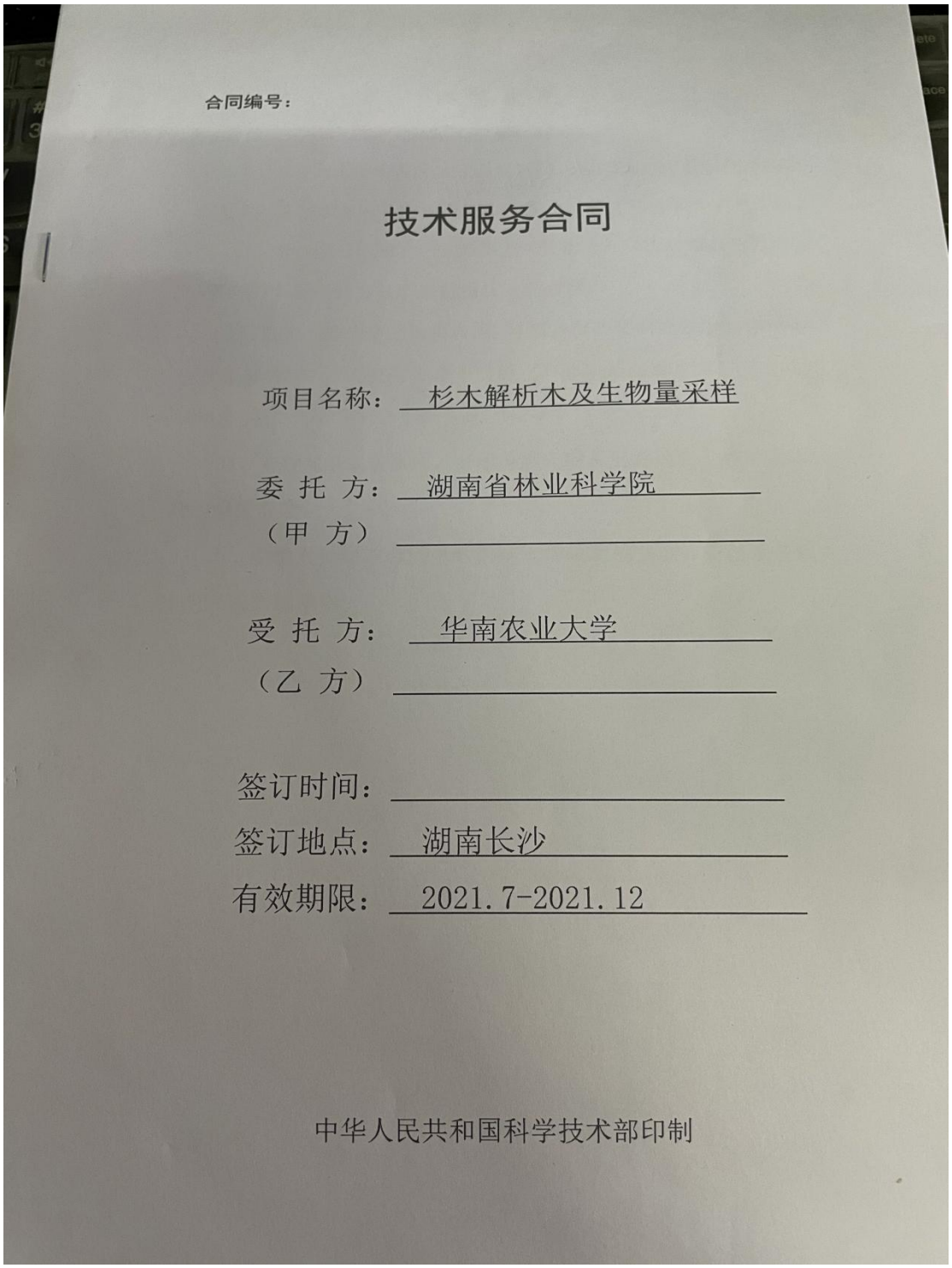
（公章）

2021 年 12 月 30 日

子课题负责人签字（签章）：

2021 年 12 月 30 日

2.4 杉木解析木及生物量采样



合同编号：

技术服务合同

项目名称：杉木解析木及生物量采样

委 托 方：湖南省林业科学院
（甲 方）

受 托 方：华南农业大学
（乙 方）

签订时间：

签订地点：湖南长沙

有效期限：2021.7-2021.12

中华人民共和国科学技术部印制

填 写 说 明

一、本合同为中华人民共和国科学技术部印制的技术服务合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（受托方）以技术知识为另一方（委托方）解决特定技术问题所订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同委托人或共同受托人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术服务合同

委托方（甲方）：湖南省林业科学院

住　所　地：湖南省长沙市韶山南路 658 号

法定代表人：陈明皋

项目联系人：马丰丰

联系方式：19973180768

通讯地址：湖南省长沙市韶山南路 658 号

电　　话：　　传真：

电子信箱：mafengfeng0403@126.com

受托方（乙方）：华南农业大学

住　所　地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：邓成

联系方式：18825090920

通讯地址：广州市天河区五山路 483 号

电　　话：　　传真：

电子信箱：154083733@qq.com

本合同甲方委托乙方就杉木解析木及生物量采样项目进行的专项技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1．技术服务的目标：获取构建杉木生长模型的数据。

2．技术服务的内容：杉木三大主产区 50 块临时样地调查、50 株标准木树干解析、生物量取样等。

3．技术服务的方式：提交解析木圆盘及生物量采样样品。

第二条：乙方应按下列要求完成技术服务工作：

1．技术服务地点：湖南杉木主产区

2．技术服务期限：2021.7-2021.12

3．技术服务进度：2021 年 11 月 30 日之前完成所有外业调查工作。如因不可抗力因素影响需要延期的，可由双方协商后延。

4．技术服务质量要求：通过甲方验收

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提供下列工作条件和协作事项：

1．提供技术资料：

（1）杉木解析木采样地点；

（2）解析木与生物量采样实施方案。

2．提供工作条件：

（1）协助乙方与相关林场协调采样事宜。

（2）为乙方在湖南杉木主产区开展临时样地调查、解析木与生物量采样等提供必要的交通工具和办公场所。

3．其他：解决外业调查期间需要由甲方出面协调解决的问题和经双方协商需要由甲方解决的其他问题。

4．甲方提供上述工作条件和协作事项的时间及方式：合同生效后5个工作日内，甲方根据乙方要求向乙方提供所需的相关技术资料或由双方协商共同赴杉木主产区所在区县相关部门进行采样协调事宜。

第四条：甲方向乙方支付技术服务报酬及支付方式为：

1．技术服务费总额为：99940元

2．技术服务费由甲方分期（一次或分期）支付乙方。

具体支付方式和时间如下：

（1）项目签订合同5日内，支付50%费用，为49970元；

（2）完成任务的一半后，支付40%费用，为39976元；

（3）验收通过后支付剩余10%费用9994元；转账支付。

乙方开户银行名称、地址和帐号为：

开户银行：广州工行五山支行

账户名称：华南农业大学

地址：广州市天河区五山路483号

帐号：3602002609000310520

第五条：本合同的变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在15日内予以答复；逾期未予答复的，视为同意：

1．乙方因甲方原因在采样实施方案获取等方面造成工作时间顺延。

2．技术服务经费不能按时到位。

第六条：双方确定以下列标准和方式对乙方的技术服务工作成果进行验收：

1．乙方完成技术服务工作的形式：提交所有解析木圆盘和生物量（干、枝、叶、皮）采样样品。

2．技术服务工作成果的验收标准：通过项目组验收。

3．技术服务工作成果的验收方法：甲方组织验收。

4．验收的时间和地点：提交样品之日在长沙或采样现场拍照验收。

第七条：双方确定：

1．在本合同有效期内，甲方利用乙方提交的技术服务工作成果所完成的新的技术成果，归双（甲、双）方所有。

2．在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归双（乙、双）方所有。

第八条：双方确定，按以下约定承担各自的违约责任：

1．甲方违反本合同第三条约定，应当按逾期天数相应的顺延技术服务进度时间（支付违约金或损失赔偿额的计算方法）。

2．甲方违反本合同第四条约定，应当按逾期天数，每日向乙方支付应付但未付款项的0.5‰作为违约金（支付违约金或损失赔偿额的计算方法）。

3．乙方违反本合同第二条约定，应当向甲方减收

或免收相应的技术服务费_____（支付违约金或损失赔偿额的计算方法）。

第九条：双方确定，在本合同有效期内，甲方指定__马丰丰__为甲方项目联系人，乙方指定__邓成__为乙方项目联系人。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 发生不可抗力；

2. 无

第十一条：双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第__1__种方式处理：

1. 提交__长沙市__仲裁委员会仲裁；

2. 依法向人民法院起诉。

第十二条：本合同一式__6__份，甲乙双方各执__3__份，具有同等法律效力。

第十七条：本合同经双方签字盖章后生效。

甲方：湖南省林业科学院（盖章）

法定代表人／委托代理人：（签名）

2021年7月25日

乙方：华南农业大学（盖章）

法定代表人／委托代理人：（签名）

2021年7月25日

1. 检索证明

检索证明

根据委托人提供的论文材料，委托人**华南农业大学林学与风景园林学院 李青粉** 9 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因子	中科院大类分区
1	Dynamics of physiological and miRNA changes after long-term proliferation in somatic embryogenesis of Picea balfouriana	TREES-STRUCTURE AND FUNCTION 出版年：2019 出版日期：APR 卷期：33 2 页码：469-480 文献类型：Article	第一作者	B 类	华南农业大学	SCI	IF2-year=2.125 IF5-year=2.256 （2019）	农林科学 3 区 Top 期刊：否 （2019）
2	Identification of novel miRNAs and miRNA expression profiling in embryogenic tissues of Picea balfouriana treated by 6-benzylaminopurine	PLOS ONE 出版年：2017 出版日期：MAY 9 卷期：12 5 页码：- 文献号：e0176112 文献类型：Article	第一作者	B 类	华南农业大学	SCI	IF2-year=2.766 IF5-year=3.352 （2017）	生物 3 区 Top 期刊：否 （2017）
3	Changes in the Metabolome of Picea balfouriana Embryogenic Tissues That Were Linked to Different Levels of 6-BAP by Gas Chromatography-Mass Spectrometry Approach	PLOS ONE 出版年：2015 出版日期：OCT 30 卷期：10 10 页码：- 文献号：e0141841 文献类型：Article	第一作者	B 类	广西壮族自治区林业科学研究院	SCI	IF2-year=3.057 IF5-year=3.535 （2015）	生物 3 区 Top 期刊：否 （2015）

4	Thinning effects on forest evolution in Masson pine (Pinus massoniana Lamb.) conversion from pure plantations into mixed forests	FOREST ECOLOGY AND MANAGEMENT 出版年：2020 出版日期：DEC 1 卷期：477 页码：- 文献号：118503 文献类型：Article	通讯作者	T2 类	华南农业大学	SCI	IF2-year=3.558 IF5-year=4.039 （2020）	农林科学 1 区 Top 期刊：是 （2020）
5	Development of improved and comprehensive growth and yield models for genetically improved stands	ANNALS OF FOREST SCIENCE 出版年：2020 出版日期：SEP 7 卷期：77 3 页码：- 文献号：89 文献类型：Review	通讯作者	A 类	华南农业大学	SCI	IF2-year=2.583 IF5-year=3.148 （2020）	农林科学 2 区 Top 期刊：否 （2020）
6	Allocation Patterns and Temporal Dynamics of Chinese Fir Biomass in Hunan Province, China	FORESTS 出版年：2023 出版日期：FEB 卷期：14 2 页码：- 文献号：286 文献类型：Article	通讯作者	A 类	华南农业大学	SCI	IF2-year=2.4 IF5-year=2.7 （2023）	农林科学 2 区 Top 期刊：否 （2023）
7	Thinning Effects on the Tree Height-Diameter Allometry of Masson Pine (Pinus massoniana Lamb.)	FORESTS 出版年：2019 出版日期：DEC 卷期：10 12 页码：-	通讯作者	B 类	华南农业大学	SCI	IF2-year=2.221 IF5-year=2.484 （2019）	农林科学 3 区 Top 期刊：否 （2019）

		文献号：1129 文献类型：Article						
8	Estimation of Biomass Dynamics and Allocation in Chinese Fir Trees Using Tree Ring Analysis in Hunan Province, China	SUSTAINABILITY 出版年：2023 出版日期：FEB 卷期：15 4 页码：- 文献号：3306 文献类型：Article	共同通讯作者	B 类	华南农业大学	SCI	IF2-year=3.3 IF5-year=3.6 （2023）	环境科学与生态学 3 区 Top 期刊：否 （2023）
9	Determining the Ecological Compensation Standard Based on Forest Multifunction Evaluation and Financial Net Present Value Analysis: A Case Study in Southwestern Guangxi, China	JOURNAL OF SUSTAINABLE FORESTRY 出版年：2020 卷期：39 7 页码：730-749 文献类型：Article	通讯作者	B 类	华南农业大学	SCI	IF2-year=1.51 IF5-year=1.755 （2020）	环境科学与生态学 4 区 Top 期刊：否 （2020）

说明：论文等级和中科院大类分区按《华南农业大学学术论文评价方案（试行）》划分。

报告免责声明:如未盖章,报告无效

检索员：刘汉忠

华南农业大学图书馆

2025-07-11

2. 以第一作者发表的论文

2.1 Dynamics of physiological and miRNA changes after long-term proliferation in somatic embryogenesis of *Picea balfouriana*

Trees
https://doi.org/10.1007/s00468-018-1793-x

ORIGINAL ARTICLE

Dynamics of physiological and miRNA changes after long-term proliferation in somatic embryogenesis of *Picea balfouriana*

Qingfen Li[1] · Cheng Deng[1] · Tianqing Zhu[2] · Juanjuan Ling[2] · Hanguo Zhang[3] · Lisheng Kong[4] · Shougong Zhang[2] · Junhui Wang[2] · Xiaoyang Chen[1]

Received: 25 February 2018 / Accepted: 11 December 2018

Abstract

Key message **We found that embryogenic tissues of *Picea balfouriana* adopt different physiological pathways for long-term proliferation with 6-benzylaminopurine treatment and identified the miRNAs closely associated with proliferation.**

Abstract The long-term maintenance of somatic embryo production capacity in conifer embryogenic tissue (ET) is essential for the production of vigorous somatic seedlings. However, this ability is often lost after several months of proliferation in many conifer species including *Picea balfouriana*. Cytokinins are known to influence several important physiological processes during plant growth and development, including somatic embryogenesis (SE). In this study, we found that the 6-benzylaminopurine (BA) concentration influenced the yields of *P. balfouriana* somatic embryos and their germination response. Only ET of *P. balfouriana* proliferated on medium supplemented with 3.6 µM BA produced somatic embryos that germinated into normal plants. Most hormone levels increased in ET after prolonged proliferation. Moreover, antioxidant enzyme activities and polyamine contents were also significantly changed after 8 months of culture, which might be modulated by accumulated zeatin riboside (ZR). Finally, some selected microRNAs and their target genes were confirmed to be involved in the proliferation of ET of *P. balfouriana* and they also might be regulated by accumulated ZR. These findings may facilitate efforts to clarify basic physiological processes after the long-term proliferation stage of SE in conifers and delay the decreased production capacity of somatic embryos.

Keywords Embryogenic tissue · BA · Hormones · Antioxidant enzymes · Polyamines · miRNA

Abbreviations

BA	6-Benzylaminopurine
ABA	Abscisic acid
ET	Embryogenic tissue
FW	Fresh weight
IAA	Indole-3-acetic acid

Communicated by Klimazewska.

Qingfen Li and Cheng Deng contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00468-018-1793-x) contains supplementary material, which is available to authorized users.

✉ Xiaoyang Chen
xychen@scau.edu.cn

Junhui Wang
wangjh@caf.ac.cn

[1] Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, Guangdong, China

[2] State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Tree Breeding and Cultivation of State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China

[3] State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin 150040, Hei Longjiang, China

[4] Department of Biology, Centre for Forest Biology, University of Victoria, Victoria, BC V8P 5C2, Canada

Published online: 14 January 2019

PGR	Plant growth regulator
POD	Peroxidase
Put	Putrescine
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
SE	Somatic embryogenesis
SOD	Superoxide dismutase
Spd	Spermidine
Spm	Spermine
ZR	Zeatin riboside

Introduction

Somatic embryo production capacity in conifers declines rapidly during proliferation (between 3 and 12 months of subculture) (Trontin et al. 2011), receiving increasing attention. Previously, the morphology, physiology, and genetic mechanisms of embryo induction and the development of somatic embryogenesis (SE) have been extensively studied (Klimaszewska et al. 2016; Abrahamsson et al. 2018), but maturation remains a critical step in the production of high-quality plants in conifers, because not all cotyledonary somatic embryos convert to plantlets. The aging of embryogenic cultures during proliferation impacts not only the yield but also the quality of cotyledonary embryos, leading to lower germination rates. Breton et al. (2006) showed that the maturation of somatic embryos is qualitatively unchanged but that it gradually and quantitatively decreases with the number of consecutive subcultures, and therefore, decreases as the chronological age of the cultures increases. The somatic embryo production potential of *Cryptomeria japonica* (Maruyama et al. 2000) and *Abies lasiocarpa* (Kvaalen et al. 2005) gradually decreased as the duration of proliferation stage progressed. In addition, the production of somatic embryos in *Pinus pinaster* and *Larix leptolepis* was observed to decrease rapidly and was lost following tissue proliferation for 6 months (Klimaszewska et al. 2009; Li et al. 2013), whereas the embryogenic tissues (ETs) of *Eucalyptus globulus* and hybrid larch could produce somatic embryos after proliferating for 3 and 9 years (Lelu-Walter and Pâques 2009; Corredoira et al. 2015). Therefore, an improved understanding of the physiological and molecular events associated with somatic embryo production capacity is essential.

The decreased and/or lost somatic embryo production capacity may be closely related to endogenous hormone contents. Plant growth regulators (PGRs) are the key substances controlling the whole process of SE through the regulation of phytohormones. Auxin and cytokinin are known to play influential roles in regulating SE. However, most studies of phytohormones have been limited to the induction and maturation of embryogenic calli (Yang et al. 2012). Klimaszewska et al. (2009) showed that zeatin riboside (ZR) had the lowest titer in young cultures compared with aged cultures. Moreover, certain ratios of hormones control morphogenetic responses; the ratio of indole-3-acetic acid (IAA) or abscisic acid (ABA) to cytokinins (Żur et al. 2015) or the ratio of ABA–IAA (Centeno et al. 1997) or to various types of cytokinins (e.g., ZR-type and isopentenyl-type) (Grieb et al. 1997) tend to be good indicators of the potential to produce mature somatic embryos. To date, few studies have addressed the dynamic changes in the physiological index during the long-term proliferation of ETs, especially when cultured on medium with different PGRs.

Cytokinins are known to influence several physiological processes during plant growth and development (Plačková et al. 2015). In recent years, increasing attention has been paid to the influence of cytokinins on the SE of plants, which have been found to be the predominant phytohormones compared with IAA in the SE process for some plants (Zhang and Finer 2016; Grzyb et al. 2017). Cytokinin responses are often tightly regulated via autoregulatory negative-feedback loops or interactions with other phytohormones to achieve the correct balance for optimal growth in vitro (Stirk and Van 2014). Previous studies have shown that the quantity of cytokinins in plants is important, and their quality is also strongly associated with in vitro rooting and phytochemicals (Montalbán et al. 2013). Furthermore, Díaz-Vivancos et al. (2011) highlighted the effects of cytokinins that modulated enzymatic antioxidant activity on the shoot growth of *Crocus sativus* L. Although cytokinins are commonly used for the maintenance of the potential to produce mature somatic embryos, little attention has been paid to this treatment.

We have reported that 6-benzylaminopurine (BA) plays an essential role in the early SE of *P. balfouriana* using genome-wide technologies, such as transcriptomics and proteomics (Li et al. 2014a, b, 2015). We showed that the potential to produce mature somatic embryos of *P. balfouriana* could be prolonged by changing the BA concentration (after 2 years), and that some miRNAs might have participated in this process. Therefore, the aim of the present study was to investigate the physiological characteristics involved in the proliferation of *P. balfouriana* ET on media with different concentrations of BA and to validate miRNA changes during this process.

Materials and methods

Plant material

ETs of *P. balfouriana* were established by following the procedures described by Li et al. (2014a). In brief, we collected thirty cell lines (1A-10A of genotype 4, 1B-10B of genotype 7 and 1C-10C of genotype 10, all genotypes were

half-sib seeds), which were initiated at the Research Institute of Forestry, Chinese Academy of Forestry using immature seeds cultured on a semi-solid half-strength LM medium (Litvay et al. 1985, ½ LM) supplemented with 10 µM 2,4-D, 5 µM BA, 500 mg/L glutamine, 1 g/L casein hydrolysate, 1% sucrose, and 2% gellan gum (Gelrite®, Sigma-Aldrich) at 24 ± 1 °C in the dark for 3 months. ETs (Fig. 1a) were proliferated on ½ LM medium (1 g L^{-1} casein hydrolysate, 500 mg·L^{-1} glutamine, and 2% gellan gum (Gelrite®, Sigma-Aldrich) at 24 ± 1 °C in the dark) containing three concentrations of BA (2.5, 3.6, or 5 µM). The embryogenic cultures were subcultured every 2 weeks with five tissue clumps, each 0.5 cm in diameter, and each was placed in one Petri dish (90 × 15 mm). This experiment was repeated two times.

After 8 months, the ETs from media with different concentrations of BA (Fig. 1b, f, j) were transferred to ½ LM without any PGRs for 1 week (prematuration). For somatic embryo maturation, the tissues were cultured on ½ LM supplemented with 61 µM ABA, 6% sucrose, 0.4% activated charcoal (Sigma-Aldrich), 500 mg·L^{-1} glutamine, 1 g L^{-1} casein hydrolysate and 4% gellan gum (Gelrite®, Sigma-Aldrich) at 24 ± 1 °C in the dark. Ten Petri dishes with 100 mg FW the ETs spread over filter papers disc (Whatman, #2) were used in this experiment (Fig. 2a), and this experiment was repeated two times. The number of somatic embryos from all thirty cell lines was counted after 3 months. After proliferation for 2 years, the somatic embryos were maturated in form of lumps (each 0.5 cm in diameter) on a Petri dish (Six lumps of ETs were cultured on each Petri dish) with filter paper (Fig. 2b).

Fifty somatic embryos from each BA treatment (Fig. 1d, h, l) of from all thirty cell lines were germinated on ¼ LM supplemented with 2% sucrose, 0.5% activated charcoal (Sigma-Aldrich), 500 mg L^{-1} glutamine, 500 mg L^{-1} casein hydrolysate and 3% gellan gum (Gelrite®, Sigma-Aldrich) at 24 ± 1 °C, in the light (30 µEm^{-2} s^{-1}, 16 h photoperiod). All experiments were replicated two times. After 3 months the number of germinants from all thirty cell lines was counted.

Different stages of cell line 4A (of the elite genotype 4) on proliferation medium supplemented with three concentrations of BA were chosen for histocytological, morphological and qRT-PCR studies. S1 was the ETs proliferated

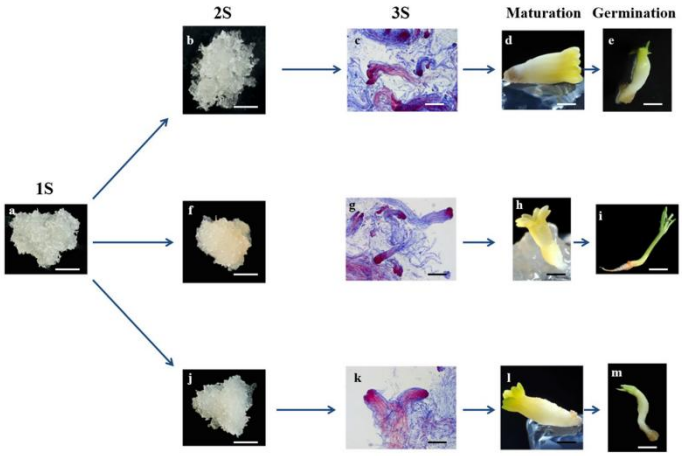


Fig. 1 Morphological and histocytological changes in response to BA treatment during ETs proliferation of *P. balfouriana*. **a** Cell line 4A, scale bars, 500 µm. **b, f, j** Proliferation of ETs on media containing different concentrations of BA. ETs proliferated on medium with 2.5 µM BA (**b**), 3.6 µM BA (**f**), and 5.0 µM BA (**j**). **c, g, k** Prematuration of ETs on media with different concentrations of BA. Somatic embryos stained red (acetocarmine) and suspensors stained blue (Evan's blue). ETs were put on 1/2 LM medium without PGRs after proliferation for 8 months on 2.5 µM BA (**c**), 3.6 µM BA (**g**), and 5.0 µM BA (**k**). Scale bars, 500 µm. **d, h, l** Somatic embryos from ETs proliferated with 2.5 µM BA (**d**), 3.6 µM BA (**h**), and 5.0 µM BA (**l**). Scale bars, 500 µm. **e, i, m** Germination of somatic embryos. **e** Somatic embryo germinated with the elongation of the primary needles and an enlarged hypocotyl and the radicle covered with a root cap. **i** Somatic embryo germinated with the elongation of the primary needles, hypocotyl and radicle. **m** Somatic embryo germinated with the elongation of the primary needles, hypocotyl and radicle covered with a root cap. Scale bars, 500 µm. S1 was collected when the ETs proliferated on initiation medium with 10 µM 2,4-D and 5 µM BA for 2 weeks; S2 was collected after the ETs proliferated on media containing different concentrations of BA for 8 months; S3 was collected when the ETs were cultured on 1/2 LM without PGRs (prematuration) for 1 week

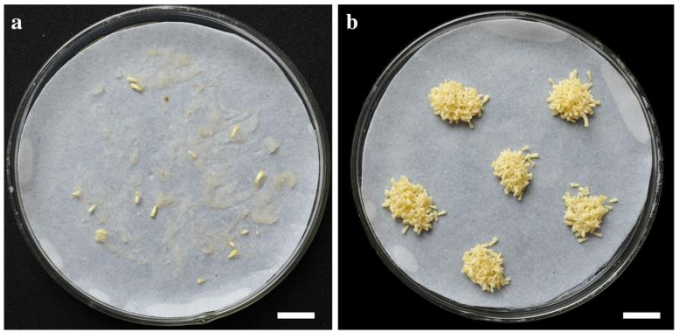


Fig. 2 Maturation of ETs in *P. balfouriana*, which had proliferated for 2 years on medium containing 3.6 µM BA (subcultured every 2 weeks). **a** Maturation of ETs, which were spread over filter paper on the maturation medium. **b** Maturation of ETs in the form of small lumps (0.5 cm in diameter). Scale bars, 500 µm

on initiation medium with 10 µM 2,4-D and 5 µM BA for 2 weeks; S2 was the ET proliferated on medium containing three different concentration of BA for 8 months; S3 was the ET cultured on ½ LM without PGRs (prematuration) for 1 week, which previously had proliferated for 8 months on medium containing either of the three concentrations of BA. ETs for hormone measurements were collected, immediately frozen in liquid nitrogen, and stored at − 80 °C.

Histocytology and morphology of somatic embryo development and germination

The progressive stages in the maturation and germination of somatic embryos were photographed under a stereomicroscope (Leica M205 FA). Three samples were double-stained with Evans blue and acetocarmine to distinguish early somatic embryos (Gupta and Holmstrom 2005). Stained cultures were then examined on glass slides with cover slips and photographed under a microscope (Leica DM6000 B). We counted the number of early somatic embryos in the field of vision under the microscope, ten fields of vision were selected for each treatment.

Quantification of hormones

ETs (500 mg FW) from three BA treatments (three replicates) were used for the determination of hormone levels, which were measured with UPLC/ESI-MS/MS using Waters ACQUITY UPLC system. The procedure for the quantification of multiple hormones were performed as described by Kong et al. (1997). This experiment was repeated two times.

Determination of the activity of superoxide dismutase (SOD) and peroxidase (POD)

ETs (500 mg FW) from three BA treatments (three replicates) were ground into a fine powder in liquid nitrogen and homogenized in 2 mL phosphate buffer (50 mM, pH 6.0) for extraction. The extract solutions were centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatants were used to determine the activity of SOD and POD according to Yang et al. (2008). This experiment was repeated three times.

Analysis of polyamines (PAs)

Putrescine (Put), spermidine (Spd), and spermine (Spm) were determined according to Santa-Catarina et al. (2006). Fresh samples of ETs (500 mg FW) from three BA concentrations and in triplicates were ground in 2 mL of 5% perchloric acid (Merck, Darmstadt, Germany). 1 h of incubation at 4 °C, they were centrifuged for 30 min at 12,000×*g* at 4 °C. The pellet was dissolved in the mobile phase used for HPLC analysis (Agilent, 150 × 3.9 mm, 4 µm), which was performed using a liquid chromatograph. The mobile phase was a mixture of acetonitrile and water (60:40, v/v) at a flow rate of 0.7 mL min^{-1}. The benzoyl PAs were detected at 230 nm. A standard mixture of Put, Spd, and Spm was used as the standard. All the analytical-grade reagents and solvents used for the determination of PAs were from Sigma-Aldrich or Merck and were used or prepared as recommended by the manufacturer. This experiment was repeated three times.

Quantitative real-time PCR analysis

Total RNA was isolated and purified from ETs (500 mg FW) treated by three concentrations BA using a Total RNA Purification kit (Norgen Biotek Corporation, Canada) according to the manufacturer's instructions and using the on-column DNA removal protocol. qRT-PCR was carried out for eighteen miRNAs (premiRNAs) and twenty target genes to estimate their expression relationship. Gene-specific primers were designed using Primer-Blast (http://

www.ncbi.nlm.nih.gov/tools/primer blast/) software and synthesized commercially (First BASE Laboratories, Seri Kembangan, Malaysia). The selected miRNAs and primer sequences are listed in Table S1. The target mRNAs and their primer sequences are listed in Table S2. First-strand cDNA was generated from 1-μg RNA samples using the iScript™ cDNA Synthesis Kit (Bio-Rad). Each qRT-PCR run was performed using 30 ng of first-strand cDNA and 300 nM of each primer in a 15-μL reaction using iTaq™ Universal SYBR® Green Supermix (Bio-Rad) in an iQ™5 real-time PCR detection system (Bio-Rad) with iQ™5 Optical system software (Bio-Rad). The relative quantification of the miRNA and target gene expression was calculated and normalized using csi-snoR14 and WS0109_C05 (peroxisomal targeting signal receptor), respectively, as references. PCR amplification was performed according to the following protocol: an initial denaturation step of 3 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, then 58 °C for 30 s and 72 °C for 30 s in 96-well optical reaction plates (Bio-Rad, USA). To assess the purity of the amplified products, a melting curve was generated for each sample. qRT-PCR analysis was performed in triplicate (three biological replicates and three technical replicates). Expression levels of the tested reference miRNAs and genes were determined by CT values and calculated by the delta–delta CT method.

Statistical analysis

All data (Variance analysis and Multiple Comparison) were analyzed and box plots designed by R-3.3.3-win with the agricolae package and boxplot package, respectively. The means were compared by the least significant difference test (95% confidence level), and standard deviations of the means were calculated.

Results

SE on media with three concentrations of BA

There were significant differences in the number of early somatic embryos and the mature somatic embryos in all thirty cell lines between the two lower concentrations and high concentration of BA (5.0 μM, Table 1). The germination rate after proliferation on 3.6 μM BA was significantly higher than with the other two treatments. There were differences in the morphology of somatic embryos produced by tissues proliferated on media with the three BA concentrations, some of which could not germinate normally. Most of the somatic embryos from cultures treated with 2.5 μM BA had inflated hypocotyls (Fig. 1e), while those treated with 5.0 μM BA did not form radicles (Fig. 1m). The majority of somatic embryos from cultures treated with 3.6 μM BA had a normal morphology (Fig. 1h). Furthermore, ETs that proliferated for 2 years on medium containing 3.6 μM BA formed only a few somatic embryos when they were spread over filter paper surface on the maturation medium (Fig. 2a). However, many somatic embryos formed after the ET was matured in the form of small lumps after 3 months (without subculture) (Fig. 2b).

Changes in the hormone levels after different durations of proliferation on media with three concentrations of BA

To characterize the physiological changes that occur after long-term proliferation of *P. balfouriana* with different BA concentrations, the levels of hormones were determined (Fig. S1). The results showed that the level of IAA in S3 tissue proliferated with 2.5 and 5.0 μM BA was significantly higher than that in S1, which did not differ from that in S2 (Fig. S1a). The gibberellic acid (GA_3) contents in 3.6 μM BA treatment in S2 and S3 were significantly lower than that in S1 (Fig. S1b). However, there were no statistically significant differences among the three stages when ETs were proliferated on 2.5 or 5.0 μM BA. The difference of ABA contents in S3 between the 2.5 and 5.0 μM BA treatments was significant (Fig. S1c). The ABA content in S2 at all three BA concentrations was significantly higher than that in S1. The ZR levels and profiles with BA treatment differed significantly among the three stages and BA concentrations. The ZR contents at 2.5 and 3.6 μM BA in S2 and S3 were significantly higher than that in S1, and there were no differences between them in S2 and S3 of these two treatments (Fig. S1d). However, there were significant differences among the three stages at 5.0 μM BA treatment.

Table 1 Maturation of somatic embryos of *P. balfouriana* after 8 month-proliferation on media with various BA concentrations

Treatments (μM)	Mean number of early somatic embryos	Mean number of mature somatic embryos per 100 mg FW	Germination per 50 somatic embryos (%)
2.5	7.80 ± 1.33a	39.8 ± 2.86b	34.2 ± 0.04b
3.6	12.0 ± 3.03a	123.4 ± 8.26a	67.4 ± 0.08a
5.0	1.0 ± 0.63b	4.8 ± 2.48c	16.4 ± 0.04b

The numbers are means of 30 lines ± standard deviations (SD); The different letters indicate there were significant difference among three treatments; ten fields of vision were used for early somatic embryo counts

To assess the hormonal balance in the ET of the three treatments, the interactions between hormones were expressed as their ratios (Fig. S2). The ABA/ZR and ABA/IAA ratio in S2 from each BA concentration were significantly higher than that in S1 (Fig. S2a, b). Moreover, the ABA/ZR ratio in S3 at 2.5 µM BA treatment was significantly higher than that in S3 at 5.0 µM BA (Fig. S2a). There was no significant difference in the IAA/ZR ratio of each stage among the three treatments or the IAA/ZR ratio of each treatment among the three stages (Fig. S2c).

Changes in the activity of antioxidant enzymes and polyamine contents in ETs before and after proliferation on media with three concentrations of BA

After long-term proliferation, SOD activity significantly differed among the three stages for all three BA treatments, with SOD activity highest in S2 at each BA concentration (Fig. S3a). POD activity at 2.5 µM BA was significantly higher in S2 than in S1 and S3, and the difference between the latter two stages was significant (Fig. S3b). In contrast, there were no significant differences between POD activities at 3.6 and 5.0 µM BA in S1 and S2, which were statistically significantly higher than those in S3. The PAs showed a similar response to the different BA concentrations, except that Spd content of S2 and S3 was significantly higher at 5.0 µM BA than that in S2 at 2.5 µM BA and S3 at 3.6 µM BA, respectively. Furthermore, the Spd levels increased after maintenance on proliferation medium and were significantly higher in S3 than those in S1 under each treatment, whereas the levels of Spm and Put did not show significant changes among the three stages (Fig. S4b, c). It was interesting to note that there were no differences in Spm and Put contents among the three proliferation times at 2.5 and 5.0 µM BA (Fig. S4a, c).

Analysis of miRNAs and their target genes

To investigate the roles of miRNAs during *P. balfouriana* SE, 18 miRNAs of known functions were selected for analysis by qRT-PCR. miRNAs had been found to be involved in the early SE of *P. balfouriana* in our transcriptome study (Li et al. 2017). The relative quantitative results demonstrated that the levels of all selected miRNAs and their target genes were different at the three concentrations of BA. Ten miRNAs and their target genes had the same expression patterns (Fig. 3). The other eight miRNAs showed the reverse expression compared with their target genes (Fig. 4). The expression levels of miRNA 5021 and 5758 and their target genes were higher at 2.5 µM than at 5.0 µM BA. miRNAs 1160 and 3633 were higher at 2.5 µM BA than at 3.6 µM BA, but their target genes showed the opposite pattern.

Discussion

Poor embryo quality often results in slow initial growth and low germination percentages, even abnormal germination. The age of embryogenic cultures maintained on a proliferation medium negatively influences their ability to generate mature somatic embryos in some conifer species (Klimaszewska et al. 2016). *P. balfouriana* ETs that have been maintained in vitro on proliferation medium for 2 years have provided an excellent opportunity for studying the effects of long-term culture on the potential to produce mature somatic embryos. In addition, we found that BA levels in the culture medium that were too low (2.5 µM) or too high (5.0 µM) not only inhibited the proliferation of ET but also disrupted maturation of early somatic embryos. There were significant differences in the yields of early somatic embryos and mature somatic embryos when ETs proliferated at 2.5 and 3.6 µM BA, but their corresponding germination rates were remarkably different. Specifically, regenerants from somatic embryos of ETs cultured on 2.5 and 5.0 µM BA often exhibited malformation of radicles and hypocotyls after several weeks. We suggest that these morphologies are linked to the age of the ET and the BA concentration in the proliferation medium. BA has often been reported to cause hyperhydricity and shoot-tip necrosis, and other effects in *Aloe polyphylla* (Bairu et al. 2007), or to adversely affect rooting and the subsequent acclimatization of micropropagated sea oats (Valero-Aracama et al. 2010).

The reduced potential to produce mature somatic embryos in long-term cultures may be partly due to the formation and accumulation of hormones, in agreement with Plačková et al. (2015). In this study, the level of endogenous ZR was highest in the S3 cultures under each BA treatment, which might lead to high levels of BA metabolites. Furthermore, ZR and ABA contents in the S2 and S3 cultures under the three BA treatments were all higher than those in the S1 culture. Changes in hormone levels are a normal phenomenon during SE, but the patterns of these changes are not entirely consistent, depending on the species and culture conditions (Grzyb et al. 2017). It is worth noting that a dramatic increase in IAA occurred after prolonged proliferation of *P. balfouriana*, but it was more stable after prolonged proliferation on medium with 3.6 µM BA, which might be important for the consistent formation ability of somatic embryos. Furthermore, lower IAA/ZR, ABA/CKs, and ABA/IAA ratios likely promote the maintenance of the potential to produce mature somatic embryos; this result agrees with the results obtained for *Cyathea delgadii* Sternb (Grzyb et al. 2017). Moreover, most hormone contents in the ETs (which proliferated for 8 months) under each BA treatment

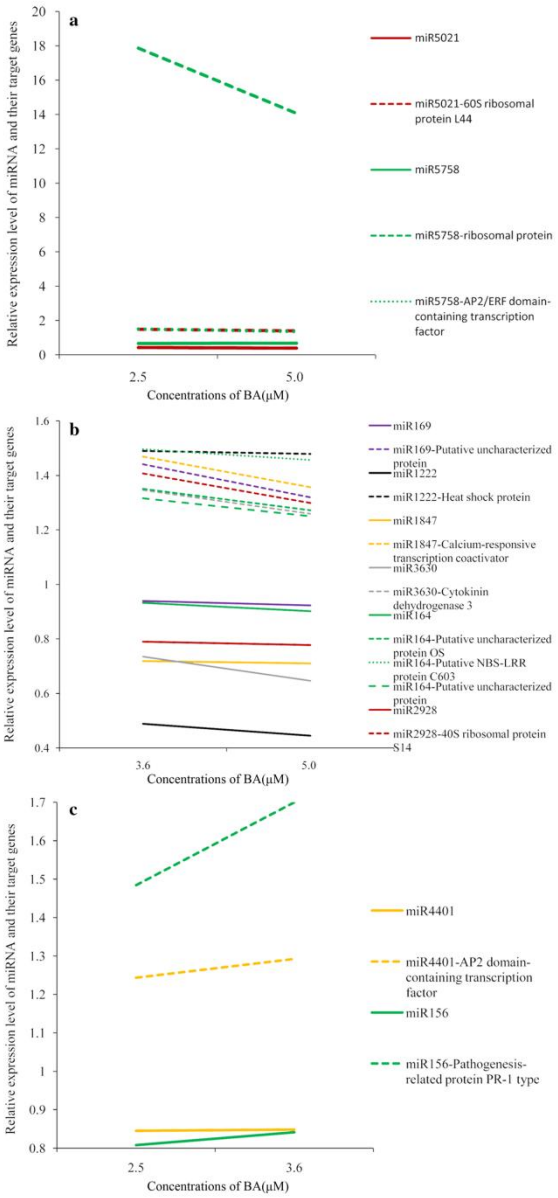


Fig. 3 MiRNAs and their target genes showed a uniform expression tendency at different BA concentrations. **a** Changes in the 5021 and 5738 miRs and their target genes. **b** Changes in the 169, 1222, 1847, 3630, 164, and 2928 miRs and their target genes. **c** Changes in the 4401 and 156 miRs and their target genes

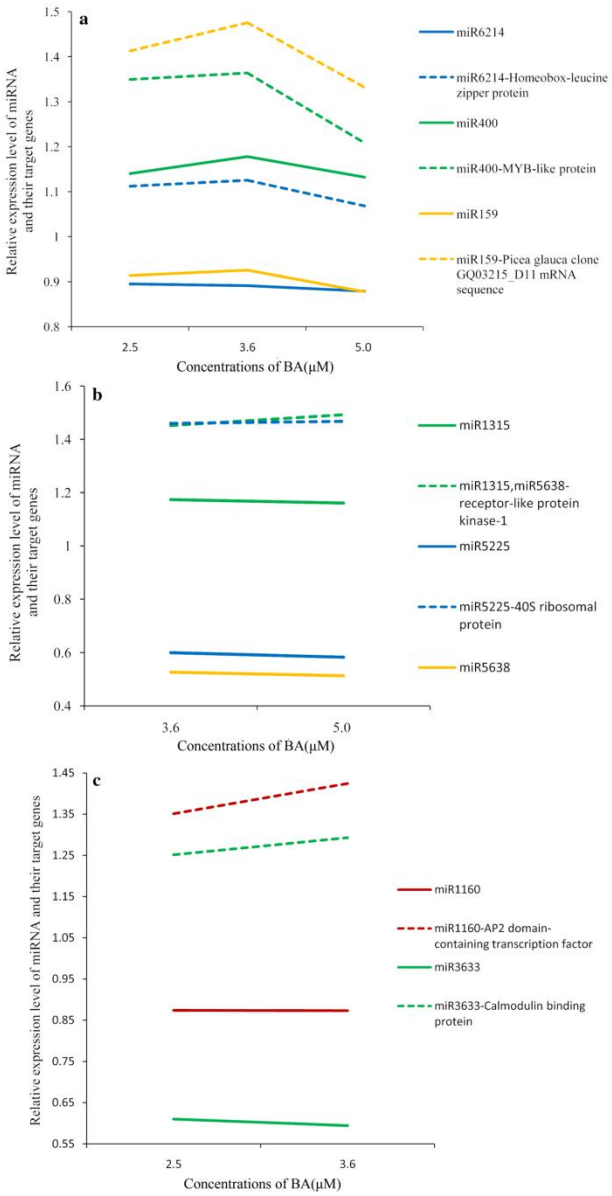


Fig. 4 MiRNAs and their target genes had opposite expression tendencies at different BA concentrations. **a** Changes in the 6214, 400, and 159 miRs and their target genes. **b** Changes in the 1315, 5225 and 5638 miRs and their target genes. **c** Changes in the 1160 and 3633 miRs and their target genes

were higher than those in the ETs of S1, which implicated endogenous hormones were accumulated when the proliferation stage was extended.

Our results confirm previous reports and clearly demonstrate that the influence of endogenous ZR level in the proliferation medium on the ability to produce mature somatic embryos of *P. balfouriana* is pronounced. Phytohormones sharing a similar chemical structure differentially affected the antioxidative system of plant tissue (Díaz-Vivancos et al. 2011), but the exact model of this action is not known and is not universal for all plant species (Tang and Newton 2005). For *P. balfouriana*, SOD and POD activities increased after 8 months of proliferation when compared with those in the ETs of S1. In this research, the activity of SOD in ETs under 5.0 μM BA treatment showed no significant differences from those in 2.5 and 3.6 μM BA. Additionally, the activities of POD in ETs at BA 2.5 and 3.6 μM showed no statistically significant differences compared with those at 5.0 μM BA. Furthermore, SOD activity was significantly higher in S2 tissues with a higher potential to produce mature somatic embryos than those in the S1 and S3 cultures (treated with 2.5 and 3.6 μM BA). Cytokinins have been reported to modulate the antioxidative system of Pssu-ipt transgenic tobacco plants with increased cytokinin contents (Synkova et al. 2006). Díaz-Vivancos et al. (2011) showed that cytokinins altered the levels of antioxidant enzymes in *Cucumis sativus* Linn explants. The role of PODs in auxin catabolism is thought to be an important factor in plant growth and development, which has been shown to be stimulated by all the assayed cytokinins (Díaz-Vivancos et al. 2011). These results suggest a possible use of antioxidant enzyme activity to evaluate the ability to produce somatic embryos, especially in long-term proliferated cultures. In addition, it is well known that POD is involved in the cross-linking of cell wall components. Sala et al. (2013) used microscopy and immunocytochemistry to show major differences in the cell wall structure between embryogenic and nonembryogenic cultures, suggesting that the loss of the potential to produce mature somatic embryos of *P. balfouriana* may be due to a change in POD. We found that the activities of SOD and POD suddenly decreased following the removal of PGRs from the medium, potentially because of the lack of PGR addition during the prematuration stage and the possible importance of low SOD and POD activities to initiate the development of somatic embryos from the ETs, which is consistent with the findings of Filipović et al. (2015) and Tian et al. (2003). Therefore, in *P. balfouriana*, maintenance of the potential to produce mature somatic embryos by their progressive seem to be related to discrete changes in some components of the antioxidant enzymatic system caused by BA.

The involvement of PAs in the control of cell division and differentiation indicates that they may play an important role in ET and early somatic embryo formation (Galston and Flores 1991; Niemi et al. 2002). Our data agree with previous findings for the proliferation of conifer ETs (Häggman et al. 2011; Jo et al. 2013; Salo et al. 2015), which showed that cell lines with blocked development of somatic embryos had higher levels of putrescine (Fig. S5). The concentration of Spd increased with a prolonged proliferation time until early somatic embryo formation. The Put:Spd ratio has been reported as a biomarker of embryogenic competence in many angiosperm species, which was lower in ETs that could form normal somatic embryos than in ETs with the blocked development of somatic embryos (Li and Burritt 2003; Santa-Catarina et al. 2006), which was in accordance with our findings. In addition, the content of Spd increased after prolonged proliferation. This phenomenon may be illustrated by the observation that Spd exerts a positive effect on antioxidant systems (Shi et al. 2010). Gemperlová et al. (2009) mentioned that several developmental processes controlled by cytokinins also appear to involve PAs, which indicates that PAs participate in the proliferation of ET in *P. balfouriana*.

Most of the miRNAs and target genes investigated herein were differentially expressed and might participate in the process of proliferation of ETs. We found that the expression patterns of 159, 1160, 3633, 1315, 5225 and 5638 miRNAs and their target genes were in accordance with our previous study (ETs proliferated for 3 months) (Li et al. 2017), indicating that there were few changes in miRNAs expression patterns when the proliferation time was prolonged. The 1160 and 5758 miRNAs may participate in the physiological variations after long-term proliferation. The AP2/ERF transcription factor (target gene of miRNA5758) and AP2 transcription factor (target gene of miRNA 1160) levels were higher in tissues that produced more somatic embryos, which was closely related to the induction and maturation of SE (Ouakfaoui et al. 2010; Wickramasuriya and Dunwell 2015). WIND1 (AP2/ERF—family transcription factor) acts via the ARR-dependent signaling pathway to promote cell dedifferentiation (Müller and Sheen 2008), which is an indispensable process for SE. Another transcription factor, MYB-like protein, was upregulated in 2.5 μM BA treatment compared with 5 μM BA treatment, which might be the target gene of miRNA 400 that was downregulated and closely related to maintenance of the embryogenic/nonembryogenic potential (Li et al. 2013). Moreover, miRNA 3633 may also be involved in the proliferation of SE through the regulation of its target gene, calmodulin binding protein, which may interconnect with auxin. Ca^{2+}-mediated signaling had been found to be involved in the induction/regulation of SE from proembryogenic cells of *Santalum album* (Anil and Rao 2000), which is a crucial second messenger for many rapid cellular processes during responses to hormones, such as auxin (Hepler 2005; Vanneste and

Friml 2013). It is interesting to note that ribosomal protein (target gene of miRNA 5021) exhibited higher levels under 3.6 μM BA treatment, which might be required for maintaining the ability to produce somatic embryos. This result is in accordance with the findings of Correia et al. (2012) and Li et al. (2015), who found that ribosomal protein genes are predominantly upregulated in ET compared within calli. Overall, these studies highlight the idea that epigenetic regulation plays an important role in maintenance of the potential to produce mature somatic embryos. As more precise and powerful tools for epigenetic analysis become available for application in a broader range of conifer species, analysis of the epigenetic landscape of plant cell cultures may be extended to more woody plants.

Conclusions

Few studies have focused on the proliferation mechanism of ET in conifers. Our findings showing the effects of BA on concentrations of hormones, PAs and antioxidant enzyme activities highlight the close relationship among the effects of hormones, polyamines and antioxidant enzymes in the maintenance of somatic embryo production capacity and suggest that the antioxidant enzymatic system and polyamine catabolism play important roles in somatic embryo formation and even germination. The integration of physiological data, together with expressed miRNAs and their target gene data, has the potential to provide the most comprehensive and informative insight into maintenance of the potential to produce mature somatic embryos in woody species. Future research in this field requires new and/or comprehensive technological methods, including more sensitive approaches for mRNA, protein, and miRNA detection and identification. These approaches will become more effective with the integration of new data from genome sequencing projects. Furthermore, functional validation of specific identified target genes/proteins is needed to use these targets as markers for the SE process. The coordination of all this knowledge will provide new insights for future studies addressing the optimization of SE protocols for large-scale propagation and conservation strategies for *P. balfouriana*.

Author contribution statement LQF and DC designed the experiments and wrote the manuscript. LQF, ZTQ, LJJ, ZHG, and KLS were responsible for carrying out the experiments, data collection, and statistical analysis. ZSG, CXY, WJH were consulted during the study and edited by them.

Acknowledgements The authors thank the Forestry Station of Xianggelila Country in Yunnan Province for cone sampling in the *P. balfouriana* seed orchard. This study was supported by grants from the General Financial Grant from China Postdoctoral Science Foundation (2017M622717) and the Fundamental Research Funds for the Central Nonprofit Research Institution of the Chinese Academy of Forestry (TGB2013011).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Abrahamsson M, Clapham D, von Arnold S (2018) Somatic embryogenesis in Scots pine (*Pinus sylvestris* L.). In: Jain S, Gupta P (eds) Step wise protocols for somatic embryogenesis of important woody plants, vol 84. Springer, Cham, pp 123–133 Forestry Sciences

Anil US, Rao KS (2000) Calcium-mediated signaling during sandalwood somatic embryogenesis. Role for exogenous calcium as second messenger. Plant Physiol 123(4):1301–1312

Bairu MW, Stirk WA, Dolezal K, Van Staden J (2007) Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can meta-topolin and its derivatives serve as replacement for benzyladenine and zeatin? Plant Cell Tissue Organ Cult 90:15–23

Breton D, Harvengt L, Trontin JF, Bouvet A, Favre JM (2006) Long-term subculture randomly affects morphology and subsequent maturation of early somatic embryos in maritime pine. Plant Cell Tissue Organ Cult 87:95–108

Centeno ML, Rodríguez R, Berros B, Rodríguez A (1997) Endogenous hormonal content and somatic embryogenic capacity of *Corylus avellana* L. cotyledons. Plant Cell Rep 17:139–144

Corredoira E, Ballester A, Ibarra M, Vieitez AM (2015) Induction of somatic embryogenesis in explants of shoot cultures established from adult *Eucalyptus globulus* and *E. saligna*×*E. maidenii* trees. Tree Physiol 35:678–690

Correia S, Vinhas R, Manadas B, Lourenço AS, Veríssimo P, Canhoto JM (2012) Comparative proteomic analysis of auxin-induced embryogenic and nonembryogenic tissues of the solanaceous tree *Cyphomandra betacea* (Tamarillo). J Proteome Res 11:1666–1675

Díaz-Vivancos P, Majourhat K, Fernández JA, Hernández JA, Piqueras A (2011) Study of the antioxidant enzymatic system during shoot development from cultured intercalar meristems of saffron. Plant Growth Regul 65:119–126

Filipović BK, Simonović AD, Trifunovic MM, Dmitrović SS, Savić JM, Jevremović SB, Subotić AR (2015) Plant regeneration in leaf culture of *Centaurium erythraea* Rafn. Part 1: the role of antioxidant enzymes. Plant Cell Tissue Organ Cult 121:703–719

Galston AW, Flores HE (1991) Polyamines and plant morphogenesis. In: Slocum R, Flores HE (eds) The biochemistry and physiology of polyamines in plants. CRC Press, Boca Raton, pp 175–186

Gemperlová L, Fischerová L, Cvikrová M, Malá J, Vondráková Z, Martincová O, Vágner M (2009) Polyamine profiles and biosynthesis in somatic embryo development and comparison of germinating somatic and zygotic embryos of Norway spruce. Tree Physiol 29:1287–1298

Grieb B, Schafer F, Imani J, Mashayekhi KN, Arnholdt-Schmitt B, Neumann KH (1997) Changes in soluble proteins and phytohormone concentrations of cultured carrot petiole explants during induction of somatic embryogenesis (*Daucus carota* L.). J Appl Bot Food Qual 71:94–103

Grzyb M, Kalandyk A, Waligórski P, Mikuła A (2017) The content of endogenous hormones and sugars in the process of early

somatic embryogenesis in the tree fern *Cyathea delgadii* Sternb. Plant Cell Tissue Organ Cult 129:387–397
Gupta PK, Holmstrom D (2005) Double staining technology for distinguishing embryogenic cultures. In: Jain SM, Gupta PK (eds) Protocol for somatic embryogenesis in woody plants. Springer, The Netherlands, pp 573–575
Häggman H, Vuosku J, Kestilä J, Suorsa M, Ruottinen M, Neubauer P, Läärä E, Sarjala T (2011) The role of spermidine and spermine in cell function and pattern formation in Scots pine embryogenesis. In: Park Y-S, Bonga JM, Park S-Y, Moon H-K (eds) Advances in somatic embryogenesis of trees and its application for the future forests and plantations. IUFRO working party 2.09.02: Somatic embryogenesis and other clonal propagation methods of forest trees, August 19–21 (Suwon, South Korea) pp 100–102
Hepler PK (2005) Calcium: a central regulator of plant growth and development. Plant Cell 17:2142–2155
Jo L, Santos AL, Bueno CA, Barbosa HR, Floh EI (2013) Proteomic analysis and polyamines, ethylene and reactive oxygen species levels of *Araucaria angustifolia* (Brazilian pine) embryogenic cultures with different embryogenic potential. Tree Physiol 34:94–104
Klimaszewska K, Noceda C, Pelletier G, Label P, Rodriguez R, Lelu-Walter MA (2009) Biological characterization of young and aged embryogenic cultures of *Pinus pinaster* (Ait.). Vitr Cell Dev Biol Plant 45:20–33
Klimaszewska K, Hargreaves C, Lelu-Walter MA, Trontin JF (2016) Chap. 7 Advances in conifer somatic embryogenesis since year 2000. In: Germanà MA, Lambardi M (eds) In Vitro embryogenesis in higher plants. Methods in molecular biology, Springer, New York, pp 131–162
Kong LS, Attree SM, Fowke LC (1997) Changes of endogenous hormone levels in developing seeds, zygotic embryos and megagametophytes in *Picea glauca*. Physiol Plant 101(1):23–30
Kvaalen H, Daehlen OG, Rognstad AT, Grønstad B, Egertsdotter U (2005) Somatic embryogenesis for plant production of *Abies lasiocarpa*. Can J For Res 35:1053–1060
Lelu-Walter MA, Pâques LE (2009) Simplified and improved somatic embryogenesis of hybrid larches (*Larix*×*eurolepis* and *Larix*×*marschlinsii*): perspectives for breeding. Ann For Sci 66:1–104
Li ZL, Burritt DJ (2003) Changes in endogenous polyamines during the formation of somatic embryos from isogenic lines of *Dactylis glomerata* L. with different regenerative capacities. Plant Growth Regul 40:65–74
Li WF, Zhang SG, Han SY, Wu T, Zhang JH, Qi LW (2013) Regulation of LaMYB33 by miR159 during maintenance of embryogenic potential and somatic embryo maturation in *Larix kaempferi* (Lamb.) Carr. Plant Cell Tissue Organ Cult 113:131–136
Li QF, Zhang SG, Wang JH (2014a) Transcriptome analysis of callus from *P. balfouriana*. BMC Genom 15:533
Li QF, Zhang SG, Wang JH (2014b) Transcriptomic and proteomic analyses of embryogenic tissues in *P. balfouriana* treated by 6-benzylaminopurine. Physiol Plant 154:95–113
Li QF, Wang JH, Pulkkinen P, Kong LS (2015) Changes in the metabolome of *P. balfouriana* ETs that were linked to different levels of 6-BAP by gas chromatography-mass spectrometry approach. PloS One 10(10):e0141841
Li QF, Deng C, Xia Y, Kong LS, Zhang HG, Zhang SG, Wang JH (2017) Identification of novel miRNAs and miRNA expression profiling in embryogenic tissues of *Picea balfouriana* treated by 6-benzylaminopurine. PloS One 12(5):e0176112
Litvay JD, Verma DC, Johnson MA (1985) Influence of loblolly pine (*Pinus taeda* L.) culture medium and its components on growth and somatic embryogenesis of the wild carrot (*Daucus carota* L.). Plant Cell Rep 4:325–328
Maruyama E, Tanaka T, Hosoi Y, Ishii K, Morohoshi N (2000) Embryogenic cell culture, protoplast regeneration, cryopreservation, biolistic gene transfer and plant regeneration in Japanese cedar (*Cryptomeria japonica* D. Don). Plant Biotechnol 17:281–296
Montalbán IA, Novák O, Rolčik J, Strnad M, Moncaleán P (2013) Endogenous cytokinin and auxin profiles during in vitro organogenesis from vegetative buds of *Pinus radiata* adult trees. Physiol Plant 148:214–231
Müller B, Sheen J (2008) Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. Nature 453:1094–1097
Niemi K, Haggman H, Sarjala T (2002) Effects of exogenous diamines on the interaction between ectomycorrhizal fungi and adventitious root formation in Scots pine in vitro. Tree Physiol 22:373–381
Ouakfaoui SE, Schnell J, Abdeen A, Colville A, Labbé H, Han SY, Baum B, Laberge S, Miki B (2010) Control of somatic embryogenesis and embryo development by AP2 transcription factors. Plant Mol Biol 74:313–326
Plačková L, Hrdlička J, Smýkalová I, Cvečková M, Novák O, Griga M, Doležal K (2015) Cytokinin profiling of long-term in vitro pea (*Pisum sativum* L.) shoot cultures. Plant Growth Regul 77:125–132
Sala K, Potocka I, Kurczynska E (2013) Spatio-temporal distribution and methyl-esterification of pectic epitopes provide evidence of developmental regulation of pectins during somatic embryogenesis in *Arabidopsis thaliana*. Biol Plant 57:410–416
Salo HM, Sarjala T, Jokela A, Häggman H, Vuosku J (2015) Moderate stress responses and specific changes in polyamine metabolism characterize Scots pine somatic embryogenesis. Tree Physiol 36:392–402
Santa-Catarina C, Silveira V, Balbuena TS, Viana AM, Estelita MEM, Handro W, Floh EI (2006) IAA, ABA, polyamines and free amino acids associated with zygotic embryo development of *Ocotea catharinensis*. Plant Growth Regul 49:237–247
Shi J, Fu XZ, Peng T, Huang XS, Fan QJ, Liu JH (2010) Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidative capacity and stomatal response. Tree Physiol 30:914–922
Stirk WA, Van SJ (2014) Plant growth regulators in seaweeds: occurrence, regulation and functions. In: Nathalie B (ed) Advances in botanical research, vol 71. Academic Press, Amsterdam, pp 125–159
Synkova H, Semoradova S, Schnablova R, Witters E, Husak M, Valcke R (2006) Cytokinin-induced activity of antioxidant enzymes in transgenic Pssu-ipt tobacco during plant ontogeny. Biol Plant 50:31–41
Tang W, Newton RJ (2005) Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus* L.) zygotic embryos. Plant Physiol Biochem 43:760–769
Tian M, Gu Q, Zhu M (2003) The involvement of hydrogen peroxide and antioxidant enzymes in the process of shoot organogenesis of strawberry callus. Plant Sci 165:701–707
Trontin J-F, Reymond I, Quoniou S, Canlet F, Debille S, Bruneau G, Harvengt L, Le Metté C, Vallance M, Teyssier C, Lable P, Lelu-Walter MA (2011) An overview of current achievements and shortcomings in developing Maritime pine somatic embryogenesis and enabling technologies in France. In: Park Y-S, Bonga JM, Park S-Y, Moon H-K (eds) Advances in somatic embryogenesis of trees and its application for the future forests and plantations. IUFRO working party 2.09.02: Somatic embryogenesis and other clonal propagation methods of forest trees, August 19–21 2010 (Suwon, South Korea) pp 132
Valero-Aracama C, Kane M, Wilson S, Philman N (2010) Substitution of benzyladenine with meta-topolin during shoot multiplication increases acclimatization of difficult- and easy-to acclimatize

sea oats (*Uniola paniculata* L.) genotypes. Plant Growth Regul 60:43–49

Vanneste S, Friml J (2013) Calcium: the missing link in auxin action. Plants 2(4):650–675

Wickramasuriya AW, Dunwell JM (2015) Global scale transcriptome analysis of Arabidopsis embryogenesis in vitro. BMC Genom 16:301

Yang Y, Han C, Liu Q, Lin B, Wang JW (2008) Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedlings. Acta Physiol Plant 30(4):433–440

Yang X, Zhang X, Yuan D, Jin F, Zhang Y, Xu J (2012) Transcript profiling reveals complex auxin signalling pathway and transcription regulation involved in dedifferentiation and redifferentiation during somatic embryogenesis in cotton. BMC Plant Biol 12:110

Zhang ZF, Finer JJ (2016) Use of cytokinin pulse treatments and micrografting to improve sunflower (*Helianthus annuus* L.) plant recovery from cotyledonary tissues of mature seeds. In Vitr Cell Dev Biol Plant 52:391–399

Żur I, Dubas E, Krzewska M, Waligórski P, Dziurka M, Janowiak F (2015) Hormonal requirements for effective induction of microspore embryogenesis in triticale (× *Triticosecale* Wittm.) anther cultures. Plant Cell Rep 34:47–62

2.2 Identification of novel miRNAs and miRNA expression profiling in embryogenic tissues of *Picea balfouriana* treated by 6-benzylaminopurine

PLOS ONE

RESEARCH ARTICLE

Identification of novel miRNAs and miRNA expression profiling in embryogenic tissues of *Picea balfouriana* treated by 6-benzylaminopurine

Qingfen Li[1,2☯], Cheng Deng[1☯], Yan Xia[2], Lisheng Kong[3], Hanguo Zhang[4], Shougong Zhang[2], Junhui Wang[2]*

1 Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China, **2** State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Tree Breeding and Cultivation of State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China, **3** Department of Biology, Centre for Forest Biology, University of Victoria, Victoria, British Columbia, Canada, **4** State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin, China

☯ These authors contributed equally to this work.
* wangjh@caf.ac.cn

Citation: Li Q, Deng C, Xia Y, Kong L, Zhang H, Zhang S, et al. (2017) Identification of novel miRNAs and miRNA expression profiling in embryogenic tissues of *Picea balfouriana* treated by 6-benzylaminopurine. PLoS ONE 12(5): e0176112. https://doi.org/10.1371/journal.pone.0176112

Editor: Yun Zheng, Kunming University of Science and Technology, CHINA

Received: October 10, 2016

Accepted: April 5, 2017

Published: May 9, 2017

Data Availability Statement: Data are available via Sequence Read Archive (SRA) with identifier PRJNA248161, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA248161.

Funding: Financial support for this study was provided by the Fundamental Research Funds for the Central Non-profit Research Institution of CAF, China (TGB2013011).

Competing interests: The authors have declared that no competing interests exist.

Abstract

Here, we compared miRNA expression profiles in embryonic cell cultures of the conifer *Picea balfouriana* following application of the synthetic cytokinin 6-benzylaminopurine (6-BAP). We used next-generation sequencing to analyze three libraries of small RNAs from the treated embryogenic cell cultures and generated 24,000,000 raw reads from each of the libraries. Over 70 differentially regulated micro RNA (miRNA) families (≥2 fold change in expression) were identified between pairs of treatments. A quantitative analysis showed that miR3633 and miR1026 were upregulated in tissues with the highest embryogenic ability. These two miRNAs were predicted to target genes encoding receptor-like protein kinase and GAMYB transcription factors, respectively. In one library, miR1160, miR5638, miR1315, and miR5225 were downregulated. These four miRNAs were predicted to target genes encoding APETALA2, calmodulin-binding protein, and calcium-dependent protein kinase transcription factors. The expression patterns of the miRNAs and their targets were negatively correlated. Approximately 181 potentially novel *P. balfouriana* miRNAs were predicted from the three libraries, and seven were validated during the quantitative analysis. This study is the first report of differential miRNA regulation in tissues treated with 6-BAP during somatic embryogenesis. The differentially expressed miRNAs will be of value for investigating the mechanisms of embryogenic processes that are responsive to 6-BAP in *P. balfouriana*.

Introduction

Somatic embryogenesis (SE) is an important method in spruce breeding programs and enables the regeneration and cloning of trees with desirable genotypes. However, the callus tissue formed from some genotypes can gradually lose embryogenic capacity. The mechanism of this effect is unclear, as the early stages of SE are insufficiently known [1–3]. This mechanism has been investigated by identifying differentially expressed genes between embryogenic and nonembryogenic tissues in order to elucidate the molecular regulatory networks that operate during SE in plant species [4], including *Picea abies* (L.) H. Karst. [5], *Picea glauca* (Moench) Voss [6], *Glycine max* (L.) Merr. [7], *Gossypium hirsutum* L. [8], *Zea mays* L. [9], *Solanum tuberosum* L. [10], *Triticum aestivum* L. [11], and *Citrus* × *sinensis* (L.) Osbeck [12]. However, the genetic regulation of these differentially expressed genes and their specific functions remain largely unknown.

Microribonucleic acids (miRNAs) are a group of endogenous small RNA (sRNA) molecules, generally 20–25 nucleotides (nt) in length, that play essential roles in most eukaryotes by regulating the expression of their target genes [13]. MiRNAs extensively affect biological processes in plants, primarily development and stress responses [14]. Notably, SE is also regulated by miRNAs and their roles have been studied [15–22] including in gymnosperms [23–24]. Wu et al. [19] found that the target genes of the miRNAs miR164, miR166, and miR397 were associated with the formation of nonembryogenic callus. Moreover, the effects of miR166a overexpression on the development of SEs in *Larix leptolepis* has been investigated [25]. Su et al. [26] reported that overexpression of miR167 inhibited SE formation, showing that miR167 negatively regulates SE induction. Zhang et al. [27] reported that miR165 was differentially expressed between embryogenic and nonembryogenic callus. Wu et al. [28] found that the conserved miRNAs csi-miR156a/b, miR164b, and 171c directly suppressed expression of a specific transcription factor, and were suggested to inactivate postembryonic growth to maintain normal SE. Zhang et al. [29] showed that miR171a/b may influence the function of proembryogenic masses (PEMs), while miR171c may have a role in SE induction in larch; miR397 and miR398 were found to be primarily involved in modulation of PEM propagation and transition to single embryos. The transcription factor gene *SQUAMOSA Promoter-Binding Protein-Like* is regulated by miR156; this transcription factor acts as a pleiotropic regulator of plant development and can promote vegetative phase transition by activating miR172 [30]. Li et al. [31] concluded that in *Larix kaempferi* (Lamb.) Carrière, the post-transcriptional regulation of *MYB33* by miR159 was associated with the maintenance of embryogenic or nonembryogenic potential and somatic embryo maturation.

Picea balfouriana Rehder & E.H. Wilson grows in many parts of the world and is prevalent in southwestern and eastern regions of China. It is a species of spruce that has ecological and economic importance, and produces high-quality wood. To date, transcriptomic and proteomic approaches have been applied to unravel the molecular mechanisms of SE in *P. balfouriana* [1, 2]. We showed that 6-BAP, a synthetic cytokinin, had a significant influence on embryogenic competence during the proliferation stage [2]. Our earlier analysis indicated that 6-BAP has a significant effect on proteins and mRNAs. However, the mRNAs of 15% of proteins have not been identified in *P. balfouriana* mRNA libraries. In the present study we sought to obtain a better understanding of the effect of 6-BAP on the molecular regulation of SE as this could be of value for spruce seedling production. To this end, we compared miRNA expression patterns among embryogenic cultures that had received different 6-BAP treatments.

Materials and methods

Plant materials

A *P. balfouriana* embryogenic cell line was initiated in 2011 using seeds from elite genotype 4 at the Research Institute of Forest, Chinese Academy of Forestry (Beijing, China). Cells from this line were placed in half-strength LM medium as an induction medium [32], which was supplemented with 10 μM 2,4-D-ichlorophenoxyacetic acid and 5.0 μM 6-BAP [33], 500 $mg{\cdot}L^{-1}$ glutamine, $1g{\cdot}L^{-1}$ casein hydrolysate, 1% sucrose, and 8% agar, at 24 ± 1˚C in the dark. The proliferation medium was half-strength LM medium with three concentrations of 6-BAP (2.5 μM, 3.6 μM, and 5.0 μM); the other supplements and culture conditions were the same as for the induction medium. After three months, embryogenic tissues with different embryogenic capabilities were produced. The embryogenic cultures were subcultured at two-week intervals. The SE culture experiment was performed twice. The embryo differentiation method has been described previously [3].

Samples of the embryogenic cultures were collected after being subcultured for 7 d. For each treatment, three and six biological replicates for physiological and sRNA profiling, respectively, were collected. All samples were transferred to cryotubes, flash frozen in liquid nitrogen (N_2), and stored at -80˚C until further processing for metabolite extraction.

Plant hormone determination

The extraction, purification, and determination of endogenous levels of indole-3-acetic acid (IAA), zeatin riboside (ZR), gibberellic acid (GA_3), and ABA were performed by an indirect enzyme-linked immunosorbent assay (ELISA) technique as described previously [34]. Briefly, a 0.5 g sample of each treatment was homogenized in liquid N_2 and extracted in cold 80% (v/v) methanol with butylated hydroxytoluene ($1\ mmol{\cdot}L^{-1}$) overnight at 4˚C. The supernatant was collected after centrifugation at 1,500 × *g* (4˚C) for 8 min, passed through a C_{18}Sep-Pak cartridge (Waters, Milford, MA), and dried under N_2. The residue was dissolved in phosphate buffered saline ($0.01\ mol{\cdot}L^{-1}$, pH 7.4) and the levels of IAA, ZR, GA_3, and ABA were determined. Microtitration plates (Nunc, Denmark) were coated with synthetic IAA, ZR, GA_3, or ABA ovalbumin conjugates in $NaHCO_3$ buffer ($50\ mmol{\cdot}L^{-1}$, pH 9.6) and left overnight at 37˚C. Then, ovalbumin solution ($10\ mg{\cdot}mL^{-1}$) was added to each well to block nonspecific binding. After incubation for 30 min at 37˚C, standard IAA, GA_3, ABA, and ZR samples and antibodies were added and incubated for a further 30 min at 37˚C. The antibodies against IAA, ZR, GA_3, and ABA were obtained as described by Weiler et al. [35]. In addition, horseradish peroxidase-labelled goat anti-rabbit immunoglobulin was added to each well and incubated for 1 h at 37˚C. Finally, the buffered enzyme substrate (ortho-phenylenediamine) was added, and the enzyme reaction was carried out in the dark at 37˚C for 15 min, then terminated using $3\ mol{\cdot}L^{-1}$ H_2SO_4. Absorbance was recorded at 490 nm. Analyses of the enzyme-immunoassay data followed the procedures described in Weiler et al. [35].

RNA isolation and purification

Total RNA was isolated and purified from tissues at the proliferation stage of SE using a Total RNA Purification kit (Norgen Biotek Corporation, Canada) according to the manufacturer's instructions, following the on-column DNA removal protocol. All RNA samples from tissues treated with three concentrations of 6-BAP were stored at -80˚C until sRNA sequencing was performed and were mixed in equal ratios to form a single RNA pool.

Sequencing and data analysis

Three sRNA libraries were generated from the different 6-BAP-treated tissues and were sequenced on a HiSeq 2000 Sequencing System (Illumina, San Diego, CA, USA) to identify conserved and novel miRNAs. First, sRNA fragments (16–30 nt) were isolated from a 15% polyacrylamide electrophoresis gel and purified. Then the sRNAs were sequentially ligated to a 5′RNA adapter (5′-GUUCAGAGUUC UACAGUCCGACGAUC-3′) and a 3′RNA adapter (5′-pUCGUAUGCCGUCUUCUGCUUGidT-3′: p, phosphate; idT, inverted deoxythymidine) using T4 RNA ligase. The resulting adaptor-ligated sRNAs were reverse transcribed to cDNA with a reverse transcription primer (5′-CAAGCAGAAGAC GGCATACGA-3′) using Superscript II reverse transcriptase (Invitrogen) and amplified by polymerase chain reaction (PCR). The cDNA was sequenced on an Illumina/Solexa sequencing platform by the Beijing Genomics Institute (Shenzhen, China).

Bioinformatics tools were used to analyze the sequencing data. The 35-nt sequence tags were first trimmed of adaptors, regions of low complexity, and low-quality sequences; then, the length distribution of clean tags was summarized. The remaining sRNA sequences (clean reads) were mapped to the transcriptomes of *P. balfouriana* embryogenic cultures (PRJNA211928 and PRJNA248161). The unique sRNA sequences were searched against known miRNA sequences in miRBase (Release 21, http://www.mirbase.org/) to identify conserved miRNAs in *P. balfouriana*. Mireap software was used to predict novel miRNAs among the sRNAs that did not match any of the sequences in miRBase, and the secondary structures of the putative novel miRNAs were predicted using Mfold 3.1 [22]. At the same time, because there was bias (5′ U) in the first-position base of miRNA, we estimated prediction accuracy by calculating this first position base bias among sRNA candidates 18–24 nt in length. Additionally, the target genes of potential novel and conserved miRNAs were predicted as described previously [36–38] and their potential roles in early SE were investigated by functional annotation using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway databases.

Selection of candidate reference miRNAs

Five miRNA genes, U6 snRNA, dlo-miR24, dlo-miR168a*, Csi-snoR14, and 5.8S ribosomal RNA (rRNA), were selected because they have been reported to be the most stable reference RNA genes for miRNA quantitative reverse-transcription (qRT)-PCR studies [20,24]. Three different gene normalization applets, geNorm [39], BestKeeper [40], and NormFinder [41], were used to analyze the expression stability of the candidate reference RNA genes. The geNorm software calculates a gene stability measure (M) for each gene in a given set of samples via stepwise exclusion of the least stable genes. The preliminary analysis of BestKeeper, based on the inspection of raw threshold cycle values, estimates the variation of all the reference genes using correlation analyses to develop a weighted index, and gives standard deviation values < 1. NormFinder is based on an analysis of variance mathematical model to estimate intra- and intergroup variation that calculates reference gene stability values. Based on the results from the three applets, we selected the most stable gene as the reference gene in this study.

Real-time quantitative PCR of miRNAs and their targets

The expression levels estimated from the high-throughput sequencing data of *P. balfouriana* miRNAs were validated by qRT-PCR. RNA samples from the 6-BAP-treated embryogenic cultures were reverse transcribed using an NCode VILO miRNA cDNA Synthesis kit (Invitrogen, USA). A total of 20 miRNAs (including conserved and novel) were examined using an NCode

Express SYBR Green ER miRNA qPCR kit (Invitrogen). All the reactions were performed in triplicate in a STEPONE PLUS Real Time PCR System (Applied Biosystems, USA), with a dissociation curve used to control for primer dimers in the reactions. Mature miRNA abundance was calculated relative to the expression of the selected reference gene. The selected miRNAs and primer sequences are listed in S1 Table.

Total RNA was extracted as described above, and qRT-PCR was carried out to assess the abundance of 30 of the miRNA target mRNAs. The mRNA qRT-PCR was performed using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Thermo Fisher, USA) and a SYBR Premix EX Taq Kit (TaKaRa Biotechnology, Japan), following the manufacturers' instructions. All expression levels were normalized to the expression of the reference gene WS0109_C05 (peroxisomal targeting signal receptor). The 30 target mRNAs and their primer sequences are listed in S2 Table. Reactions were performed on an ABI Step-One Plus Real Time PCR System (Applied Biosystems) with three replicates for each sample. After PCR, dissociation curves and amplification curves were analyzed to verify the specificity of the amplification. The data were analyzed with Microsoft Excel2007 using Student's t-test, and P-values of less than 0.05 were considered significant.

Results

Development of *P. balfouriana* somatic embryos

During the maturation stage, embryogenic tissue from the *P. balfouriana* cell line exhibited different embryogenic abilities after treatment with 2.5 μM, 3.6 μM, or 5.0 μM 6-BAP (Table 1). The highest rates of embryogenic tissue proliferation occurred in medium containing the intermediate concentration (3.6 μM) of 6-BAP, which yielded the most fully mature embryos with a normal set of cotyledons (113 ± 6 per 100 mg tissue). This culture also had the highest germination rate (48.47% ± 0.03) of the three treatments. Tissue in medium containing 5.0 μM 6-BAP produced the lowest number of somatic embryos (23 ± 2 per 100 mg tissue) and had the lowest germination rate (9.42% ± 0.02). Tissues treated with 2.5 μM or 3.6 μM 6-BAP had higher embryogenic competence than those treated with 5.0 μM 6-BAP.

In the proliferation stage, superoxide dismutase (SOD) activity in the 2.5 μM and 3.6 μM treatments was significantly higher than in the 5.0 μM treatment, whereas in the maturation stage, SOD activity was significantly lower with 2.5 μM and 3.6 μM than with 5.0 μM 6-BAP (Fig 1a). Peroxidase (POD) activity was contrary to that of SOD (Fig 1b). Interestingly, there were significant differences in SOD and POD activities between embryogenic tissues treated with different concentrations of 6-BAP in the proliferation and maturation stages.

In the proliferation stage, IAA and ZR levels in tissues with the highest embryogenic ability (3.6 μM 6-BAP) were significantly higher and lower, respectively, than in the other two treatments (Fig 2). There were no significant differences in the contents of GA_3 and ABA among the three treatments in the proliferation stage; however, there were significant differences in

Table 1. Number of mature cotyledonary embryos generated from each 6-BAP treatment and their germination rates.

6-BAP concentrations	Mature embryos per 100 mg of embryogenic tissue	Germination rate
2.5 μM	89 ± 3^{b}	$31.78\% \pm 0.02^{b}$
3.6 μM	113 ± 6^{a}	$48.47\% \pm 0.03^{a}$
5.0 μM	23 ± 2^{c}	$9.42\% \pm 0.02^{c}$

Superscript letters (a, b, and c) indicate significant differences ($p \leq 0.05$).

https://doi.org/10.1371/journal.pone.0176112.t001

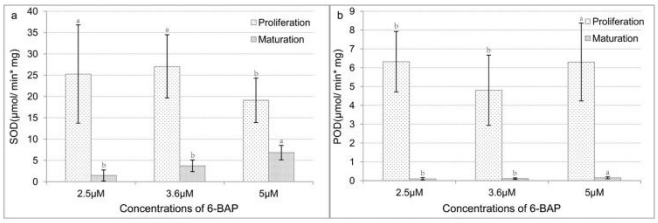


Fig 1. Activities of antioxidant enzymes in 6-BAP-treated tissues in proliferation and maturation stages. (a) Activity of superoxide dismutase (SOD). **(b)** Activity of peroxidase (POD). Tissues in proliferation and maturation stages were collected after being transferred to new media for 7 d. Lowercase letters (a, b, c) indicate significant differences ($p \leq 0.05$).

https://doi.org/10.1371/journal.pone.0176112.g001

the maturation stage. The contents of GA_3 and ABA in the 3.6 μM and 2.5 μM treatments were significantly higher than in the 5.0 μM treatment.

High-throughput sequencing of sRNAs

The Illumina/Solexa sequencing generated 25,518,179 reads from the 2.5 μM library, 25,588,136 reads from the 3.6 μM library, and 24,417,174 reads from the 5.0 μM library after removing the empty adapters and low-quality sequences (data are available via Sequence Read Archive [SRA] with identifier PRJNA248161, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA248161). Only 3.18%, 21.18%, and 4.23% of the unique sRNA sequences from the 2.5 μM, 3.6 μM, and 5.0 μM libraries, respectively, mapped to the *P. balfouriana*

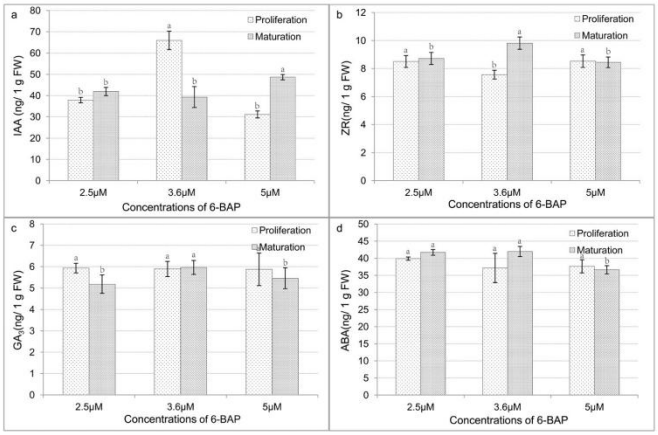


Fig 2. Levels of plant hormones in 6-BAP-treated tissues in proliferation and maturation stages. (a) Levels of 3-acetic acid (IAA). **(b)** Levels of zeatin-riboside (ZR). **(c)** Levels of gibberellic acid (GA_3). **(d)** Levels of abscisic acid (ABA). Tissues in proliferation and maturation stages were collected after being transferred to new media for 7 d. Lowercase letters (a, b, c) indicate significant differences ($p \leq 0.05$).

https://doi.org/10.1371/journal.pone.0176112.g002

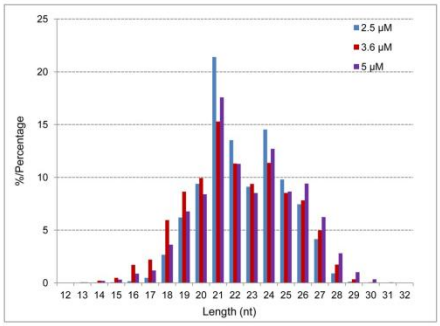


Fig 3. Size distribution analysis of the sRNA sequences in three *Picea balfouriana* libraries.

https://doi.org/10.1371/journal.pone.0176112.g003

transcriptomes (Fig 3). The unique sRNAs were then compared against all plant precursor and mature miRNAs listed in miRBase and 27,362 (0.85%), 30,165 (1.05%), and 27,479 (1.02%) of the unique sequences from the 2.5 μM, 3.6 μM, and 5.0 μM libraries, respectively, were found to be similar to known miRNAs. A BLASTN search against the Rfam database identified rRNAs, small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and tRNAs among the unique sRNA sequences in our datasets. However, most unique sRNA sequences (96.82%, 78.82%, and 95.77% in the 2.5 μM, 3.6 μM, and 5.0 μM libraries, respectively) could not be annotated; this difficulty is consistent with results obtained in similar studies in other plants [24, 42–45]. The primary reason for the high percentage of unannotated sequences may be the limited number of species-level genomes and expressed sequence tags in these databases.

The length distributions of the sRNAs in the three libraries were similar (Fig 3). The unique sRNAs were 12–32 nt long with 21 nt predominating (21.4%, 15.28%, and 17.57%), followed by 24 nt (14.63%, 11.37%, and 12.70%), and 22 nt (13.52%, 11.31%, and 11.28%). This pattern of length distribution is consistent with those reported for tomato [46], wheat [47], *Populus* [48], and most other angiosperms [43,45,49]. The ratio of 21 to 24 nt lengths is highly variable among plants, indicating that significant differences exist in sRNA biogenesis pathways among different species [50].

Conserved miRNAs in *P. balfouriana* callus

By comparing the sRNA sequences in the three libraries to known mature plant miRNAs in miRBase, we obtained 6,050 miRNAs (250 families), 4,984 miRNAs (214 families), and 5,381 miRNAs (229 families) in the 2.5 μM, 3.6 μM, and 5.0 μM libraries, respectively. Notably, more than 2,000 of the miRNAs matched in miRBase had no annotation in each library. Approximately 90% of the identified miRNAs were shared in the three libraries, and the same nine miRNA families (miR156, 166, 396, 951, 950, 946, 3712, 1312, and 1314) were shared among the first 10 most abundant miRNA families in each library.

Prediction and classification of targets of differently regulated miRNAs in *P. balfouriana*

More than 4% of the miRNAs appeared in only one of the libraries and more than 70 differentially expressed miRNA families ($\geq$ 2 fold change) were identified between any two of the

Table 2. Numbers of differentially expressed miRNAs and their targets among the three 6-BAP-treated libraries.

	2.5 μM vs 5.0 μM	3.6 μM vs 2.5 μM	3.6 μM vs 5.0 μM
miRNA	79	91	87
Target gene	689	462	547

https://doi.org/10.1371/journal.pone.0176112.t002

libraries. The numbers of differentially expressed miRNAs among the three libraries and the numbers of their predicted targets are shown in Table 2. The GO analysis and KEGG pathway enrichment of most of the target genes of the conserved miRNAs are listed in Table 3. Intriguingly, miR400 was predicted to target genes that encode two MYB-like proteins that might be involved in the early developmental stage of *P. balfouriana* tissues. Several miRNAs (miR156, 166, and 172) were more abundant in the 3.6 μM library, which had a higher maturation rate, than in the other two libraries. Both miR1315 and miR5638, which targeted a gene encoding a receptor-like protein kinase, were upregulated in the 2.5 μM and 5.0 μM treatments compared to the 3.6 μM treatment. MiR164, which was predicted to target a gene encoding the AP2 domain-containing transcription factor, was downregulated in the 3.6 μM library.

Prediction of potentially novel *P. balfouriana* miRNAs

In addition to the conserved miRNAs, 70, 54, and 57 potentially novel *P. balfouriana* miRNAs were predicted from the remaining unique unannotated sRNA sequences in the 2.5 μM, 3.6 μM, and 5.0 μM libraries, respectively (Table 4). The lengths of the putative novel miRNAs varied from 19 nt to 25 nt, of which 51.61% were 21 nt long. Based on the secondary structures predicted using Mfold, the precursor sequences had negative folding free energies ranging from -18.00 kcal mol^{-1} to -160.16 kcal mol^{-1} with an average free energy of -44.22 kcal mol^{-1}, which is lower than the folding free energies reported for Arabidopsis (-59.5 kcal mol^{-1}) and rice (-71.0 kcal mol^{-1}) precursor miRNAs [47], but higher than for *Dimocarpus longan* Lour.

Table 3. GO analysis and KEGG pathway enrichment of target genes of known miRNAs.

	First[a]	Second[b]	Third[c]
Cellular component[d]	cell	intracellular	organelle
Molecular function[d]	binding	catalytic activity	hydrolase activity
Biological process[d]	cellular process	metabolic process	cellular metabolic process
Pathway enrichment[e]	metabolic pathways	spliceosome	RNA transport

[a, b, c]First, second, and third most abundant terms.
[d]GO categories.
[e]Kegg pathways.

https://doi.org/10.1371/journal.pone.0176112.t003

Table 4. Predicted novel miRNAs of *Picea balfouriana*.

Treatment	Types of predicted miRNAs	Number of miRNAs	Predicted miRNAs	Target sites	Length (nt)	miRNA sequences matched to miRbase[a]
2.5 μM	94	86,595	70	180	21	18 (6, 33.3%)
3.6 μM	78	38,408	54	144	21	15 (8, 53.3%)
5.0 μM	77	50,498	57	152	21	13 (7, 53.8%)

[a]. The superscript letter a indicates that the novel miRNA sequence had orthologs in other species

https://doi.org/10.1371/journal.pone.0176112.t004

Table 5. Orthologs of putative novel miRNAs conserved in other species.

miRNA	Location[a]	Sequence (5′–3′)	Length (nt)	Count	Homolog	MFE[b] (kcal mol^{-1})	5′/3′
pba-miR1	**Spruce91_Unigene_BMK.6580:145:356:+**	CAGCCCTTCTGCTATCCACAAC	22	605	pta-miR946a	−76.7	3
pba-miR2	**Spruce93_Unigene_BMK.18933:170:269:-**	TGCCTGGCTCCCTGTATGCCA	21	8	ath-miR160a	−47.4	5
pba-miR3	**Spruce93_Unigene_BMK.27721:104:213:+**	TGAAGCTGCCAGCATGATCTGG	22	39	ath-miR167d	−56.2	5
pba-miR4	**Spruce93_Unigene_BMK.27721:101:215:+**	AGATCATGCGGCAGTTTCACC	21	44	ptc-miR167f	−59.6	3
pba-miR5	**Spruce93_Unigene_BMK.25825:32:161:-**	GGCAAGTTGTCTTTAGCTACA	21	6	zma-miR169r	−53.2	3
pba-miR6	**Unigene12051_C1907:73:212:+**	CAGCCAAGGATGACTTGCCGG	21	11	ath-miR169b	−51.5	5
pba-miR7	**Unigene29362_C1907:111:204:+**	CGCTATCCATCCTGGGCTTCA	21	22	aly-miR390a	−50.1	3
pba-miR8	**Unigene1519_C1907:71:158:+**	TCGCAGGATAGATGGCGCCGGCC	23	129	mdm-miR391	−46.1	5
pba-miR9	**Unigene42283_C1907:36:134:+**	TATGGGAGGAATGGGCAAAGCT	22	18	gma-miR482b	−41.1	3

[a]. The superscript letter a indicates the location of the miRNA on their precursors.

[b]. The superscript letter b means minimum free energy.

https://doi.org/10.1371/journal.pone.0176112.t005

(44.01 kcal mol^{-1}) precursor miRNAs [24]. The numbers of reads obtained for those candidate novel miRNAs of *P. balfouriana* varied from 5 to 13,021. For example, ptc-miR167f (44 reads), mdm-miR391 (101 reads), and pta-miR946a (605 reads) were highly expressed, while zma-miR169r (6 reads), ath-miR160a (8 reads), and ath-miR169b (11 reads) were expressed at low levels. When nucleotide bias was analyzed, the nucleotide U (53.08%) was most frequent, followed by G (27.53%), A (16.01%), and C (3.38%).

To determine whether the novel miRNAs were conserved in other plants, we compared their sequences with the miRNA sequences of other organisms present in miRBase 21. The nine miRNAs listed in Table 5 had orthologs in other species.

Putative functions of predicted miRNA targets in *P. balfouriana*

GO terms for biological process, molecular function, and cellular component categories were assigned as targets of the novel miRNAs by BLAST searches against the *P. balfouriana* transcriptome databases. Under biological process, the predominant terms were primary metabolic process, metabolic process, cellular process, and other developmental processes. Under molecular function, the predominant terms were transferase activity, nucleic acid binding, binding, and other functions. Under cellular component, the predominant terms included cell, organelle, intracellular, and other components. Most of the predicted novel miRNA target genes were annotated as function unknown. The KEGG pathway enrichment showed that these target genes were involved in ribosome, metabolic pathways, glutathione metabolism, calcium signaling pathway, and other pathways.

Validation of suitable reference genes for studying miRNA expression

geNorm. For each tissue, the gene-stability value (M) was calculated by geNorm for each candidate gene based on non-normalized expression levels (Q). The candidate genes were ranked according to the M value. An M value of 1.5 was used as a cutoff to assess gene stability [51–53]. For the 2.5 μM, 3.6 μM, and 5.0 μM sample groups, all the candidates had an M value lower than 0.8. The candidate reference genes csi-snoR14 and 5.8S rRNA were the least stable across the three samples (Table 6).

BestKeeper. The main parameters used to evaluate a potential reference gene in BestKeeper are "std dev [± CP]" (recommend < 1) or "set dev [± x-fold]" (recommend < 2). These

Table 6. Expression stability and ranking of reference genes as calculated by geNorm.

Samples	U6 snRNA	dlo-miR24	dlo-miR168a*	csi-snoR14	5.8S rRNA
2.5 μM	0.0079	0.0232	0.0001	0.0016	1.0000
3.6 μM	0.0041	0.0283	0.0001	0.0018	0.7013
5.0 μM	0.0056	0.0306	0.0000	0.0014	0.9188
M < 1.5	0.5900	0.5370	0.7350	0.4480	0.4750

https://doi.org/10.1371/journal.pone.0176112.t006

two parameters were the smallest for csi-snoR14 and dlo-miR24, indicating that they were the two most stable reference genes for the 2.5 μM, 3.6 μM, and 5.0 μM samples (Table 7).

NormFinder. Expression stability of the candidate reference genes was reanalyzed with NormFinder. Expression variation of the candidate genes among the 2.5 μM, 3.6 μM, and 5.0 μM samples was estimated using a model-based approach. Intragroup variation was calculated and converted into a stability value for each candidate, and the candidates were ranked accordingly (Table 8). Among the five candidate reference genes, csi-snoR14 was the most stable with a value of 0.1167, and dlo-miR168a* was the least stable (0.4729).

Validation of miRNAs and their potential targets

The analyses of the five candidate reference genes identified csi-snoR14 as the optimal reference miRNA, and therefore all the miRNAs were normalized to csi-snoR14. The validation results showed that some miRNAs were upregulated in the 3.6 μM sample, which had the highest embryogenic competence, while others were downregulated. Eleven mRNAs were predicted as potential targets for eight miRNAs; both miR5225 and miR1160 had two potential targets. A gene encoding a receptor-like protein kinase was the predicted target of miR5638 and miR1315. Our results showed that five targets (receptor-like protein kinase,

Table 7. Stability assessment of the candidate reference genes by BestKeeper.

	U6 snRNA	dlo-miR24	dlo-miR168a*	csi-snoR14	5.8Sr RNA
n	9	9	9	9	9
geo Mean [CP]	30.46	28.19	36.76	32.29	23.20
ar Mean [CP]	30.46	28.19	36.77	32.29	23.20
min [CP]	29.84	27.80	35.29	31.89	22.86
max [CP]	31.88	28.69	37.81	32.66	24.10
std dev [± CP]	0.46	0.28	0.58	0.19	0.30
CV [% CP]	1.50	0.98	1.58	0.59	1.31
min [x-fold]	-1.54	-1.31	-2.78	-1.32	-1.27
max [x-fold]	2.69	1.41	2.06	1.29	1.87
std dev [± x-fold]	1.37	1.21	1.49	1.14	1.23

https://doi.org/10.1371/journal.pone.0176112.t007

Table 8. Stability assessment of the candidate reference genes by NormFinder.

Gene name	Stability value
U6 snRNA	0.3409
dlo-miR24	0.2486
dlo-miR168a*	0.4729
csi-snoR14	0.1167
5.8S rRNA	0.1812

https://doi.org/10.1371/journal.pone.0176112.t008

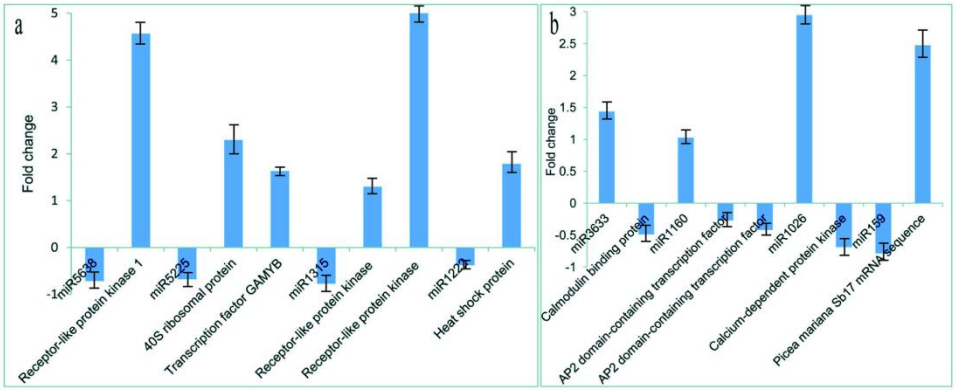


Fig 4. Validation of differentially regulated miRNAs and their targets by qRT-PCR. (a)The x-axis shows the miRNAs validated in this study. The y-axis shows the $\log_2$ ratio of their expression in the 3.6 μM 6-BAP versus the 5.0 μM 6-BAP libraries. Three biologically independent replicates were analyzed for each qRT-PCR; **(b)**The x-axis shows the miRNAs validated in this study. The y-axis shows the $\log_2$ ratio of their expression in the 3.6 μM 6-BAP versus the 2.5 μM 6-BAP libraries. Three biologically independent replicates were analyzed for each qRT-PCR.

https://doi.org/10.1371/journal.pone.0176112.g004

40S ribosomal protein, transcription factor GAMYB, and heat shock protein) were clearly less abundant in the tissue with the lowest embryogenic ability (5.0 μM 6-BAP) compared with the tissue with the highest embryogenic ability (3.6 μM 6-BAP) (Fig 4a), while the abundances of the corresponding miRNAs (miR5638,-5225, -159, -1315, and -1222) were the opposite. Furthermore, miR3633, -1160, and -1026 were downregulated in tissues treated with 3.6 μM 6-BAP compared with those treated with 2.5 μM 6-BAP (Fig 4b), while the expression of their targets, AP2, calmodulin-binding protein, and calcium-dependent protein kinase, respectively, showed the opposite pattern. Finally, of seven potential novel miRNAs that were present in at least two of the 6-BAP treatments, six had homologous sequences in other species; the exception was spruce91-m0017, for which no homologous sequence was found, although a homologous sequence (lysine-rich arabinogalactan protein) was found for its putative target (Unigene58367).

Discussion

Our results indicated that 6-BAP affected the production of somatic embryos and their germination rates. Moreover, 6-BAP influenced plant hormone levels and antioxidant enzyme activities in both the proliferation and maturation stages, showing that early embryo differentiation without plant growth regulators did not eliminate the influence of 6-BAP on the plant hormone levels and antioxidant enzyme activities in callus tissue.

Three receptor-like protein kinases were upregulated in the 3.6 μM 6-BAP treatment. Kinases such as SOMATIC EMBRYO RECEPTOR KINASE (SERK) have been identified previously in SE [54]. These kinases constitute a special subgroup of receptor protein kinases that are associated with SE. SERK1 is highly expressed during embryogenic cell formation in culture and during early embryogenesis in Arabidopsis. Hecht et al. [55] showed that overexpression of SERK1 not only did not result in any obvious plant phenotypes, it also gave a 3- to 4-fold increase in embryogenic competence, indicating that SERK1 enhanced embryogenic

competence and promoted the transition of somatic cells to an embryogenic state. Thus, higher embryogenic ability may result from increased expression of receptor-like protein kinases by 6-BAP through miR5638 and miR1315. Previous studies have reported the effect of 6-BAP on *SERK* genes. For example, 6-BAP alone induced SE in *Medicago truncatula* Gaertn. and promoted *MtSERK*1 expression [56]. Nolan et al. [56] reported that auxin and naphthalene-1-acetic acid application together with 6-BAP could significantly stimulate embryogenic cell formation and proliferation, which was accompanied by increased *MtSERK1* expression. However, our results differ from those obtained in *M*. *truncatula*, but are consistent with those of Zhang et al. [57], who demonstrated that 6-BAP inhibited SE and reduced ZmSERK1 and ZmSERK2 expression in a maize culture.

Notably, a gene targeted by miR5225 that encodes the transcription factor GAMYB was upregulated in the 3.6 μM 6-BAP treatment. GAMYB is involved in programmed cell death in both aleurone and tapetal tissues, and in both tissues this process is mediated by a gibberellin (GA_3) [58]. Conversely, members of the miR159 family repressed conserved GAMYB-like genes [59–61]. Noma et al. [62] found lower GA (probably GA_1) levels in embryogenic lines of carrot and anise, but Jiménez and Bangerth [63] found higher GA (GA_1, GA_3, GA_{20}) levels in embryogenic maize lines. Furthermore, in carrot [64], wheat [65], and grapevine [66], no differences in GA levels were found among cultures showing different embryogenic characteristics. A few researchers have studied the relationships among GAs and cytokinins. We found that the GA_3 content in the tissues with highest embryogenic ability (treated with 3.6 μM 6-BAP) was higher than in those treated with 5.0 μM 6-BAP. On the other hand, GAMYB has been identified as an activator of GA-regulated genes [59]. Together, these findings indicate that miR159 may repress GAMYB expression in the 5.0 μM treatment, leading to reduced GA_3.

Some members of the AP2 domain-containing transcription factor family, which contains 173 members [67], were reported to play a major role in embryogenesis and organ development [68,69]; for example, BABY BOOM (BBM) was shown to be involved in cell proliferation and morphogenesis [70,71]. The embryogenic tissue of Arabidopsis, which can induce SE, had elevated BBM expression [72]. Piyatrakul et al. [73] identified 11 AP2/ERF genes as very early markers that could predict the regeneration potential of proliferating callus lines. However, how these genes regulated early SE was unclear. Furthermore, 12 miRNAs (miR156, -159, -172, -393, -395, -396, -408, -894, -1511, -n11, -n12, and -n14) were predicted to inhibit the transcripts of 29 *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. HbAP2/ERF genes [67]. In our study, we identified two AP2 domain-containing transcription factors that may be regulated by miR1160, which were downregulated in the tissues with higher embryogenic ability, indicating that the overexpression of AP2 triggered by miR1160 may lead to a decrease or even a loss of embryogenic competence.

Calmodulin-binding protein and calcium-dependent protein kinase (CDPK) were downregulated in the 3.6 μM treatment compared to the 2.5 μM treatment. In carrot, the role of calcium in SE is essential for morphogenesis of undifferentiated cells into somatic embryos at a threshold of 200 mM [74]. Higher concentrations of calcium have no effect on either the viability or embryogenic potential of the culture. At lower concentrations, or after chelation of residual calcium with ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, SE is inhibited and the calcium channel blockers, verapamil and nifedipine, exert an inhibitory influence on embryogenic capacity [75]. Two CDPKs of 55 and 60 kDa have been identified in soluble protein extracts of sandalwood embryogenic cultures; these have Ca^{2+}-dependent and calmodulin-independent protein kinase activity and a developmentally regulated, tissue-specific soluble CDPK (swCDPK) accumulates in all stages of embryo development [76,77]. This

indicates that swCDPK is a Ca^{2+} modulator that can act alone or in conjunction with calmodulin during sandalwood SE.

Conclusions

In the present study, sRNA libraries were constructed by high-throughput sequencing of *P. balfouriana* callus cultures treated with three 6-BAP concentrations. After processing the sequencing data, we identified more than 4,000 conserved and 50 novel miRNAs in each library. The expression levels of more than 70 miRNAs showed significantly different regulation between pairs of treatments. The expression patterns for eight selected miRNAs and their targets were examined in detail, and there was a negative correlation between the expression patterns of the miRNAs and their targets. Notably, these targets have been reported to be involved in SE, suggesting that the associated miRNAs might act as regulators of embryogenic ability. The characterization and expression profile comparisons of the *P. balfouriana* miRNA libraries provide a good foundation for elucidating the complex miRNA-mediated regulatory network of SE in callus tissue treated with 6-BAP.

Supporting information

S1 Table. Selected miRNAs and primer sequences.
(DOC)

S2 Table. The 30 target mRNAs and their primer sequences.
(DOCX)

Acknowledgments

Financial support for this study was provided by the Fundamental Research Funds for the Central Nonprofit Research Institution of CAF, China (TGB2013011).

Author Contributions

Conceptualization: HGZ JHW.

Data curation: QFL CD.

Formal analysis: QFL CD.

Funding acquisition: SGZ JHW.

Investigation: HGZ LSK.

Methodology: QFL YX.

Project administration: SGZ JHW.

Resources: QFL CD.

Software: CD.

Supervision: LSK.

Validation: QFL YX.

Visualization: QFL.

Writing – original draft: QFL.

Writing – review & editing: QFL CD.

References

1. Li QF, Zhang SG, Wang JH. Transcriptomic and proteomic analyses of embryogenic tissues in *Picea balfouriana* treated by 6-Benzylaminopurine. Physiol Plantarum. 2015; 154: 95–113.
2. Li QF, Zhang SG, Wang JH. Transcriptome analysis of callus from *Picea balfouriana*. BMC Genomics. 2014; 15: 533.
3. Li QF, Wang JH, Pulkkinen P, Kong LS. Changes in the metabolome of *Picea balfouriana* embryogenic tissues that were linked to different levels of 6-BAP by gas chromatography-mass spectrometry approach. Plos One. 2015; 10: e0141841. https://doi.org/10.1371/journal.pone.0141841 PMID: 26517840
4. Ledwoń A, Gaj MD. *LEAFY COTYLEDON2* gene expression and auxin treatment in relation to embryogenic capacity of *Arabidopsis* somatic cells. Plant Cell Rep. 2009; 28: 1677–1688. https://doi.org/10.1007/s00299-009-0767-2 PMID: 19763577
5. van Zyl L, Bozhkov PV, Clapham DH, Sederoff RR, von Arnold S. Up, down and up again is a signature global gene expression pattern at the beginning of gymnosperm embryogenesis. Gene Expr Patterns. 2003; 3: 83–91. PMID: 12609608
6. Stasolla C, van Zyl L, Egertsdotter U, Craig D, Liu W, Sederoff RR. The effects of polyethylene glycol on gene expression of developing white spruce somatic embryos. Plant Physiol. 2003; 131: 49–60. https://doi.org/10.1104/pp.015214 PMID: 12529514
7. Nissen FT, Shealy RT, Khanna A, Vodkin LO. Clustering of microarray data reveals transcript patterns associated with somatic embryogenesis in soybean. Plant Physiol. 2003; 132: 118–136. https://doi.org/10.1104/pp.103.019968 PMID: 12746518
8. Zeng F, Zhang X, Zhu L, Tu L, Guo X, Nie Y. Isolation and characterization of genes associated to cotton somatic embryogenesis by suppression subtractive hybridization and macroarray. Plant Mol Biol. 2006; 60: 167–183. https://doi.org/10.1007/s11103-005-3381-x PMID: 16429258
9. Che P, Love TM, Frame BR, Wang K, Carriquiry AL, Howell SH. Gene expression pattern during somatic embryo development and germination in maize Hi II callus cultures. Plant Mol Biol. 2006; 62: 1–14. https://doi.org/10.1007/s11103-006-9013-2 PMID: 16845483
10. Sharma SK, Millam S, Hedley PE, McNicol J, Bryan GJ. Molecular regulation of somatic embryogenesis in potato: an auxin led perspective. Plant Mol Biol. 2008; 68: 185–201. https://doi.org/10.1007/s11103-008-9360-2 PMID: 18553172
11. Singla B, Tyagi AK, Khurana JP, Khurana P. Analysis of expression profile of selected genes expressed during auxin induced somatic embryogenesis in leaf base system of wheat (*Triticum aestivum*) and their possible interactions. Plant Mol Biol. 2007; 65: 677–692. https://doi.org/10.1007/s11103-007-9234-z PMID: 17849219
12. Ge XX, Chai LJ, Liu Z, Wu XM, Deng XX, Guo WW. Transcriptional profiling of genes involved in embryogenic, non- embryogenic calluses and somatic embryogenesis of Valencia sweet orange by SSH-based microarray. Planta. 2012; 236: 1107–1124. https://doi.org/10.1007/s00425-012-1661-7 PMID: 22622359
13. Ghildiyal M, Zamore PD. Small silencing RNAs: an expanding universe. Nat Rev Genet. 2009; 10: 94–108. https://doi.org/10.1038/nrg2504 PMID: 19148191
14. Mallory AC, Vaucheret H. Functions of microRNAs and related small RNAs in plants. Nat Genet. 2006; 38: S31–S36. https://doi.org/10.1038/ng1791 PMID: 16736022
15. Vernoux T, Benfey PN. Signals that regulate stem cell activity during plant development. Curr Opin Genet Dev. 2005; 15: 388–394. https://doi.org/10.1016/j.gde.2005.06.008 PMID: 15967658
16. Williams L, Grigg SP, Xie M, Christensen S, Fletcher JC. Regulation of Arabidopsis shoot apical meristem and lateral organ formation by microRNA miR166g and its AtHD-ZIP target genes. Development. 2005; 132: 3657–3668. https://doi.org/10.1242/dev.01942 PMID: 16033795
17. Luo YC, Zhou H, Li Y, Chen JY, Yang JH, Chen YQ, et al. Rice embryogenic calli express a unique set of microRNAs, suggesting regulatory roles of microRNAs in plant post-embryogenic development. FEBB Lett. 2006; 58: 5111–5116.
18. Szittya G, Moxon S, Santos DM, Jing RC, Fevereiro MPS, Moulton V, et al. High-throughput sequencing of *Medicago truncatula* short RNAs identifies eight new miRNA families. BMC Genomics. 2008; 9: 593–601. https://doi.org/10.1186/1471-2164-9-593 PMID: 19068109
19. Wu XM, Liu MY, Ge XX, Xu Q, Guo WW. Stage and tissue-specific modulation of ten conserved miRNAs and their targets during somatic embryogenesis of Valencia sweet orange. Planta. 2011; 233: 495–505. https://doi.org/10.1007/s00425-010-1312-9 PMID: 21103993

20. Kou SJ, Wu XM, Liu Z, Liu YL, Xu Q, Guo WW. Selection and validation of suitable reference genes for miRNA expression normalization by quantitative RT-PCR in citrus somatic embryogenic and adult tissues. Plant Cell Rep. 2012; 31: 2151–2163. https://doi.org/10.1007/s00299-012-1325-x PMID: 22865195

21. Li TT, Chen JH, Qiu S, Zhang YJ, Wang PK, Yang LW, et al. Deep sequencing and microarray hybridization identify conserved and species-specific microRNAs during somatic embryogenesis in hybrid yellow poplar. Plos One. 2012; 7: e43451. https://doi.org/10.1371/journal.pone.0043451 PMID: 22952685

22. Yang XY, Wang LC, Yuan DJ, Lindsey K, Zhang XL. Small RNA and degradome sequencing reveal complex miRNA regulation during cotton somatic embryogenesis. J Exp Bot. 2013; 64: 1521–1536. https://doi.org/10.1093/jxb/ert013 PMID: 23382553

23. Oh TJ, Wartell RM, Cairney J, Pullman GS. Evidence for stage-specific modulation of specific microRNAs (miRNAs) and miRNA processing components in zygotic embryo and female gametophyte of loblolly pine (*Pinus taeda*). New Phytol. 2008; 179: 67–80. https://doi.org/10.1111/j.1469-8137.2008.02448.x PMID: 18433430

24. Lin YL, Lai ZX. Comparative analysis reveals dynamic changes in miRNAs and their targets and expression during somatic embryogenesis in longan (*Dimocarpus longan* Lour.). Plos One. 2013; 8: 1–11.

25. Li ZX, Li SG, Zhang LF, Han SY, Li WF, Xu HY, et al. Over-expression of miR166a inhibits cotyledon formation in somatic embryos and promotes lateral root development in seedlings of Larix leptolepis. Plant Cell Tiss Org Cult. 2016; 1–13.

26. Su YH, Liu YB, Zhou C, Li XM, Zhang XS. The microRNA167 controls somatic embryogenesis in Arabidopsis through regulating its target genes ARF6 and ARF8. Plant Cell Tiss Org Cult. 2016; 124(2):1–13.

27. Zhang SG, Zhou J, Han SY, Yang WH, Li WF, Wei HL, et al. Four abiotic stress-induced miRNA families differentially regulated in the embryogenic and non-embryogenic callus tissues of *Larix leptolepis*. Biochem Biophys Res Commun. 2010; 398: 355–360. https://doi.org/10.1016/j.bbrc.2010.06.056 PMID: 20599742

28. Wu XM, Kou SJ, Liu YL, Fang YN, Xu Q, Guo WW. Genomewide analysis of small RNAs in nonembryogenic and embryogenic tissues of citrus: microRNA- and siRNA-mediated transcript cleavage involved in somatic embryogenesis. Plant Biotechnology Journal. 2015; 13(3):383–394. https://doi.org/10.1111/pbi.12317 PMID: 25615015

29. Zhang JH, Zhang SG, Han SY, Wu T, Li XM, Li WF, et al. Genome-wide identification of microRNAs in larch and stage-specific modulation of 11 conserved microRNAs and their targets during somatic embryogenesis. Planta. 2012; 236: 647–657. https://doi.org/10.1007/s00425-012-1643-9 PMID: 22526500

30. Yang X, Wang L, Yuan D, Lindsey K, Zhang X. Small RNA and degradome sequencing reveal complex miRNA regulation during cotton somatic embryogenesis. Journal of Experimental Botany. 2013: 64 (6):1521–1536. https://doi.org/10.1093/jxb/ert013 PMID: 23382553

31. Li WF, Zhang SG, Han SY, Wu T, Zhang JH, et al. Regulation of LaMYB33 by miR159 during maintenance of embryogenic potential and somatic embryo maturation in Larix kaempferi (Lamb.) Carr. Plant Cell Tiss Org Cult. 2013; 113: 131–136.

32. Litvay JD, Johnson MA, Verma DC, Einspahr D, Weyrauch K. Conifer suspension culture medium development using analytical data from developing seeds. IPC Tech Pap. 1981; 115: 1–17.

33. Wang JH, Li QF, Zhang SG. Somatic embryogenesis and plantlet regeneration of Picea balfouriana. 2012. pp. 1–10. ZL 201210274334.9.

34. He Z. A laboratory guide to chemical control technology on field crop. Beijing: Beijing Agricultural University Press; 1993.

35. Weiler EW, Jourdan PS, Conrad W. Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by a specific and highly sensitive solid-phase enzyme immunoassay. Planta. 1981; 153: 561–571. https://doi.org/10.1007/BF00385542 PMID: 24275876

36. Prabu GR, Mandal AK. Computational identification of miRNAs and their target genes from expressed sequence tags of tea (*Camellia sinensis*). Genomics Proteomics Bioinform. 2010; 8: 113–121.

37. Dai X, Zhuang Z, Zhao PX. Computational analysis of miRNA targets in plants: current status and challenges. Brief Bioinform. 2011; 12: 115–121. https://doi.org/10.1093/bib/bbq065 PMID: 20858738

38. Jeyaraj A, Chandran V, Gajjeraman P. Differential expression of microRNAs in dormant bud of tea [*Camellia sinensis* (L.) O. Kuntze]. Plant Cell Rep. 2014; 33: 1053–1069. https://doi.org/10.1007/s00299-014-1589-4 PMID: 24658841

39. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002; 3: 0034.1–0034.11.

40. Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper-Excel-based tool using pair-wise correlations. Biotechnol Lett. 2004; 26: 509–515. PMID: 15127793

41. Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription- PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004; 64: 5245–5250. https://doi.org/10.1158/0008-5472.CAN-04-0496 PMID: 15289330

42. Liang CW, Zhang XW, Zou J, Xu D, Su F, Ye NH. Identification of miRNA from *Porphyra yezoensis* by high-throughput sequencing and bioinformatics analysis. Plos One. 2010; 5: e10698. https://doi.org/10.1371/journal.pone.0010698 PMID: 20502668

43. Zhao CZ, Xia H, Frazier TP, Yao YY, Bi YP, Li AQ, et al. Deep sequencing identifies novel and conserved microRNAs in peanuts (*Arachis hypogaea* L.). BMC Plant Biol. 2010 10: 3. https://doi.org/10.1186/1471-2229-10-3 PMID: 20047695

44. Qiu DY, Pan XP, Wilson IW, Li FL, Liu M, Teng WJ, et al. High throughput sequencing technology reveals that the taxoid elicitor methyl jasmonate regulates microRNA expression in Chinese yew (*Taxus chinensis*) Gene. 2009; 436: 37–44. https://doi.org/10.1016/j.gene.2009.01.006 PMID: 19393185

45. Song CN, Wang C, Zhang CQ, Korir NK, Yu HP, Ma ZQ, et al. Deep sequencing discovery of novel and conserved microRNAs in trifoliate orange (*Citrus trifoliata*). BMC Genomics. 2010; 11: 431. https://doi.org/10.1186/1471-2164-11-431 PMID: 20626894

46. Moxon S, Jing R, Szittya G, Schwach F, Rusholme Pilcher RL, Moulton V, et al. Deep sequencing of tomato short RNAs identifies microRNAs targeting genes involved in fruit ripening. Genome Res. 2008; 18: 1602–1609. https://doi.org/10.1101/gr.080127.108 PMID: 18653800

47. Yao YY, Guo GG, Ni ZF, Sunkar R, Du J, Zhu JK, et al. Cloning and characterization of microRNAs from wheat (*Triticum aestivum* L.). Genome Biol. 2007 8: R96. https://doi.org/10.1186/gb-2007-8-6-r96 PMID: 17543110

48. Barakat A, Wall PK, Diloreto S, Depamphilis CW, Carlson JE. Conservation and divergence of microRNAs in *Populus*. BMC Genomics. 2007; 8: 481. https://doi.org/10.1186/1471-2164-8-481 PMID: 18166134

49. Joshi T, Yan Z, Libault M, Jeong DH, Park S, Green PJ, et al. Prediction of novel miRNAs and associated target genes in *Glycine max*. BMC Bioinformatics. 2010; 11(Suppl 1): S14.

50. Wu L, Zhou H, Zhang Q, Zhang J, Ni F, Liu C, et al. DNA methylation mediated by a microRNA pathway. Molecular Cell. 2010; 38: 465–475. https://doi.org/10.1016/j.molcel.2010.03.008 PMID: 20381393

51. Davoren PA, McNeill RE, Lowery AJ, Kerin MJ, Miller N. Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. BMC Mol Biol. 2008; 9: 76. https://doi.org/10.1186/1471-2199-9-76 PMID: 18718003

52. Maroufi A, Van Bockstaele E, De Loose M. Validation of reference genes for gene expression analysis in chicory (*Cichorium intybus*) using quantitative real-time PCR. BMC Mol Biol. 2010; 11: 15. https://doi.org/10.1186/1471-2199-11-15 PMID: 20156357

53. Gimenez MJ, Piston F, Atienza SG. Identification of suitable reference genes for normalization of qPCR data in comparative transcriptomics analyses in the Triticeae. Planta. 2011; 233: 163–173. https://doi.org/10.1007/s00425-010-1290-y PMID: 20960006

54. Yang XY, Zhang XL. Regulation of somatic embryogenesis in higher plants. Critical Reviews in Plant Sci. 2010; 29: 1, 36–57.

55. Hecht V, Vielle-Calzada JP, Hartog MV, Schmidt EDL, Boutilier K, Grossniklaus U, et al. The Arabidopsis *SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1* gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol. 2001; 127: 803–816. PMID: 11706164

56. Nolan KE, Kurdyukov S, Rose RJ. Expression of the *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1* (*SERK1*) gene is associated with developmental change in the life cycle of the model legume *Medicago truncatula*. J Exp Bot. 2009; 60: 1759–1771. https://doi.org/10.1093/jxb/erp046 PMID: 19305022

57. Zhang S, Liu XG, Lin YA, Xie GN, Fu FL, Liu HL, et al. Characterization of a *ZmSERK* gene and its relationship to somatic embryogenesis in a maize culture. Plant Cell Tiss Org Cult. 2011; 105: 29–37.

58. Guo WJ, Ho THD. An abscisic acid-induced protein, HVA22, inhibits gibberellin-mediated programmed cell death in cereal aleurone cells. Plant Physiol. 2008; 147: 1710–1722. https://doi.org/10.1104/pp.108.120238 PMID: 18583533

59. Woodger FJ, Millar A, Murray F, Jacobsen JV, Gubler F. The role of GAMYB transcription factors in GA-regulated gene expression. J Plant Growth Regul. 2003; 22: 176–184.

60. Tsuji H, Aya K, Ueguchi-Tanaka M, Shimada Y, Nakazono M, Watanabe R, et al. GAMYB controls different sets of genes and is differentially regulated by microRNA in aleurone cells and anthers. Plant J. 2006; 47: 427–444. https://doi.org/10.1111/j.1365-313X.2006.02795.x PMID: 16792694

61. Alonso-Peral MM, Li JY, Li YJ. The miR159 regulated *GAMYB-like* genes inhibit growth and promote programmed cell death in Arabidopsis. Plant Physiol. 2010; 154: 757–771. https://doi.org/10.1104/pp.110.160630 PMID: 20699403

62. Noma M, Huber, Ernst D, Pharis RP. Quantitation of gibberellins and the metabolism of [^{3}H] gibberellin A_1, during somatic embryogenesis in carrot and anise cell cultures. Planta. 1982; 155: 369–376. https://doi.org/10.1007/BF00394464 PMID: 24271967

63. Jiménez VM, Bangerth F. Hormonal status of maize initial explants and of the embryogenic and non-embryogenic callus cultures derived from them as related to morphogenesis in vitro. Plant Sci. 2001; 160: 247–257. PMID: 11164596

64. Jiménez VM, Bangerth F. Endogenous hormone levels in explants and in embryogenic and non-embryogenic cultures of carrot. Physiol Plant. 2001; 111: 389–395. PMID: 11240924

65. Jiménez VM, Bangerth F. Endogenous hormone levels in initial explants and in embryogenic and non-embryogenic callus cultures of competent and non-competent wheat genotypes. Plant Cell Tiss Org Cult. 2001; 67: 37–46.

66. Jiménez VM, Bangerth F. Relationship between endogenous hormone levels in grapevine callus cultures and their morphogenetic behaviour. Vitis. 2000; 39: 151–157.

67. Duan CF, Argout X, Gébelin V, Summo M, Dufayard JF, Leclercq J, et al. Identification of the *Hevea brasiliensis* AP2/ERF superfamily by RNA sequencing. BMC Genomics. 2013; 14: 30. https://doi.org/10.1186/1471-2164-14-30 PMID: 23324139

68. Ouakfaoui SE, Schnell J, Abdeen A, Colville A, Labbe H, Han S, et al. Control of somatic embryogenesis and embryo development by AP2 transcription factors. Plant Mol Biol. 2010; 74: 313–326. https://doi.org/10.1007/s11103-010-9674-8 PMID: 20798978

69. Lau S, Slane D, Herud O, Kong J, Jurgens G. Early embryogenesis in flowering plants: setting up the basic body pattern. Annu Rev Plant Biol. 2012; 63: 483–506. https://doi.org/10.1146/annurev-arplant-042811-105507 PMID: 22224452

70. Boutilier K, Offringa R. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. Plant Cell. 2002; 14: 1737–1749. https://doi.org/10.1105/tpc.001941 PMID: 12172019

71. Morcillo F, Gallard A, Pillot M, Jouannic S, Aberlenc-Bertossi F, Collin M, et al. EgAP2-1, an AINTEGUMENTA-like (AIL) gene expressed in meristematic and proliferating tissues of embryos in oil palm. Planta. 2007; 226: 1353–1362. https://doi.org/10.1007/s00425-007-0574-3 PMID: 17628826

72. Tsuwamoto R, Yokoi S, Takahata Y. Arabidopsis EMBRYOMAKER encoding an AP2 domain transcription factor plays a key role in developmental change from vegetative to embryonic phase. Plant Mol Biol. 2010; 73: 4811–492.

73. Piyatrakul P, Putranto RA, Martin F, Rio M, Dessailly F, Leclercq J, et al. Some ethylene biosynthesis and AP2/ERF genes reveal a specific pattern of expression during somatic embryogenesis in *Hevea brasiliensis*. BMC Plant Biol. 2012; 12: 244. https://doi.org/10.1186/1471-2229-12-244 PMID: 23268714

74. Jansen MAK, Booij H, Schel JHN, de Vries SC. Calcium increases the yield of somatic embryos in carrot embryogenic suspension cultures. Plant Cell Rep. 1990; 9: 221–223. https://doi.org/10.1007/BF00232184 PMID: 24226707

75. Overvoorde PJ, Grimes HD. The role of calcium and calmodulin in carrot somatic embryogenesis. Plant Cell Physiol. 1994; 35: 135–144.

76. Anil VS, Rao KS. Calcium-mediated signaling during sandalwood somatic embryogenesis. Role for exogenous calcium as second messenger. Plant Physiol. 2000; 123: 1301–1311. PMID: 10938349

77. Anil VS, Harmon AC, Rao KS. Spatio-temporal accumulation and activity of calcium-dependent protein kinases during embryogenesis, seed development, and germination in sandalwood. Plant Physiol. 2000; 122: 1035–1043. PMID: 10759499

2.3 Changes in the Metabolome of *Picea balfouriana* Embryogenic Tissues That Were linked to Different Levels of 6-BAP by Gas Chromatography-Mass Spectrometry Approach

PLOS | ONE

RESEARCH ARTICLE

Changes in the Metabolome of *Picea balfouriana* Embryogenic Tissues That Were Linked to Different Levels of 6-BAP by Gas Chromatography-Mass Spectrometry Approach

Q. F. Li[1,2], J. H. Wang[1]*, P. Pulkkinen[3], L. S. Kong[4]

1 State Key Laboratory of Forest Genetics and Tree Breeding, Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China, **2** Guangxi Key Laboratory of Special Non-wood Forest Cultivation & Utilization, GuangXi Zhuang Autonomous Region Forestry Research Institute, Nanning, China, **3** Finnish Forest Research Institute, Haapastensyrjä, Finland, **4** Unit Centre for Forest Biology, Department of Biology, University of Victoria, Victoria, Canada

* wangjh@caf.ac.cn

OPEN ACCESS

Citation: Li QF, Wang JH, Pulkkinen P, Kong LS (2015) Changes in the Metabolome of *Picea balfouriana* Embryogenic Tissues That Were Linked to Different Levels of 6-BAP by Gas Chromatography-Mass Spectrometry Approach. PLoS ONE 10(10): e0141841. doi:10.1371/journal.pone.0141841

Editor: Mohana Krishna Reddy Mudiam, CSIR-Indian Institute of Toxicology Research, INDIA

Received: July 21, 2015

Accepted: October 13, 2015

Published: October 30, 2015

Data Availability Statement: Our data are all contained within the paper and Supporting Information files.

Funding: This research was funded by the Advanced Generation Breeding Technological Research and Demonstration of Northern Conifer Species (2012BAD01B01).

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Embryogenic cultures of *Picea balfouriana*, which is an important commercial species for reforestation in Southern China, easily lose their embryogenic ability during long-term culture. Embryogenic tissue that proliferated at lower concentrations (3.6 μM and 2.5 μM) of 6-benzylaminopurine (6-BAP) were more productive, and generated 113 ± 6 and 89 ± 3 mature embryos per 100 mg embryogenic tissue, respectively. A metabolomic approach was used to study the changes in metabolites linked to embryogenic competence related to three different 6-BAP concentrations (2.5 μM, 3.6 μM, and 5 μM). A total of 309 compounds were obtained, among which 123 metabolites mapped to Kyoto Encyclopedia of Genes and genomes (KEGG) pathways. The levels of 35 metabolites were significantly differentially regulated among the three 6-BAP treatments, and 32 metabolites differed between the 2.5 μM and 5 μM treatments. A total of 17 metabolites appeared only once among the three comparisons. The combination of a score plot and a loading plot showed that in the samples with higher embryogenic ability (3.6 μM and 2.5 μM), up-regulated metabolites were mostly amino acids and down-regulated metabolites were mostly primary carbohydrates (especially sugars). These results suggested that 6-BAP may influence embryogenic competence by nitrogen metabolism, which could cause an increase in amino acid levels and higher amounts of aspartate, isoleucine, and leucine in tissues with higher embryogenic ability. Furthermore, we speculated that 6-BAP may affect the amount of tryptophan in tissues, which would change the indole-3-acetic acid levels and influence the embryogenic ability.

Introduction

The progressively diminishing embryogenic ability of embryogenic tissues has been well characterized in conifer trees. A primary example of such recalcitrance in spruce species is the inability or decreased competence of established embryogenic tissue to generate early stage embryos in response to suitable maturation conditions and to develop fully mature embryos. Although technology for the initiation and proliferation of somatic tissues and subsequent generation of mature cotyledonary embryos in spruces and other conifers has improved [1–10], some embryogenic tissues from many conifer species continue to exhibit a high degree of variability and others have lost embryogenic ability.

Picea balfouriana is an evergreen spruce tree that is distributed mostly in the southwest and northern regions of the Tibetan plateau. Because of the high quality of the wood and its fast growth, *P. balfouriana* is a major species of choice for afforestation. However, there are several drawbacks to using *P. balfouriana* for afforestation, including the fact that it reproduces primarily sexually, its seedlings initially grow slowly, and it sets seed late [11]. In our laboratory we have established the whole somatic embryogenesis system of *P. balfouriana* and applied for a patent [12]. We found that the embryogenic ability of the system was easily decreased when the amount of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (6-BAP) in proliferation stage were maintained or removed, and realized there was an urgent need to study the early stage of somatic embryogenesis [13]. We found that if the level of 2,4-D was decreased during proliferation, the embryogenic tissue would hardly generate somatic embryos. The amount of the cytokinin 6-BAP that added in the proliferation stage would influence the final yield of mature embryos from tissues, especially during long-term culture. It is well known that cytokinins play important roles in the control of cell division in plants, and the cytokinin signaling pathway has been studied recently [14,15]. However, the mechanism of action of 6-BAP in plant somatic embryogenesis is hardly known. In a previous study, we found that the influence of 6-BAP on embryogenic capacity was through relevant mRNAs and proteins [16] and, therefore, we inferred that 6-BAP may affect the corresponding metabolites.

Metabolomics, the global analysis of cellular metabolites, is a powerful tool based on functional proteomics that can be applied to gain insights into biological functions, which may be an effective approach for the functional characterization of genes, and may help in the description and elucidation of physiological responses in plants under different environmental conditions [17–27]. Gas chromatography-mass spectrometry (GC-MS) is generally performed using electron-impact quadrupole or time-of-flight mass spectrometry [28] and is one of the most developed analytical platforms for plant metabolite profiling [29]. Using GC-MS, it is possible to profile several hundred compounds belonging to diverse chemical classes, including sugars, organic acids, amino acids, sugar alcohols, aromatic amines, and fatty acids. For example, the regulation of developmental events has been elucidated at the metabolic level using metabolic profiling [30, 31] and different capabilities of embryogenic cell lines of *Pinus taeda* L. [32] and *Picea abies* (L.) [33] had been explained by a model based on the combined data for metabolic profiles. Further, the combination of GC-MS and OPLS-DA (orthogonal projections to latent structures discriminant analysis) [34–37] has been used to visualize and discriminate interesting metabolites.

In the present study, we investigated the metabolic profiles of embryonic tissues using three 6-BAP concentrations and identified important metabolites that were affected by 6-BAP and associated with embryogenic competence. The objective was to use the GC-MS approach to investigate changes in the metabolome that were linked to embryogenic ability, which was related to different levels of 6-BAP.

Materials and Methods

Plant material and sampling

One selected embryogenic cell line of *P. balfouriana* (Fig 1A) was used in this study. This cell line was established in 2011 and was initiated at the Research Institute of Forest, Chinese Academy of Forestry using seeds from elite genotype 4 that were induced on solidified half-strength LM medium [38] supplemented with 10 μM 2,4-D and 5 μM 6-BAP[12], 1% sucrose, 500 mg/L glutamine, 1 g/L casein hydrolysate, and 2% Gelrite at 24 ± 1°C in the dark. This cell line was proliferated on solidified half-strength LM medium with three concentrations of 6-BAP (2.5 μM, 3.6 μM, and 5 μM) and with other supplements kept unchanged at 24 ± 1°C in the dark. This produced embryogenic tissues with different embryogenic capabilities after 3 months. The embryogenic cultures were sub-cultured at 2-week intervals. This somatic embryogenesis culture experiment was performed twice. Both experimental series yielded similar results with respect to embryo development, and samples from one of the experimental series were selected for metabolomic profiling.

Samples of embryogenic culture were collected after subculturing for 7 days. For each treatment, six biological replicates were collected. The samples were denoted as 2.5 μM-, 3.6 μM-, and 5 μM-treated samples. All samples were transferred to cryotubes, flash frozen in liquid nitrogen, and stored at −80°C until further processing for metabolite extraction.

Early embryo differentiation from tissues was stimulated by transferring the cultures to half-strength LM medium lacking plant growth regulators for 1 week. Promotion of late embryo development and maturation (Fig 1B) was performed by transferring cultures to half-strength LM medium supplemented with 61 μM abscisic acid and 0.4% active charcoal, 6% sucrose, 500 mg/L glutamine, 1 g/L casein hydrolysate, and 4% Gelrite, and culturing at 24 ± 1°C in the dark. There were 10 replicates of each treatment. Then, somatic embryos (at least 3 mm long) generated from 100 mg embryogenic tissue that had 3–5 cotyledons and could germinate were counted after 8 weeks (Fig 1C). Twenty somatic embryos from each of the three 6-BAP treatments were put on one quarter-strength LM medium with 0.5% active charcoal, 2% sucrose, 500 mg/L glutamine, 500 mg/L casein hydrolysate, and 3% Gelrite for germination at 24 ± 1°C in the light (30 $\mu Em^{-2}s^{-1}$, 16 h photoperiod). There were 10 replicates for each treatment. After 6 weeks, the numbers of germinated somatic embryos with elongated root and hypocotyl were counted.

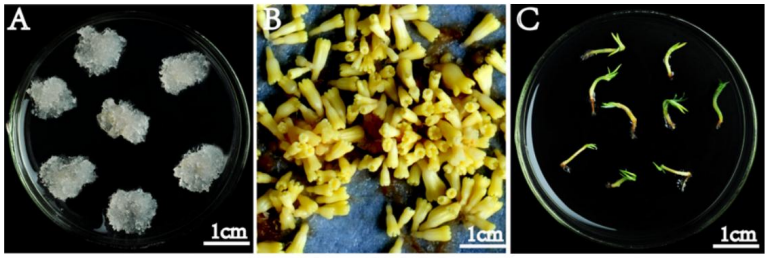


Fig 1. Somatic embryogenesis of *Picea balfouriana*. (A) Embryogenic tissues. **(B)** Somatic embryos. **(C)** Germination of somatic embryos.

doi:10.1371/journal.pone.0141841.g001

Histological analysis

Another cell line that was induced from elite genotype 3 in 2013 and treated in the same way as the test group was used as the control to validate the influence of 6-BAP. The numbers of mature somatic embryos and germinated somatic embryos of the control line were counted in the same way as in the test group. Histological differences between the test and control lines were analyzed. Proliferated tissues on medium supplemented with different levels of 6-BAP were treated and stained for general light microscopy observations according to Gupta and Holmstrom [39] with some modification. The embryonal head cells were stained bright red (acetocarmine) and suspensor cells were stained blue. These two sections together were considered as the early stage embryos and the numbers of early stage embryos of each 6-BAP concentration were counted.

Statistical analysis

To compare the influence of different levels of 6-BAP on the maturation of tissues and the germination of their generated somatic embryos, the numbers of early somatic embryos, the numbers of mature somatic embryos, and the germination rates were subjected to analysis of variance (ANOVA) using SPSS20 software(http://www-01.ibm.com/software/analytics/spss/downloads.html). The level of significance was $P < 0.05$.

Metabolite extraction and gas chromatography–time-of-flight mass spectrometry (GC/TOF/MS) analysis

Metabolites from embryogenic tissue (100-mg fresh weight) were extracted according to Lisec et al. [40] with minor modifications. Briefly, embryogenic tissue stored at −80°C was ground in a mortar using liquid nitrogen, and transferred into 2-mL centrifuge tubes. Ribitol (60 μL) was added and vortexed for 10 s, followed by the addition of 0.35 mL 100% methanol and vortexing for 10 s. The tubes were placed into an ultrasound machine at 70°C for 10 min, and then centrifuged for 10 min at 12,000 rpm at 4°C. Next, 0.35 mL supernatant was transferred into new Eppendorf tubes and samples were blow-dried using moderate nitrogen. Methoxamine hydrochloride (80 μL) was added, vortexed for 30 s, and allowed to react for 2 h at 37°C. Finally, 100 μL BSTFA reagent (containing 1% TMCS, v/v) was added to the mixture and allowed to react for 1 h at 70°C.

The GC/TOF/MS analysis was performed using an Agilent 7890A gas chromatograph system coupled with a Pegasus four-dimensional time-of-flight mass spectrometer (Agilent, USA). The system used a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m × 250-μm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). Next, a 1-μL aliquot of the analyte was injected in splitless mode. Helium was used as the carrier gas, the front inlet purge flow was 3 mL min^{-1}, and the gas flow rate through the column was 1 mL min^{-1}. The initial temperature was maintained at 90°C for 0.25 min, and then raised to 240°C at a rate of 5°C min^{-1}, and finally to 285°C at a rate of 20°C min^{-1} for 11.5 min. The injection, transfer line, and ion source temperatures were 280°C, 250°C, and 220°C, respectively. The energy was −70 eV in electron-impact mode. The MS data were acquired in full-scan mode with the m/z range of 20–600 at a rate of 100 spectra per second after a solvent delay of 492 s.

Multivariate and statistical analyses of raw signals, data baseline filtering, and peak identification and integration were performed using the Simca software (http://www.umetrics.com/products/simca). The data were then imported into the TagFinder software [41] with default parameters for correction of retention time to mass debris, peak alignment, and deconvolution

analysis [42]. The total mass of the signal integration area was normalized for each sample; that is, the total integral area of each sample was set as 1000. Then, principal component analysis (PCA) of internal standard peak areas was performed to provide sample weights and *t1* scores to normalize the data before multivariate analysis. In addition, metabolite data were mean centered and UV scaled. To obtain an overview of the metabolite data, a PCA model was calculated initially on the X-matrix [43]. In the PCA, a few latent variables were calculated, which described the largest systematic variation in the X-matrix. Thus, both the influence of noise and dimensionality on the data was greatly reduced. Using the resultant PCA score scatters, clusters and outliers within samples can be identified [44]. Finally, the normalized data were imported into SIMCA-P + 12.0.1 (Umetrics AB, Umeå, Sweden) using the OPLS-DA model with the first principal component of VIP (variable importance in the projection) values (VIP >1) combined with the Student's t-test (t-test) ($P < 0.05$) to identify differentially expressed metabolites and to search for metabolites in commercial databases such as those provided by the National Institute of Standards and Technology (NIST; http://www.nist.gov/index.html) and the publicly available KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www.genome.jp/kegg/). To characterize the physiological mechanisms of early somatic embryogenesis underlying the effects of the 6-BAP treatments, we examined the metabolic changes in embryogenic tissue at three 6-BAP concentrations. For each treatment, three comparisons were made: 2.5 μM *vs* 5 μM, 3.6 μM *vs* 2.5 μM, and 3.6 μM *vs* 5 μM. In each comparison, the sample with the lower embryo production was always made the control group. Metabolites that differed between samples were identified using OPLS-DA loading plots and a t-test of the respective metabolite peak areas. In all cases, models were judged for quality using goodness of fit (R^2X) and goodness of prediction parameters.

Visualization

If interesting metabolites are selected based solely on the correlation, a number of biochemical compounds that are present in very low concentrations also will be selected, and the risk of selecting false positives will be high. Potentially biochemically interesting compounds can be better selected based on a combination of covariance and correlation information, which is the purpose of the score plot (S-plot). The farther along the x-axis (covariance), the greater the contribution to the variance between the groups, while the farther the y-axis (correlation), the higher the reliability of the analytical result.

OPLS-DA together with the S-plot and loading plot allows complex data to be mined for metabolites that are statistically and potentially biochemically interesting compounds. The use of appropriate visualization tools helps in the communication and interpretation of scientific data. The metabolic data were analyzed as described by Wiklund *et al.* [36]. Briefly, the two vectors used in the S-plot are calculated as

$$Cov(t, Xi) = \frac{t^T X_i}{N - 1} \quad (1)$$

$$Corr(t, Xi) = \frac{Cov(t, Xi)}{s_t s_{X_i}} \quad (2)$$

where t is the score vector in the OPLS-DA model, i is the centered variable in data matrix X, and s is the estimated standard deviation. Therefore, $Cov(t,X)$ and $Corr(t,X)$ are vectors with the same length as the number of variables in the mode. These vectors are plotted in a scatter plot and are S-shaped unless the variable variance is uniform. The x-axis ($Cov(t,X)$) in the S-plot is a visualization of contribution (covariance), and the y-axis ($Corr(t,X)$) spans a

theoretical minimum (−1) and maximum (+1), where 1 is the correlation (reliability). The statistical S-plot was used to identify possible biochemically interesting compounds for both the predictive and orthogonal variations. A complementary tool for identifying interesting compounds is the loading plot where the vector $Cov(tp,X)$, which includes the corresponding jackknifed confidence intervals, provides additional information about metabolite variability.

Results

Development of *P. balfouriana* somatic embryos

During the maturation stage, embryogenic tissue of the cell lines exhibited different embryogenic ability after being treated with 2.5 μM, 3.6 μM, or 5 μM 6-BAP. Embryogenic tissue proliferated in medium containing 3.6 μM 6-BAP and yielded the most fully mature somatic embryos with a normal set of cotyledons (96±7/per 100 mg tissue), which had higher germination rates (48.47%±0.06)) than the mature somatic embryos from the other two 6-BAP treatments. Medium containing 2.5 μM 6-BAP yielded the second highest number of mature embryos (64±4/per 100 mg tissue) with germination rates of 28.39±0.04 and medium containing 5 μM 6-BAP yielded the lowest number of mature somatic embryos (11±1/per 100 mg tissue) with the lowest germination rates (9.42%±0.03).

ANOVA of the control line also revealed significant differences among the samples treated with the three concentrations of 6-BAP (Table 1). In addition, a micro-examination showed that the numbers of early somatic embryos in the different treatments were significantly different (Fig 2); that is, 31±3, 47±4, and 5±2 early somatic embryos per 50 mg tissue in the 2.5 μM, 3.6 μM, and 5 μM groups, respectively.

Metabolic changes in response to 6-BAP

Analysis of the three 6-BAP-treated culture samples yielded 309 compounds, among which 123 metabolites were assigned to KEGG pathways. A PCA model with two principal components explained 52% of the variation across the three samples (Fig 3A).

S-plot for the first two principal components of each two samples (2.5 μM versus 5 μM, 3.6 μM versus 2.5 μM and 3.6 μM versus 5 μM), explained 54%, 59% and 58% of the total variation, respectively, and was used for an overview of the data. The S-plot showed that each 6-BAP-treated sample was separated from the main cluster and distributed. OPLS-DA was used to discriminate between the two samples, and the OPLS-DA showed that the samples were separated according to the 6-BAP concentration (Fig 3B, 3C and 3D). Metabolites that distinguished the sample classes are presented in S1 Table (2.5 μM versus 5 μM), S2 Table (3.6 μM versus 2.5 μM) and S3 Table (3.6 μM versus 5 μM), respectively.

2.5 μM versus 5 μM 6-BAP-treated samples

Most of the differentially regulated metabolites (32) were present in the 2.5 μM group compared with the 5 μM group, both of which had lower embryogenic competence than the 3.6 μM

Table 1. Number of mature cotyledonary embryos generated from each treatment (per 100 mg of embryogenic tissue) and their germination rates compared with the control line.

Sample	Mature embryos / per 100 mg of embryogenic tissue	Germination rate
2.5 μM	64 ± 4^{b}	$28.39\% \pm 0.04^{b}$
3.6 μM	96 ± 7^{a}	$48.47\% \pm 0.06^{a}$
5 μM	11 ± 1^{c}	$9.42\% \pm 0.03^{c}$

$^{a, b, c}$ indicate the significance of difference ($P \leq 0.05$).

doi:10.1371/journal.pone.0141841.t001

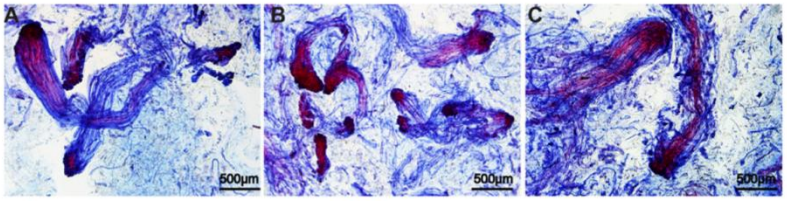


Fig 2. Early stage embryos in tissues treated with 6-BAP by micro-examination. The bars in each of the panels indicate 500 μm. **(A)** Early somatic embryos in tissue treated with 2.5 μM 6-BAP. **(B)** Early somatic embryos in tissue treated with 3.6 μM 6-BAP. **(C)** Early and mature somatic embryos in tissue treated with 5 μM 6-BAP.

doi:10.1371/journal.pone.0141841.g002

group. The majority of the regulated metabolites (14 up-regulated, four down-regulated) were amino acids or their derivatives associated with various biosynthetic pathways. Among the regulated metabolites that were carbohydrates, the number of up- and down-regulated metabolites was approximately equal. A total of eight organic acids (five up-regulated, three

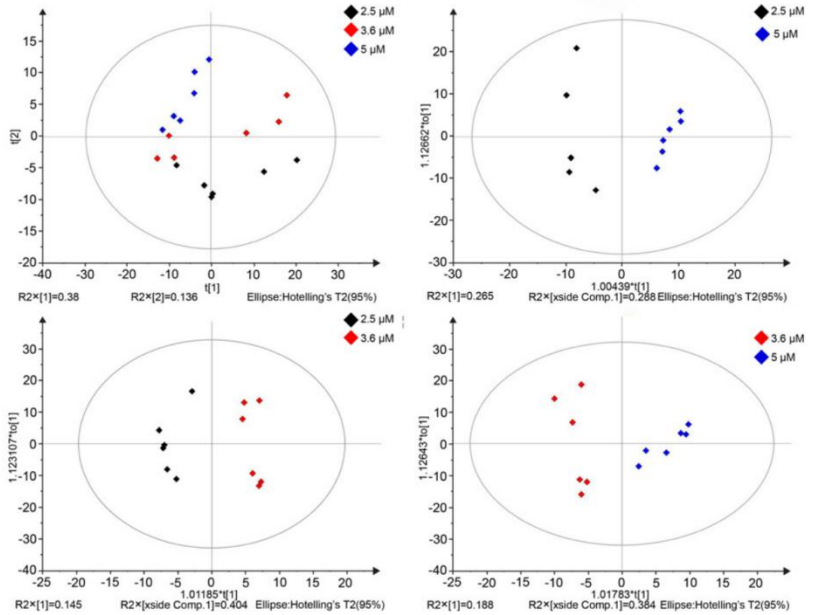


Fig 3. Score plots for the first two principal components by multivariate analysis. (A) PCA *t1*/*t2* score scatter (N = 18, R^2X [1] = 0.38, R^2X [2] = 0.14, and Q^2Y [cum] = 0.36). OPLS-DA score scatters. **(B)** 2.5 μM versus 5 μM (R^2X = 0.553, R^2Y = 0.96 and Q^2 = 0.834). **(C)** 3.6 μM versus 2.5 μM (R^2X = 0.548, R^2Y = 0.95 and Q^2 = 0.742). **(D)** 3.6 μM versus 5 μM (R2X = 0.573, R2Y = 0.89 and Q2 = 0.676). Samples are colored according to the 6-BAP concentrations.

doi:10.1371/journal.pone.0141841.g003

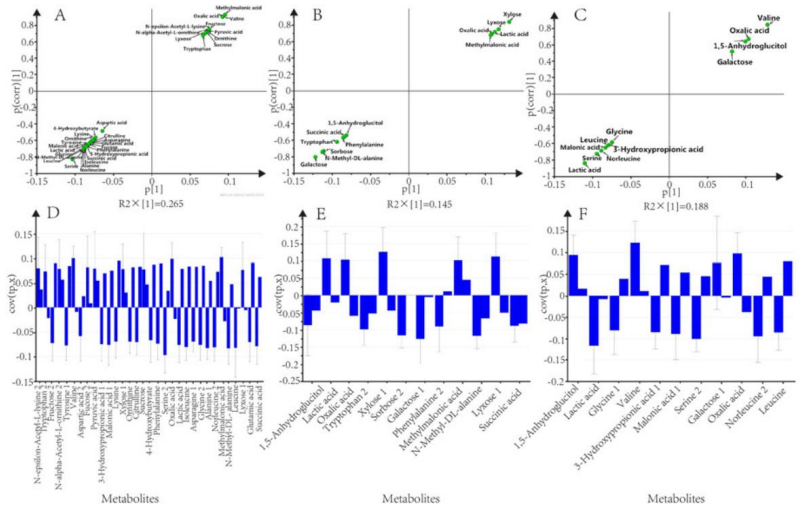


Fig 4. Strategy for identification of interesting metabolites. (A), **(B)**, and **(C)** show selected significant metabolites related to the differences between each pairwise comparison (see Fig 3). **(D)**, **(E)**, and **(F)** show the loading plots derived from each pairwise comparison. The plots mainly show the selected metabolites from the S-plot (see Fig 3).

doi:10.1371/journal.pone.0141841.g004

down-regulated) were identified in the 2.5 μM group compared with the 5 μM group; notably, lyxose 1 was significantly increased (19-fold) in the 5 μM 6-BAP group.

The separation between the 32 differentially regulated metabolites between the 2.5 μM and 5 μM groups was highlighted in the S-plot (Fig 4A). In the raw data plot both groups overlapped, while only 11 metabolites had a high correlation in the S-plot and a high reliability in the loading plot (Fig 4D) and were deemed reliable for separating the groups.

3.6 μM versus 2.5 μM 6-BAP-treated samples

Three differentially regulated metabolites were up-regulated and two were down-regulated in the 3.6 μM group compared with the 2.5 μM group, and three were amino acids, of which one was down-regulated and two were up-regulated in the 2.5 μM group compared with the control cells. Among the regulated metabolites that were organic acids, the number of up- and down-regulated metabolites was equal.

In the S-plot, 12 metabolites showed a high correlation (Fig 4B); however, in the loading plot, most of them showed low reliability because the confidence interval did not support the S-plot selection (Fig 4E). Finally, only tryptophan 2 and sorbose 2 showed reasonable reliability were selected for further investigation of their biochemical significance.

3.6 μM versus 5 μM 6-BAP-treated samples

Three of the differentially regulated metabolites were carbohydrates; one was up-regulated and two were down-regulated in 3.6 μM cultures compared with 5 μM. A common pattern was observed for the organic acid and amino acid groups, both of which comprised four

compounds with one down-regulated and three up-regulated in the 5 μM culture compared with the control cells. The differentially regulated metabolites with the highest levels in the 3.6 μM group were oxalic acid, galactose and, leucine, among which the leucine level was significantly up-regulated compared with the 5 μM group.

Significant differences between the 3.6 μM and 5 μM samples were identified using the mass peak intensities of all the detected metabolites, which were expressed in an S-plot, OPLS-DA (Fig 4C, and loading plot (Fig 4F). Both up-regulated and down-regulated metabolites were found in the 3.6 μM samples compared with 5 μM samples, but only 1,5-anhydroglucitol, 3-hydroxypropionic acid 1 and leucine were selected as significant regulated markers based on the statistical analyses.

Discussion

In the present study, we followed the metabolic events in one embryogenic cell line of *P. balfouriana* that displayed different embryogenic activities after being treated with three 6-BAP concentrations. Of the embryogenic tissues, those treated with 3.6 μM 6-BAP generated the greatest number of somatic embryos, which also had higher germination rates than the somatic embryos generated from embryogenic tissues proliferated on medium with lower (2.5 μM) or higher (5 μM) levels of 6-BAP. Furthermore, micro-examination showed that the embryonal heads and suspensors of early somatic embryos in the 5 μM-treated cultures were bigger than those in the 2.5 μM- and 3.6 μM-treated cultures. These results together with the ANOVA of mature embryos and histological analysis of early somatic embryos in the control cell line demonstrated that 6-BAP influenced the maturation of tissues and showed that the influence had universality. Many possible mechanisms could be proposed to explain these results; for example, 6-BAP may have influenced other plant growth regulators or genes involved in metabolite regulation. In the present study, we investigated this phenomenon using a metabolomics approach and found that metabolite profiles were altered significantly in response to different concentrations of 6-BAP. The preferential differential regulation of metabolites may trigger adaptive responses during somatic embryogenesis.

Although the t-test is widely used for selecting interesting compounds, we found that some of the regulated metabolites identified using the t-test may not be reliable [45–47]. For example, lyxose1 was significantly up-regulated (19-fold) in the 5 μM 6-BAP group compared with the 2.5 μM group and was selected as a reliably regulated metabolite based on the loading plot, but would not have been selected based on the t-test. The major dissent is that the t-test gives no consideration to variable intensity, which is often related to metabolite concentration [48]. In the present study, we used a loading plot because it reduces the impact of artifacts and noise in the models.

Leucine was up-regulated in embryogenic tissues proliferated in the 3.6 μM culture compared with the 5 μM culture, while sorbose 2 was significantly down-regulated in the 3.6 μM culture compared with the 2.5 μM culture. The aspartic acid, alanine, asparagine, serine, glycine, and phenylalanine amino acids were up-regulated in the 2.5 μM culture compared with the 5 μM culture, which did not efficiently generate embryos. This finding is in good agreement with the results of Richard et al. [49]. Broeckling et al. [50] reported a negative correlation between amino acid and sugar levels in embryogenic tissues, which is in agreement with our results. We found that amino acid metabolites were mostly up-regulated, whereas most of the sugars were down-regulated. All the metabolites that were differentially regulated by 6-BAP resulted in a different embryogenic capacity of the cultures.

6-BAP may affect two channels involved in early somatic embryogenesis: nitrogen metabolism and/or the IAA concentration in tissues. In nitrogen metabolism, NH_4^+ and NO_3^- are

both essential for the proliferation of embryogenic tissue and development of somatic embryos [51–53]. Ikram and Yusuf [54] found that when both KNO_3 and NH_4NO_3 were added to the embryo-induction medium, there was a considerable increase in the rates of cell growth and somatic embryogenesis. We found that the asparagine level was higher in the 2.5 μM group compared with the 5 μM group. Asparagine was shown to be a key component of nitrogen metabolism in conifers [55]. Furthermore, isoleucine and norleucine levels were also higher in the 2.5 μM group compared with the 5 μM group, and these two amino acids are synthesized by aspartate. Aspartate is synthesized from glutamate in the plastid and channeled through the aspartate metabolic pathway for the biosynthesis of lysine, threonine, isoleucine, methionine, and other essential nitrogen compounds [56]. Taken together, our data suggest that 2.5 μM 6-BAP increased the levels of several amino acids that are involved in nitrogen metabolism, which is indispensable for the proliferation of embryogenic cultures.

Another channel that we propose could be involved in early somatic embryogenesis may be affected by the influence of 6-BAP on the amount of IAA in tissues. The tryptophan level was lowest in cultures treated with 3.6 μM 6-BAP. Tryptophan can be converted to indole-3-pyruvic acid, which is then converted to indole-3-acetaldehyde from which IAA is synthesized [57]. IAA can also be synthesized by converting tryptophan to indole-3-acetaldoxime, which is then converted to indole-3-acetonitrile from which IAA is synthesized [33]. Furthermore, the interaction between auxin and cytokinin has been found to control cell proliferation and differentiation [58–60]. Therefore, our results indicated that the embryogenic ability of *P. balfouriana* embryogenic tissue could be maintained during the long proliferation stage of somatic embryogenesis by adding moderate concentrations of 6-BAP to the media. This technique for somatic embryogenesis could be applied to promote afforestation by *P. balfouriana*. In addition, the differentially regulated metabolites (asparagine, tryptophan, and others) may be used as markers to detect the embryogenic competence of tissues.

Supporting Information

S1 Table. Levels of regulated metabolites in groups 2.5 μM and 5 μM ($P<0.05$).
(DOCX)

S2 Table. Levels of regulated metabolites in groups 2.5 μM and 3.6 μM ($P < 0.05$).
(DOCX)

S3 Table. Metabolites differentially regulated in 3.6 μM and 5 μM ($P < 0.05$).
(DOCX)

Acknowledgments

This paper is dedicated to JW who founded by the Advanced Generation Breeding Technological Research and Demonstration of Northern Conifer Species (2012BAD01B01), and did so much to research somatic embryogenic mechanism of spruce. PP and LK critically read the manuscript and corrected the final draft.

Author Contributions

Conceived and designed the experiments: QL. Performed the experiments: QL JW. Analyzed the data: QL JW. Contributed reagents/materials/analysis tools: PP LK. Wrote the paper: QL.

References

1. Kliaszewska K, Park YS, Overton C, Maceacheron I, Bonga JM. Optimized somatic embryogenesis in *Pinus strobus* L. In Vitro Cell Dev Biol Plant, 2001; 37: 392–399. doi: 10.1007/s11627-001-0069-z
2. Stasolla C, Yeung EC. Recent advances in conifer somatic embryogenesis: improving somatic embryo quality. Plant Cell Tiss Org Cult, 2003; 74: 15–35. doi: 10.1023/A:1023345803336
3. Pullman GS, Namjoshi K, Zhang Y. Somatic embryogenesis in loblolly pine (*Pinus taeda* L.): improving culture initiation with abscisic acid and silver nitrate. Plant Cell Rep, 2003; 22: 85–95 doi: 10.1007/s00299-003-0673-y PMID: 12879261
4. Pullman GS, Chase KM, Skryabina A, Bucalo K. Conifer embryogenic tissue initiation: improvements by supplementation of medium with D-xylose and D-chiro-inositol. Tree Physiol. 2009; 29: 147–156. doi: 10.1093/treephys/tpn013 PMID: 19203940
5. Lipavska H, Konradova H. Somatic embryogenesis in conifers: the role of carbohydrate metabolism. In Vitro Cell Dev-An, 2004; 40: 23–30. doi: 10.1079/IVP2003482
6. MacKay JJ, Becwar MR, Park YS, Corderro JP, Pullman GS. Genetic control of somatic embryogenesis initiation in loblolly pine and implications for breeding. Tree Genet. Genomes. 2006; 2: 1–9. doi: 10.1007/s11295-005-0020-2
7. Park YS, Lelu-Walter MA, Harvengt L, Trontin JF, MacEacheron I, Klimaszewska K, Bonga JM. Initiation of somatic embryogenesis in *Pinus banksiana*, *P. strobus*, *P. pinaster*, and *P. sylvestris* at three laboratories in Canada and France. Plant Cell Tiss Org Cult. 2006; 86: 87–101. doi: 10.1007/s11240-006-9101-7
8. Lelu-Walter MA, Bernier CM, Klimaszewska K. Simplified and improved somatic embryogenesis for clonal propagation of *Pinus pinaster* (Ait.). Plant Cell Rep. 2006; 25:767–776. doi: 10.1007/s00299-006-0115-8 PMID: 16518635
9. Lelu-Walter MA, Bernier CM, Klimaszewska K. Clonal plant production from self- and cross-pollinated seed families of *Pinus sylvestris* (L.) through somatic embryogenesis. Plant Cell Tiss Org Cult. 2008; 92: 31–45. doi: 10.1007/s11240-007-9300-x
10. Vales T, Feng XR, Ge L, Xu NF, Cairney J, Pullman GS, Peter GF. Improved somatic embryo maturation in loblolly pine by monitoring ABA-responsive gene expression. Plant Cell Rep, 2007; 26: 133–143. doi: 10.1007/s00299-006-0221-7 PMID: 16941104
11. Wang JH, Zhang SG, Ma CG. Actualities and expectation of techniques for accelerating Picea seedling growth. Journal of Zhejiang Forestry College. 2005; 22: 350–354
12. Wang JH, Li QF, Zhang SG. Somatic embryogenesis and plantlet regeneration of *Picea balfouriana*. ZL 201210274334.9, pp 1–10
13. Li QF, Zhang SG, Wang JH. Transcriptome analysis of callus in *Picea balfouriana*. BMC Genomics. 2014; 15: 533. doi: 10.1186/1471-2164-15-553 PMID: 24993107
14. Argueso CT, Raines T, Kieber JJ. Cytokinin signaling and transcriptional networks. Curr Opin Plant Biol. 2010; 13(5):533–539. doi: 10.1016/j.pbi.2010.08.006 PMID: 20851038
15. Gupta S, Rashotte AM. Down-stream components of cytokinin signaling and the role of cytokinin throughout the plant. Plant Cell Rep. 2012; 31(5): 801–812. doi: 10.1007/s00299-012-1233-0 PMID: 22315145
16. Li QF, Zhang SG, Wang JH. Transcriptomic and proteomic analyses of embryogenic tissues in Picea balfouriana treated by 6-Benzylaminopurine. Physiol Plantarum, 2015; 154: 95–113. doi: 10.1111/ppl.12276
17. Chen YZ, Pang QY, He Y, Zhu N, Branstrom I, Yan XF, Chen SX. Proteomics and metabolomics of arabidopsis responses to perturbation of glucosinolate biosynthesis. Molecular Plant. 2012; 5(5): 1138–1150. doi: 10.1093/mp/sss034 PMID: 22498773
18. Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN, Willmitzer L. Metabolite profiling for plant functional genomics. Nat Biotechnol. 2000; 18: 1157–1161. doi: 10.1038/81137 PMID: 11062433
19. Roessner U, Luedemann A, Brust D, Fiehna O, Linkeb T, Willmitzera L, Femiea AR. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. Plant Cell. 2001a; 13, 11–29. doi: 10.1105/tpc.13.1.11 PMID: 11158526
20. Roessner U, Willmitzer L, Fernie AR. High-resolution metabolic phenotyping of genetically and environmentally diverse potato tuber systems identification of phenocopies. Plant Physiol. 2001b; 127:749–764. doi: 10.1104/pp.010316 PMID: 11706160
21. Fiehn O. Metabolomics–the link between genotypes and phenotypes. Plant Mol Biol, 2002; 48: 155–171 PMID: 11860207
22. Nicholson J K, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. Nat Rev Drug Discov. 2002; 1(2):153–161. doi: 10.1038/nrd728 PMID: 12120097

23. Sumner LW, Mendes P, Dixon RA. Plant metabolomics: large-scale phytochemistry in the functional genomics era. Phytochemistry. 2003; 62:817–836. doi: 10.1016/S0031-9422(02)00708-2 PMID: 12590110

24. Weckwerth W. Metabolomics in systems biology. Annu. Rev. Plant Biol. 2003; 54: 669–689. doi: 10.1146/annurev.arplant.54.031902.135014 PMID: 14503007

25. Alexandra L, Christophe J, Yannick P, Gilles L, Jean C T, Eric E, Jean L. Combined proteome and metabolite-profiling analyses reveal metabolism surprising insights into Yeast Sulfur metabolism. J Biol Chem. 2005; 280:24723–24730. doi: 10.1074/jbc.M502285200 PMID: 15855158

26. Meyer RC, Steinfath M, Lisec J, Becher M, Wall HW, Törjék O, Fiehn Oliver, Eckardt Ä, Willmitzer L, Selbig J, Altmann T. The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*. Proc Natl Acad Sci. 2007; 104: 4759–4764. doi: 10.1073/pnas.0609709104 PMID: 17360597

27. Wu DZ, Cai SG, Chen MX, Chen ZH, Zhang HT, Dai F, Wu FB, Zhang GP. Tissue metabolic responses to salt stress in wild and cultivated barley. PLoS one. 2013; 8(1): e55431. doi: 10.1371/journal. pone.0055431 PMID: 23383190

28. Roessner U, Wagner C, Kopka J,Trethewey RN, Willmitzer L. Technical advance: simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. Plant J. 2000; 23: 131–142. doi: 10.1046/j.1365-313x.2000.00774.x PMID: 10929108

29. Shulaev V, Cortes D, Miller G, Mittler R. Metabolomics for plant stress response. Physiol Plantarum. 2008; 132: 199–208. doi: 10.1111/j.1399-3054.2007.01025.x

30. Hosp J, Tashpulatov A, Roessner U, Barsova E, Katholnigg H, Steinborn R, Melikant B, Lukyanov S, Heberle-Bors E, Touraev A. Transcriptional and metabolic profiles of stress-induced embryogenic tobacco microspores. Plant Mol Biol. 2007; 63: 137–149. doi: 10.1007/s11103-006-9078-y PMID: 17016740

31. Weigelt K, Kuster H, Radchuk R, Muller M, Weichert H, Fait A, Fernie AR, Saalbach I, Weber H. Increasing amino acid supply in pea embryos reveals specific interactions of N and C metabolism, and highlights the importance of mitochondrial metabolism. Plant J. 2008; 55: 909–926. doi: 10.1111/j.1365-313X.2008.03560.x PMID: 18494854

32. Robinson AR, Dauwe R, Ukrainetz NK, Cullis IF, White R, Mansfield SD. Predicting the regenerative capacity of conifer somatic embryogenic cultures by metabolomics. Plant Biotechnol J. 2009; 7:952–963. doi: 10.1111/j.1467-7652.2009.00456.x PMID: 19906246

33. Businge E, Klaus B, Thomas M, Egertsdotter U. Metabolite profiling reveals clear metabolic changes during somatic embryo development of Norway spruce (*Picea abies*). Tree Physiol. 2012; 32: 232–244. doi: 10.1093/treephys/tpr142 PMID: 22310018

34. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). J Chemometr. 2002; 16:119–128. doi: 10.1002/cem.695

35. Bylesjö M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. J Chemometr. 2006; 20: 341–351. doi: 10.1002/cem.1006

36. Wiklund S, Johansson E, Snowstorm L, Mellerowicz EJ, Edlund U, Schockcor JP, Gottfries J, Moritz T, Trygg J. Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. Anal Chem. 2008; 80:115–122. doi: 10.1021/ac0713510 PMID: 18027910

37. Srivastava V. Active oxygen involvement in developmental processes in *Populus*. PhD thesis, Swedish University of Agricultural Sciences, Umeå, Sweden. 2009

38. Litvay JD, Johnson MA, Verma DC, Einspahr D, Weyrauch K. Conifer suspension culture medium development using analytical data from developing seeds. IPC Tech Pap. Paper 114, Appleton, WI.

39. Gupta PK, Holmstrom D. Double staining technology for distinguishing embryogenic cultures. Protocol for Somatic Embryogenesis in Woody Plants. 2005; 77: 573–575. doi: 10.1007/1-4020-2985-3_45

40. Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR. Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat Protoc. 2006; 1: 387–396. doi: 10.1038/nprot.2006.59 PMID: 17406261

41. Luedemann A, von Malotky L, Erban A, Kopka J. TagFinder: preprocessing software for the fingerprinting and the profiling of gas chromatography-mass spectrometry based metabolome analyses. Methods Mol Biol. 2012; 860: 255–286. doi: 10.1007/978-1-61779-594-7_16 PMID: 22351182

42. Luasd edemann A, Strassburg K, Erban A, Kopka J. TagFinder for the quantitative analysis of gas chromatography-mass spectrometry (GC-MS)-based metabolite profiling experiments. Bioinform Appl Note. 2008; 24: 732–737. doi: 10.1093/bioinformatics/btn02

43. Wold S, Esbensen K, Geladi P. Principal Component Analysis. Chemometrics and Intelligent Laboratory Systems. 1987; 2: 37–52

44. Madsen A, Leheny RL, GuoHY, Sprung M, Czakkel O. Beyond simple exponential correlation functions and equilibrium dynamics in x-ray photon correlation spectroscopy. New J. Phys. 2010; 12(5): 0550017. doi: 10.1088/1367-2630/12/5/055001

45. Alvarez S, Marsh EL, Schroeder SG, Schachtman DP. Metabolomic and proteomic changes in the xylem sap of maize under drought. Plant Cell and Environ. 2008; 31:325–340. doi: 10.1111/j.1365-3040.2007.01770.x

46. Widodo Patterson JH, Newbigin E, Tester M, Bacic A, Ute Roessner. Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. J Exp Bot. 2009; 60(14): 4089–4103. doi: 10.1093/jxb/erp243 PMID: 19666960

47. Hochberg U, Degu A, Toubiana D, Gendler T, Nikoloski Z, Rachmilevitch S, Fait A. Metabolite profiling and network analysis reveal coordinated changes in grapevine water stress response. BMC Plant Biol. 2013; 13: 184. doi: 10.1186/1471-2229-13-184 PMID: 24256338

48. Jiye A, Trygg J, Gullberg J, Johansson AI, Jonsson P, Antti H, Marklund SL, Moritz T. Extraction and GC/MS analysis of the human blood plasma metabolome. Anal Chem. 2005; 77(24): 8086–8094. doi: 10.1021/ac051211v PMID: 16351159

49. Richard WJ, Deane DM, Hans JV, Thorpe TA. Stage-specific nitrogen metabolism in developing carrot somatic embryos. Physiol Plantarum. 1996; 97(1):149–159. doi: 10.1111/j.1399-3054.1996.tb00491.x

50. Broeckling CD, Huhman DV, Farag MA, Smit JT h, May GD, Mendes P, Richard A. Dixon, Sumner LW. Metabolic profiling of *Medicago truncatula* cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. J Exp Bot. 2005; 56 (410): 323–336. doi: 10.1093/jxb/eri058 PMID: 15596476

51. Gleddie S, Keller W, Setterfield G. Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of *Solanum melongena* (eggplant). Can J Bot. 1983; 61(3): 656–666. doi: 10.1139/b83-074

52. Izabela MM, Janusz Z. NH_4^+ and NO_3^- requirement for wheat somatic embryogenesis. Acta Physiol Plant. 2001; 23(1): 37–42. doi: 10.1007/s11738-001-0020-2

53. Kim S, Kim S. Effect of nitrogen source on cell growth and anthocyanin production in embryogenic tissue and cell suspension culture of 'Sheridan' Grapes. J Plant Biotechnol. 2006; 4(2): 83–89

54. Ikram H, Yusuf Z. Effect of nitrates on embryo induction efficiency in cotton (*Gossypium hirsutum* L.). Afr J Biotechnol. 2004; 3(6): 319–323

55. Canovas MF, Concepcion A, Canton FR, Can ñ as RA, Torre FD. Ammonium assimilation and amino acid metabolism in conifers. J Exp Bot. 2007; 58: 2307–2318. doi: 10.1093/jxb/erm051 PMID: 17490997

56. Azevedo RA, Lancien M, Lea PJ. The aspartic acid metabolic pathway, an exciting and essential pathway in plants. Amino Acids. 2006; 30: 143–162. doi: 10.1007/s00726-005-0245-2 PMID: 16525757

57. Sitbon F, Åstot C, Edlund A, Crozier A, Sandberg G. The relative importance of tryptophan-dependent and tryptophan-independent biosynthesis of indole-3-acetic acid in tobacco. Planta. 2000; 211:715–721. doi: 10.1007/s004250000338 PMID: 11089685

58. Moubayidin L, Perilli S, Dello Ioio R, Di Mambro R, Costantino P, Sabatini S. The rate of cell differentiation controls the arabidopsis root meristem growth phase. Curr Biol. 2010. 20(12): 1138–1143. doi: 10.1016/j.cub.2010.05.035 PMID: 20605455

59. Lynch JP. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiol. 2011; 156 (3): 1041–1049. doi: 10.1104/pp.111.175414 PMID: 21610180

60. Daniele M, Helen B, John K, Malcolm B. The role of auxin and cytokinin signalling in specifying the root architecture of *Arabidopsis thaliana*. J Theor Biol. 2013; 317: 71–86. doi: 10.1016/j.jtbi.2012.08.032 PMID: 23026765

3. 以通讯作者发表的论文

3.1 Thinning effects on forest evolution in Masson pine (*Pinus massoniana* Lamb.) conversion from pure plantations into mixed forests

Forest Ecology and Management 477 (2020) 118503

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Thinning effects on forest evolution in Masson pine (*Pinus massoniana* Lamb.) conversion from pure plantations into mixed forests

Cheng Deng[a], Shougong Zhang[b], Yuanchang Lu[c], Robert E. Froese[d], Xiaojun Xu[a], Ji Zeng[e], Angang Ming[e,f], Xianzhao Liu[c], Yangsheng Xie[c], Qingfen Li[a,*]

[a] College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China
[b] Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China
[c] Research Institute of Forest Resource Information Techniques, Chinese Academy of Forestry, Beijing 100091, China
[d] College of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA
[e] Experimental Center of Tropical Forestry, Chinese Academy of Forestry, Pingxiang 532600, China
[f] Guangxi Youyiguan Forest Ecosystem Research Station, Pingxiang 532600, China

ARTICLE INFO

Keywords:
Ecological restoration
Pure forest replacement
Mixed forest
Seedling establishment
Understory vegetation development
Vegetation diversity

ABSTRACT

Monocultural coniferous plantations have prevailed worldwide in recent decades, which supplied much of the world's timber, but also exerted some negative effects on local ecologies and environmental systems. Continuous development has increased the various demands of human society for forests and it is necessary to balance concerns for the ecological and economic functions. Ample evidence indicates that mixed forests are an ideal option for providing more diversified ecological services and forest goods. Converting pure forests into mixed forests by introducing broad-leaved hardwood species below coniferous plantations has become an increasingly popular forest management strategy. Yet, there has been not enough research to date on suitable management methods for enhancing forest diversity and resilience in the context of forest conversion. To comprehensively examine how the intensity in Masson pine thinning influences forest evolution, seedlings of two native hardwood species were introduced below unthinned and thinned (varying intensity) Masson pine plantations. The effects of thinning on residual tree growth, seedling survival and growth, and understory vegetation development were analyzed using a generalized additive mixed model (GAMM). Monitoring results over 10 years indicate that thinning is a necessary management measure to accelerate forest succession in the conversion process; thinning exerts significant effects on the growth of residual trees, the survival and growth of seedlings, and the development of understory vegetation. Intense thinning results in more residual tree growth and enhances the richness and diversity of herbaceous species. However, excessive thinning can reduce the likelihood of seedling survival and growth as well as the richness and diversity of shrubs. Optimal thinning intensities appear to fall between 50% and 60% depending on the specific introduced species; light-demanding species may need higher overstory thinning intensity than shade-tolerant species. The biological characteristics of the introduced species must be taken into account to design an effective thinning strategy for pine plantations conversion.

1. Introduction

Single-species or pure forest plantations prevail worldwide over the last few decades(Evans, 1999; FAO, 2001; Evans and Turnbull, 2004; Malkamäki et al., 2017). The majority of plantations use coniferous tree species, as coniferous stands are highly profitable and comparatively modest in their ecological requirements (Kenk and Guehne, 2001). Native deciduous forests across the globe have been extensively replaced by pioneer conifer stands (Augusto et al., 2002). Among these conifer species, pine species is the most common, which dominates large areas of the Northern Hemisphere and accounts for 42% of the world's industrial forest plantations (Farjon, 2010; Payn et al., 2015). However, conversion from native deciduous forests to monocultural coniferous stands has been criticized for negative impact on local ecologies and environmental systems (Lamb et al., 2005; Chirino et al., 2006; Erskine et al., 2006), and for a general lack of traditional forest goods (Evans, 1999). Most coniferous species are highly flammable and facilitate catastrophic or stand-replacing spread of fires (Pausas et al., 2004). Pines and other coniferous pure forests are also highly vulnerable to diseases and insect pests such as nematodes (Haynes et al.,

* Corresponding author.
E-mail address: li63757416@163.com (Q. Li).

https://doi.org/10.1016/j.foreco.2020.118503
Received 12 May 2020; Received in revised form 9 August 2020; Accepted 11 August 2020

2014), beetles (Xie et al. 2020), and root diseases, which are exacerbated by climate change (Venalainen et al 2020). These adverse side effects do not align with modern concepts of forestry management and societal development (Chauvat et al., 2011).

By contrast, many studies have shown that mixed forests are an ideal approach to providing more diversified ecological services and forest goods (Keenan et al., 1999; Cusack and Montagnini, 2004; Bravo-Oviedo, 2018; Liu et al., 2018). Mixed forests may have strong biodiversity (Cavard et al., 2011; Heinrichs et al., 2018) and can produce greater product volumes, biomass, soil fertility, and carbon sequestration per unit area (Montagnini, 2000; Pretzsch et al., 2013; Koutika et al., 2014; Zhang et al., 2019). Mixed forests are generally less vulnerable to fire (Pausas et al., 2004; Schelhaas et al., 2010) and less sensitive to pest outbreaks and herbivory issues (Montagnini et al., 1995; Jactel and Brockerhoff, 2007), although the effects are usually context dependent (i.e., affected by species composition, density, etc.; Jactel et al., 2017). Thus, mixed forests are often more resistant to and resilient against external disturbances and environmental changes (Jactel et al., 2009; Bauhus et al., 2017). Re-establishing mixed forests has grown increasingly popular for these reasons. Many forest managers have sought to convert monocultures to mixed stands in recent decades (Pausas et al., 2004; Keskitalo, 2011). The conversion is also considered as part of the broader concept of forest restoration, which is an active restoration to speed up the recovery process (Stanturf et al., 2014). Some studies indicate that active restoration approaches can have higher (or similar) plant diversity to natural regeneration approaches, but often at a high cost to establish structural features of the vegetation, reassemble local species composition, and/or catalyze ecological succession (Crouzeilles et al., 2017).

Conversion usually includes the manipulation of disturbance regimes (for example, thinning and burning) and species mixing (planting of nursery-grown seedlings, direct seeding) into the resultant gaps (Zhang et al., 2019). The success of the conversion depends on the plantation design and the appropriate selection of the tree species to be used, taking into consideration silvicultural and ecological factors (Wormald, 1992; Chauvat et al., 2011). Introducing broad-leaved hardwood species seedlings into existing thinned stands can mitigate seed limitations, and is currently the most common conversion practice (Gavinet et al., 2015). Successful seedling introduction hinges on the selected tree species adapting properly to local climate and environmental conditions (Padilla et al., 2009; Vallejo et al., 2012). Native broad-leaved hardwood species are widely recommended because they have strong adaptability to local conditions, in addition to being well-accepted socially and ecologically (Ghorbani et al., 2018).

Thinning is also considered vital for the conversion of monocultural (or “pure”) forests to mixed forests. Thinning of a forest stand is a fundamental silvicultural tool for variety of management objectives (David et al., 2016; Medeiros et al., 2017). Traditionally, thinning has been used to increase production and improve the quality of residual trees by removing inferior trees, minimizing pest damage, and reducing wildfire risk (Nishizono, 2010; Hood et al., 2016). Thining is now recognized to play a role in both timber production and forest restoration. Thinning can promote stand composition and vertical structural characteristics while potentially accelerating natural regeneration (Bradford and Palik, 2009; Miller and Emmingham, 2001; Swinfield et al., 2016), increasing the growth rate of residual trees (Deng et al., 2019) and enhancing understory biodiversity (Ishii et al., 2008; Spake et al., 2019; Trentini et al., 2017; Ulvcrona et al., 2014; Ferraz et al., 2018). Thinning also can increase the tree’s intrinsic water-use efficiency and drought resistance (Sohn et al., 2016; Navarro-Cerrillo et al., 2019) while altering microenvironmental factors such as light availability, temperature, evaporative demand, and soil properties (Aussenac, 2000; Gavinet et al., 2015). These qualities altogether may improve the establishment of seedlings in the understory (Gavinet et al., 2015). Further, thinning can affect carbon dioxide fluxes, soil–plant carbon and nitrogen dynamics, and improve carbon sequestration (Bai et al., 2017; Lindroth et al., 2018; Zhang et al., 2019).

Conversion of monocultures into mixed forests has garnered a great deal of research interest in recent years, and existing studies have yielded much useful information (Spiecker et al., 2004; Ammer et al., 2008; Löf et al., 2010; Heine et al., 2019). However, there are many practical difficulties impeding the effective establishment of mixed forests (Mason et al., 2018; Coll et al., 2018). There is a widespread lack of guidance as managers seek to tackle the complicated silviculture of mixed forests (Mason et al., 2018). Much work is still needed including comprehensive studies on suitable management methods to strengthen understanding of forest diversity and resilience in the context of forest conversion (Bardat and Aubert, 2007; Salamon et al., 2008; Chauvat et al., 2011; Gavinet et al., 2015; Coll et al., 2018).

Masson pine (*Pinus massoniana* Lamb.) is an important tree species of *Pinus* (*Pinaceae*), with height up to 45 m and diameter at breast height up to 1.5 m. A high-quality timber species, Masson pine has been widely planted and occupies 13.2 percent of all forested land in China covering 14.2 million ha, and is also widely distributed in southern Africa. However, > 90% of Masson pine has been managed in monoculture plantations, which is often associated with ecosystem degradation, including reduction of soil fertility and biodiversity, the reduction of stand resistance to environmental perturbations, the frequent occurrence of diseases and insect pests such as nematodes, and the high risk of fire. In recent years, these problems have attracted attention of forest managers, who began to seek methods to effectively transform these pure plantations into more stable and resistant mixed forests (Wang et al., 2001). Studying the thinning effect on forest evolution in Masson pine conversion from pure plantations into mixed forests is also of great reference value for other Pinaceae species.

In this study, we comprehensively examined the effect of Masson pine thinning on the growth of overstory residual trees, the establishment (survival and growth) of two underplanted native broad-leaved hardwood species, and the development of understory vegetation below various thinning intensities. Our primary goal is to determine whether overstory thinning helps accelerate succession in hardwood species establishment and/or enhances vegetation development to increase biodiversity in Masson pine stands.

2. Materials and methods

2.1. Experimental sites

Experiments were conducted in Masson pine plantations at the Experimental Center of Tropical Forestry, located in the southwest of Pingxiang City, in the Guangxi Zhuang Autonomous Region of China (21°57′–22°19′ N, 106°39′–106°59′ E). The experimental area belongs to the country’s south subtropical zone and has a semi-humid/humid climate. Its mean annual rainfall is 1200–1500 mm, relative humidity is 80%-84%, and annual average temperature is 20.5–21.7°C. The landform type is mainly low hills with an elevation of 400–450 m above sea level. Soils are latosolic red loams developed from granite with a clay-loam texture, stoniness in the range 2–14%, medium to high fertility, and a mean depth of 90 cm.

The Masson pine plantations assessed in this study were established in 1993 with an initial planting density of 2500 trees per hectare. Weeding was the main maintenance activity after field planting of the trees, and a release thinning was conducted in 2000. The understory is composed of sparse dwarf scrubs (*Phyllanthus emblica*, *Melastoma candidum*) and scattered herbs (*Microstegium nodosum*, *Narenga fallax (Balansa) Bor.*).

2.2. Thinning treatments and understory species introduction

The thinning experiment was installed with uniform site conditions and implemented in a randomized block design with four blocks and four thinning treatments per block, totaling 16 experimental units. The

horizontal distance between blocks is about 54.9 m in the north–south direction and 38.1 m in the east–west direction. In each block, four permanent plots were installed. Each plot had an area of 2500 m^2 (50 m × 50 m) and was randomly assigned into one of four treatment categories: (1) T_0, unthinned control plot, (2) T_1, moderately thinned plot (approximately 30% of the Masson pine basal area removed), (3) T_2, heavily thinned plot (approximately 50% of the Masson pine basal area removed), and (4) T_3, very heavily thinned plot (approximately 70% of the Masson pine basal area removed). The horizontal distance between plots is about 20 m, and the maximum elevation difference between plots is about 40.5 m. In each plot, the inner 400 m^2 of which was the measurement plot (fixed observation plot) and the rest of the area around the 400 m^2 plot being occupied by buffer trees. The thinnings were performed during the dormant seasons of 2007, at the age of 15 years. All thinning was from below and the suppressed trees were removed while also following the principle to achieve as uniform spacing between reserved trees as possible.

Two local tree species, *Erythrophleum fordii Oliv.* and *Quercus griffithii Hook. f. et Thoms ex Miq.*, occurring in the surroundings of the study area were selected and introduced to the understory of the Masson pine plantations. The two species are the main late-successional dominant species of local hardwood forests. *Erythrophleum fordii* is a shade-requiring tree species and *Quercus griffithii* is a medium light-demanding tree species. The hardwood seedlings were cultivated in nurseries and transplanted into the field at 1 year old, in January 2008; about 1662 seedlings per hectare were evenly intercropped (831 per species, planting density approximately 2 m × 3 m) in holes dug manually.

2.3. Monitoring of forest dynamics

A fixed observation plot (basic unit of data organization) of 400 m^2 (20 m × 20 m) for each plot was established to monitor the growth of Masson pine stands, underplanted trees and understory vegetation. In each subplot, three 4 m × 4 m square plots were set (evenly distributed on the diagonal of the fixed observation plot) to monitor shrubs and three 1 m × 1 m square plots were used to monitor herbaceous species (located in the upper right corner of 4 m × 4 m plot and their observation results were integrated into one fixed observation plot respectively). Each seedling (around 66 per observation subplot) was individually tagged. The survivorship, ground diameter, and total height (H) were measured and recorded every two years. The total height and diameter at breast height of all overstory trees in the observation subplot as well as the species, quantity, relative coverage, and average height of shrubs and herbs were inventoried in August 2007 prior to thinning. These plots were inventoried in August every two years from 2008 to 2018. The overstory stand characteristics for each treatment in 2007 and 2018 are shown in Table 1, and the underplanted tree characteristics for each treatment in 2008 and 2018 are shown in Table 2.

2.4. Data statistics and analysis

Height and diameter measurements of Masson pine and underplanted trees were used to compile a relative growth rate (RGR) in basal diameter and height as follows (Hoffmann and Poorter, 2002):

$$RGR = \frac{\ln(y_i) - \ln(y_0)}{t_i - t_0} \quad (1)$$

where y_i is the performance indicator (diameter or tree height) at the last measurement date t_i and y_0 is the same indicator at the first measurement date t_0 (2007 in this study).

Richness index (S), the Shannon (H'), and the Pielou evenness (E) variables were used as indicators of understory species diversity (Magurran, 1988), and they were calculated for shrubs and herbaceous species separately.

$$H' = -\sum_{i=1}^{s} p_i \ln p_i \quad (2)$$

$$E = \frac{H'}{\ln S} \quad (3)$$

$$P_{is} = \left(\frac{n_i}{N} + \frac{c_i}{C} + \frac{h_i}{H}\right)/3 \quad (4)$$

$$P_{ih} = \left(\frac{c_i}{C} + \frac{h_i}{H}\right)/2 \quad (5)$$

where S is the richness index (number of species), P_i represents the relative importance of species i, P_{is} and P_{ih} represents the relative importance of shrubs species *and* herbaceous species respectively, n_i, c_i, and h_i are the average quantity, coverage, and height of individuals of species i, respectively, and N, C, and H are the sum of the number, coverage, and height of all individuals in the community, respectively.

A one-way analysis of variance (ANOVA) and Tukey multiple comparisons were used to separate the significance differences ($P < 0.05$) among treatment means, after the homogeneity of variance and normal distribution tests were passed. The effects of thinning on overstory tree growth, seedling survivorship and growth, and understory vegetation development were analyzed using a generalized additive mixed model (GAMM) from the "gamm4" package in R software (Pinheiro et al., 2019). The GAMM is a semi-parametric model with a linear predictor involving a sum of smooth functions of covariates, which allows flexible functional dependence of an outcome variable on covariates via nonparametric regression while accounting for correlation among observations using random effects (Lin et al., 2010). GAMM is increasingly applied in ecological and environmental researches (Groll and Tutz, 2012) as follows:

$$E_{ijk} = K_0 + f_j(T_{ijk}) + f_j(t_{ijk}) + R_{ijk} + \varepsilon_{ijk} \quad (6)$$

where E_{ijk} is the dependent variable (relative growth rate in diameter or height of Masson pine and underplanted trees, underplanted tree survival rate, indicators of understory species diversity corresponding to the jth measurement of thinning treatment i), K_0 is the overall intercept, $f_j(T_{ijk})$ is a smooth function of thinning treatment (T) corresponding to the jth measurement of plot k, $f_j(t_{ijk})$ is a smooth function of time (t) corresponding to the jth measurement in thinning treatment i of plot k, R_{ijk} is a random effect assumed to be distributed as $N(0, \sigma^2)$ with a variance component σ^2, and ε_{ijk} is an error vector.

Table 1
Overstory stand characteristics for each treatment in 2007 and 2018.

Thinning treatment	Stand density (stems/ha)			Quadratic mean diameter at breast height (cm)			Average total tree height (m)		
	2007b	2007a	2018	2007b	2007a	2018	2007b	2007a	2018
T_0	1425	1425	1353	16.9	16.9	25.6	12.1	12.1	16.2
T_1	1275	800	765	17.8	18.7	30.7	12.5	13.1	18.5
T_2	1350	575	553	17.2	18.6	32.8	12.2	13.0	19.1
T_3	1325	300	285	17.1	19.6	36.1	12.2	13.3	20.1

T_0, unthinned; T_1, moderately thinned; T_2, heavily thinned; T_3, very heavily thinned; b represents before thinning; a represents after thinning for the residual stand.

Table 2
Underplanted trees characteristics for each treatment in 2008 and 2018.

Thinning treatment	Number (stems/ha)				Quadratic mean ground diameter (cm)				Average total tree height (m)			
	2008Q	2018Q	2008E	2018E	2008Q	2018Q	2008E	2018E	2008Q	2018Q	2008E	2018E
T_0	831	438	844	538	0.5	15.6	0.4	7.3	0.6	14.8	0.5	8.8
T_1	825	542	842	583	0.5	17.8	0.4	7.5	0.6	15.9	0.5	9.0
T_2	806	606	844	644	0.5	19.8	0.4	8.1	0.6	17.2	0.5	9.7
T_3	781	544	875	620	0.5	18.7	0.4	7.9	0.6	16.2	0.5	9.4

T_0, unthinned; T_1, moderately thinned; T_2, heavily thinned; T_3, very heavily thinned; Q, *Quercus griffithii*; E, *Erythrophleum fordii*.

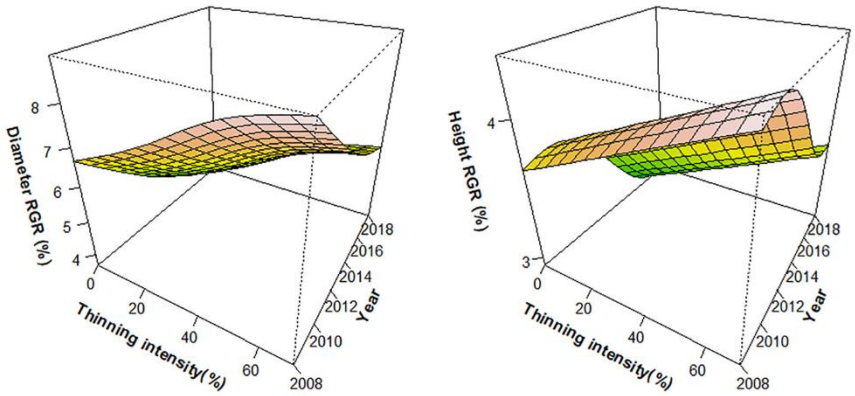


Fig. 1. Observed relative Masson pine growth rate of diameter at breast height and total tree height with thinning and time interactions.

3. Results

3.1. Masson pine stand growth

The results of the generalized additive mixed models (Table S1) show that thinning had a significant positive effect on stem diameter growth and height growth for residual Masson pine trees ($P < 0.001$). Thinning strongly promoted the relative growth rate of stem diameter and total height. A higher thinning intensity resulted in higher relative diameter and height growth rates (Fig. 1). The effect of thinning on diameter growth was greater than on height growth. There were significant differences in relative growth rates of diameter between different treatments ($p < 0.05$), but there was no significant difference in relative growth rates of height between unthinning and moderately thinned treatments ($p > 0.1$) as well as heavily thinning and very heavily thinning treatments ($P > 0.05$). As time progressed post-thinning, the relative growth rates of tree diameter and height decreased gradually; in other words, the effects of thinning on tree height and diameter growth gradually weakened over time.

3.2. Underplanted tree survival and growth

Underplanted tree survival rate was influenced by thinning treatment ($P < 0.001$), with similar performance between the *Erythrophleum fordii* and *Quercus griffithii* (Fig. 2). Significant and strong nonlinearities were observed among seedling survival rate and thinning intensity indicators (Table S1). The survival of the two underplanted tree species increased first and then decreased as thinning intensity increased (Fig. 3). For *Quercus griffithii*, there were significant differences in survival rate between different treatments ($P < 0.05$), and for *Erythrophleum fordii*, there were also significant difference in survival rate between different treatments ($P < 0.05$) except between moderately thinning and very heavily thinning treatments ($p > 0.1$).

Thinning also had a significant effect on diameter growth and height growth in underplanted *Erythrophleum fordii* and *Quercus griffithii* ($P < 0.001$). Significant nonlinearities were observed among the relative growth rate and thinning intensity (Fig. 4). For *Quercus griffithii*, there were significant differences in growth rates of diameter and height between different treatments ($P < 0.01$). While for *Erythrophleum fordii*, there was no significant difference in growth rates of diameter and height except between unthined and heavily thinned treatments ($p < 0.01$).The mean ground diameters and the average height of underplanted *Erythrophleum fordii* and *Quercus griffithii* of different thinning treatments in 2018 were shown in Table 2. The diameter growth and height growth of both underplanted trees seem to improve to the greatest extent under the moderate stand thinning regimen. As time progressed after thinning, the effects of thinning on diameter growth and height growth trends gradually weakened.

3.3. Understory vegetation development

Thinning had a significant effect on the richness index (number of species) for both shrubs and herbs in our observation area (Table S1). As shown in Fig. 5, for shrubs, the number of species observed varied from 2 to 17 in different plots in different years; there were significant differences in richness between different treatments ($P < 0.05$), and richness increased first and then decreased as thinning intensity increased. For herbs, the number of species observed varied from 4 to 12 in different plots in different years and increased with thinning intensity, and there were significant differences in richness between different treatments ($P < 0.01$) except between unthinning and moderately thinning treatments ($p > 0.1$).

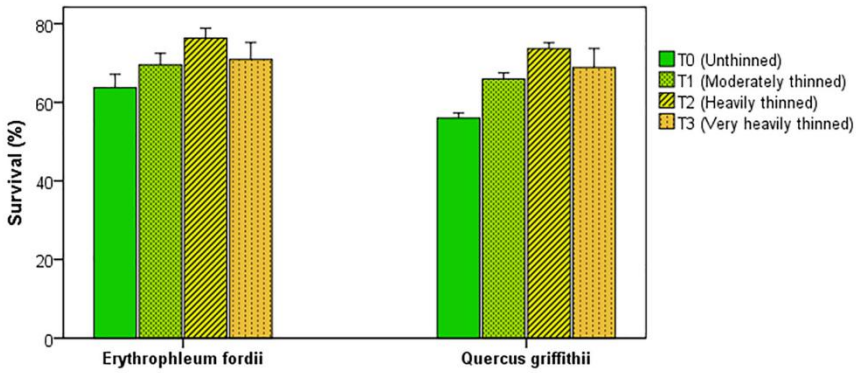


Fig. 2. Seedling survival performance under different thinning treatments.

There was no significant difference in the height of shrubs and herbs between different treatments, but with the increase of stand age, the coverage of shrubs and herbs has increased, and there were significant differences between different treatments ($P < 0.05$). Moreover, the higher the thinning intensity was, the higher the average coverage of shrubs and herbs was (Table S2).

For shrubs, thinning had a significant effect on the Shannon index ($P < 0.001$) but did not affect the Pielou evenness index, and there were significant differences in Shannon index between different treatments ($P < 0.05$). For herbs, thinning also had a significant effect on the Shannon index ($P < 0.05$) but did not affect the Pielou evenness index; there were significant differences in Shannon index between different treatments ($P < 0.05$) except between unthinned and moderately thinned treatments as well as between heavily thinning and very heavily thinning treatments. The richness index of shrubs and herbs increased with thinning intensity (Fig. 6).

4. Discussion

4.1. Thinning effect on overstory Masson pine growth

Many researchers have indicated that thinning immediately stimulates diameter growth in residual trees (Juodvalkis et al., 2005; Ulvcrona et al., 2014; Ferraz et al., 2018). However, the dynamics and relationship between height growth and stand density post-thinning are complex. Some investigators assert that height growth and total height are relatively unaffected by thinning (Fedorová et al., 2016; Swift et al., 2016; Medeiros et al., 2017). However, others have found that thinning exerts an obvious effect on tree height growth (Sharma et al., 2006; Russell et al., 2010; Missanjo and Kamanga-Thole, 2015). Our results show that thinning has positive effects on both Masson pine height and diameter growth. The extent to which thinning influences tree height and tree diameter growth is related to the intensity of the thinning. In this study, we tested moderately and heavily thinning, the results suggest that thinning has a relatively greater effect on diameter growth than tree height growth, which is consistent with certain previous studies (Zhang et al., 1997; Mäkinen and Isomäki, 2004; Rio et al., 2017). In addition, we tested a very heavy thinning, and the result seems to follow the same rule, which is also consistent with certain previous studies (Kerr, 1996). The difference significance in relative growth rates of diameter and height among different treatments also showed that diameter growth was more sensitive to thinning than height growth.

The different magnitude of thinning effect on tree height and tree diameter growth reflected the change of height-diameter allometric relationship. Our previous research showed that the allometric relationship of Masson pine was significantly affected by thinning (Deng et al., 2019). In living systems, biological traits can confer the ability to alter their phenotypes to better respond to environmental change (Botero et al., 2015). Better height growth for a given diameter endows a tree with an advantage when competing for sunlight, while more rapid diameter growth for a given tree height can promote the maintenance of this competitive advantage (Moles et al., 2009). After obtaining a spatial advantage, trees maintain this advantage through

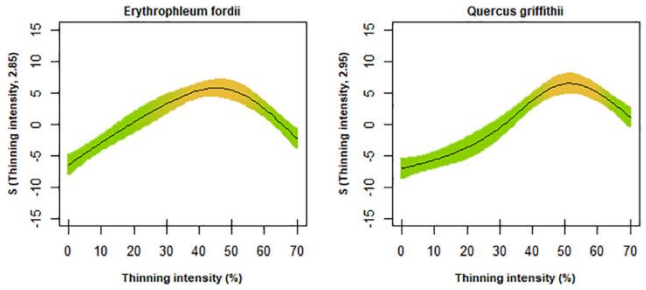


Fig. 3. Estimated degrees of freedom with smooth spline functions on observed underplanted tree survival rate.

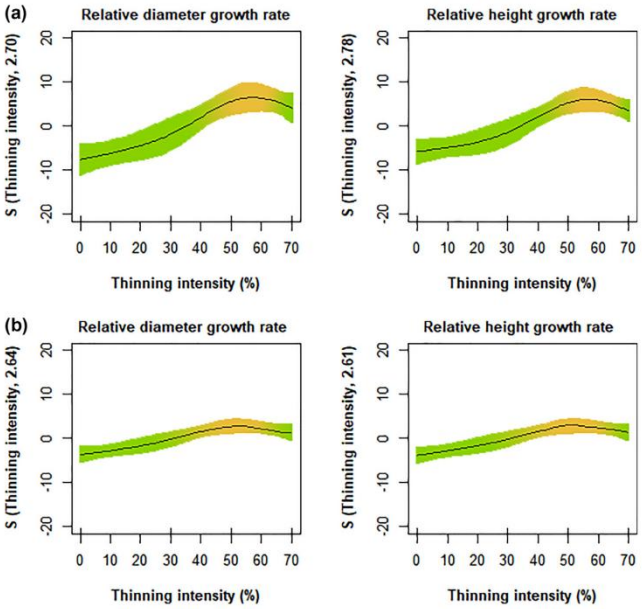


Fig. 4. Estimated degrees of freedom with smooth spline functions on observed relative diameter and height growth rates of seedling. (a) *Erythrophleum fordii*; (b) *Quercus griffithii.*

strengthening their mechanical support (Mcmahon, 1973; Niklas, 1993) and water transport capacity (Bullock, 2000) in order to maximize fitness.

Masson pine is a typical light-demanding species. Silvicultural thinning changes the canopy status of residual trees rapidly, which causes the remaining trees to suddenly have more growing space (Canellas et al., 2004). Consequently, these trees change their original growth pattern and use more growth resources for diameter growth to maintain their spatial advantage until the trees begin the next stage of space competition (Bonser and Aarssen, 1994). The sudden increase in diameter growth of retained trees after thinning supports this explanation.

4.2. Thinning effect on underplanted tree survival and growth

Thinning exerted significant effects on the survival and growth of underplanted trees, so we sought to determine the best combination of management approaches for promoting their growth and survival. Because it is unrealistic to set a series of continuous thinning intensity in the actual experiment (for example, there is not a treatment with

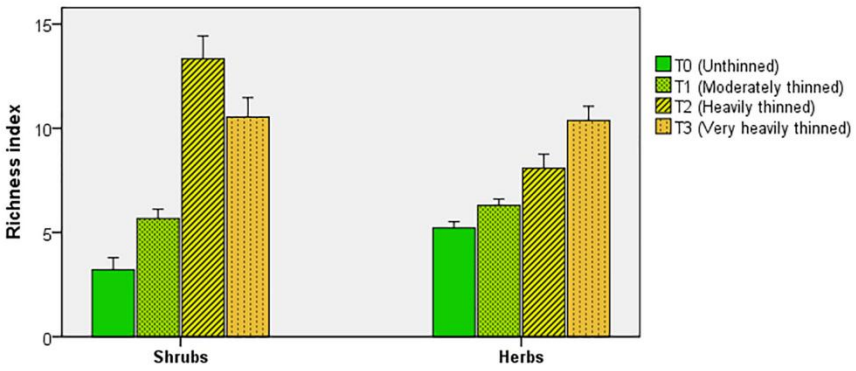


Fig. 5. Richness index for understory vegetation.

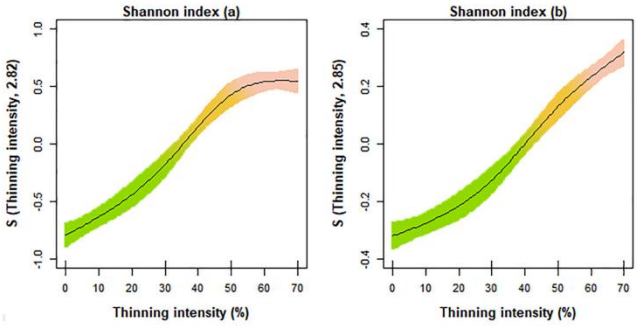


Fig. 6. Estimated degrees of freedom with smooth spline functions on observed species diversity indicators; (a) represents shrubs, (b) represents herbs.

60% of the basal are removed in our experiment), we use the generalized additive mixed model (GAMM) to determine the optimal thinning intensity interpolation interval. The peak survival of *Erythrophleum fordii* and *Quercus griffithii* through age 11 years after planting occurred at thinning intensity of 40–50% and 50–60%, respectively. The relative growth rates of diameter and height seem to have improved to the greatest extent at thinning intensities of 45–55% and 55–65% for *Erythrophleum fordii* and *Quercus griffithii*, respectively. The optimal thinning intensity to promote *Erythrophleum fordii* thus falls between 45% and 50%, at which point both the survival rate and relative diameter and height growth rates are optimal. For *Quercus griffithii*, the optimal thinning intensity falls between 55% and 60%.

The different survival rates response to thinning between species indicated that *Quercus griffithii* is marginally more sensitive than *Erythrophleum fordii*, which may be related to the shade tolerance of the two tree species (Yan et al., 2016). For *Quercus griffithii*, there were significant differences in growth rates of diameter and height among different treatments ($P < 0.01$), but for *Erythrophleum fordii*, there was no significant difference in growth rates of diameter and height except between unthinned and heavy thinning treatments ($p < 0.01$), which indicated that the growth change of *Erythrophleum fordii* was less sensitive to thinning than that of *Quercus griffithii*, which may be because *Quercus griffithii* is a fast growing species and *Erythrophleum fordii* is a slow growing species.

Residual dominant conifers compete with underplanted trees for resources, thereby reducing underplanted tree survival (Brandeis et al., 2001). Our results showed that the survival rate of underplanted trees increased as moderate thinning intensity increased. These surviving underplanted trees grew more effectively in diameter and tree height with increase in thinning intensity due to greater resource availability (Knapp et al., 2016). In unthinned and moderately thinned stands, an abundance of overstory Masson pine trees induced a decrease in seedling survival and relative diameter and height growth rates. The dense overstory canopy was well-established at the time of planting, and would have reduced light penetration and increased rain interception (Sampson and Smith, 1993; Fleischbein et al., 2005; Frank et al., 2018), which reduced available light and water sources in the understory and is likely the main cause of poor performance in the underplanted trees.

The survival rate, relative diameter growth rate, and relative height growth rate of the underplanted trees were also likely to decrease in very heavily thinned stands. The study area is hot in summer and sees little rain in the autumn, so it is possible that higher evaporative demand, temperatures, and light levels increased drought stress and directly damaged understory seedling tissues and the photosynthetic machinery (Chaves et al., 2002; Valladares et al., 2004; Sevillano et al., 2016), despite a reduction in competitive pressure from overstory trees, thereby overriding the benefits of further thinning (Gavinet et al., 2015).

Species selection is crucial when conducting an underplanting regimen in shelterwood systems (Löf et al., 2007). We found that the optimal thinning intensity (interpolation interval) of *Erythrophleum fordii* was slightly lower than that of *Quercus griffithii*, which may be related to the shade tolerance of the tree species (Mason et al., 2004; Lu et al., 2018): *Erythrophleum fordii* is shade-requiring while *Quercus griffithii* is medium-light-demanding. These observations suggest that the light demand of target tree species should be taken into account when designing a thinning strategy in the conversion of pure into mixed plantations.

It could be argued that the overall survival and growth of underplanted trees reported in many studies mostly occurred in the early years of establishment, and a majority of studies show that survival and growth improve as stand density decreases to an intermediate level because it can development the optimal microclimate, light and competition conditions (Bardon et al., 1999; Barg and Edmonds, 1999; Agestam et al., 2003). In the early stage, our result appears to be consistent with these studies. The study that in large time span was few reported under different overstory levels (Paquette et al., 2006). In this study, the planted trees of *Quercus griffithii* are reaching pine height in 2018 (in general < 2 m lower), which mean that they are highly competing for light and space with overstory Masson pine, but their survival and growth still maintain the early trend; that is, the maximum improvement can be achieved under heavy thinning, which may be due to the relatively stable survival rate of the underplanted trees after they reach a certain size, while the competition between overstory and underplanted trees has not been fully reflected. More long-term research needs to be done to identify optimal residual density for the balanced growth between overstory residual and underplanted trees.

4.3. Thinning effect on understory vegetation development

Many studies have shown that thinning influences the community structure of understory vegetation and can be an effective way to increase its diversity (Paquette et al., 2006; Ishii et al., 2008; Ares et al., 2009; Gavinet et al., 2015; Trentini et al., 2017). Our results suggest that thinning fosters the development of understory shrubs and herbaceous species, which increases stand complexity and could potentially enhance biodiversity.

Thinning increased the canopy openness and light transmittance in the short term in our observation area, which can have large effects on understory plant diversity and community composition (Ishii et al., 2008; Trentini et al., 2017). However, such effects are not a simple function of canopy cover or estimated light availability (Thomas et al.,

1999). We found that understory shrubs species richness and diversity are strongly nonlinearly correlated with thinning intensity, and to be more precise, are higher at about 50% to 60% of the thinning intensity. This indicates that too low or too high thinning intensity will fail to produce an optimal microenvironment for shrub recruitment (Trentini et al., 2017). We also found that understory herbaceous species richness and diversity were nearly linearly positive-correlated with thinning intensity..

The effect of thinning on shrubs was mainly reflected in species number and coverage rather than height, which may be because most shrubs are slow-growing species and herbs produce new growth annually. The difference significance of Shannon index among different treatments showed that the development of shrubs was more sensitive to thinning than that of herbs.

4.4. Comprehensive effect of thinning on forest evolution

Thinning has significant effects on the growth of the residual overstory trees, the survival and growth of underplanted trees, and the development of understory vegetation, thus accelerating forest succession. However, these effects are not independent. Residual overstory trees compete with underplanted trees for resources, thereby affecting underplanted tree survival and growth (Brandeis et al., 2001; Caldeira et al., 2014). Understory vegetation also appears to exert a distinct influence on seedling establishment depending on morphology, growth rates, and the capability to consume resources (Balandier et al., 2005). Herbaceous species are highly competitive with seedlings as well (Cuesta et al., 2010; Caldeira et al., 2014). However, our results show that the increase of herbage species and coverage can promote the survival and growth of underplanted trees, which may be due to the limited competition of herbage for seedlings when we transplant large seedlings. On the contrary, the increase of coverage will reduce the evaporation of water, thus forming an environment conducive to the growth of underplanted trees. Shrubs may have positive effects on seedlings by mitigating any increase in extreme temperatures and creating a lateral shadow which favors height growth (Prévosto et al., 2011; Valladares et al., 2016). Our analysis showed that the increase of species diversity and shrub coverage was beneficial to the survival and growth of underplanted trees. The outcome of plant-plant interactions can vary widely with various target species characteristics and strategies (Liancourt et al., 2005; Gavinet et al., 2015; Zhang and Tielbörger, 2019), it may need to develop a better understanding of the mechanism of plant-plant interaction.

The optimal forest succession is a result of comprehensive balance in overstory trees, underplanted trees, and understory vegetation. For residual overstory trees and herbaceous species, a higher thinning intensity favors growth and development; excessive thinning, however, leads to a decline in underplanted trees, shrub richness, and diversity. The thinning intensity which produces the optimal combination of survival and growth for underplanted trees is also roughly the thinning intensity with the highest shrub richness and diversity. To this effect, the optimal thinning intensity can be determined according to seedling survival and growth performance. The underplanted trees of the two species we observed in this study responded to thinning in the same direction but with varying magnitude, likely reflecting different shade tolerances and competitive abilities (Gavinet et al., 2015).

5. Conclusion

Planting native broad-leaved hardwood species under Masson pine pure plantations is a feasible approach to transforming pure plantations into mixed plantations, but requires the precise and proper management of overstory stands. Thinning is a necessary measure to accelerate forest succession in the conversion process; it can improve residual overstory tree growth, enhance seedling survival and growth, and promote understory vegetation development in dense Masson pine stands. However, excessive thinning can drive down seedling survival and vegetation (mainly shrubs) recruitment.

Our results suggest that heavily thinning intensities of Masson pine, about 50% to 60%, yields suitable conditions for enhancing the growth and later survival of newly introduced underplanted trees while improving the growth of residual overstory trees and promoting understory vegetation development. The survival and growth of underplanted trees appears to be the primary basis for determining the optimal thinning intensity, and depends on the specific tree species being targeted; light-demanding tree species require higher overstory thinning intensity than shade-tolerant species. Biological characteristics such as the light demand of the tree species at hand must be properly accounted for when designing an effective overstory thinning strategy.

CRediT authorship contribution statement

Cheng Deng: Conceptualization, Methodology, Investigation, Writing - original draft. **Shougong Zhang:** Conceptualization, Supervision. **Yuanchang Lu:** Conceptualization, Funding acquisition. **Robert E. Froese:** Writing - review & editing. **Xiaojun Xu:** Validation, Writing - review & editing. **Ji Zeng:** Investigation, Funding acquisition. **Angang Ming:** Investigation, Project administration. **Xianzhao Liu:** Investigation, Validation. **Yangsheng Xie:** Investigation, Data curation. **Qingfen Li:** Software, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by the Central Public-Interest Scientific Institution Basal Research Fund for Chinese Academy of Forestry China (No. IFRIT201501) and the National Natural Science Foundation of China (31971655). We also appreciate the staff of the Experimental Center of Tropical Forestry for the help during the field survey and the editors and anonymous reviewers for their constructive comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118503.

References

Agestam, E., Eko, P., Nilsson, U., Welander, N.T., 2003. The effects of shelterwood density and site preparation on natural regeneration of Fagus sylvatica in southern Sweden. For. Ecol. Manage. 176 (1), 61–73.
Ammer, C., Bickel, E., Christian, K., 2008. Converting norway spruce stands with beech - a review of arguments and techniques. Austrian J. For. Sci. 125 (1), 3–26.
Ares, A., Berryman, S.D., Puettmann, K.J., 2009. Understory vegetation response to thinning disturbance of varying complexity in coniferous stands. Appl. Veg. Sci. 12 (4), 472–487.
Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of european temperate forests on soil fertility. Annals of forest science 59 (3), 233–253.
Aussenac, G., 2000. Interactions between forest stands and microclimate: ecophysiological aspects and consequences for silviculture. Annals of Forest Science 57 (3), 287–301.
Bai, S.H., Dempsey, R., Reverchon, F., Blumfield, T.J., Ryan, S., Cernusak, L.A., 2017. Effects of forest thinning on soil-plant carbon and nitrogen dynamics. Plant Soil 411 (1–2), 437–449.
Balandier, P., Collet, C., Miller, J.H., Reynolds, P.E., Zedaker, S.M., 2005. Designing forest vegetation management strategies based on the mechanisms and dynamics of crop tree competition by neighbouring vegetation. Forestry 79 (1), 3–27.
Bardat, J., Aubert, M., 2007. Impact of forest management on the diversity of corticolous bryophyte assemblages in temperate forests. Biol. Conserv. 139 (1–2), 47–66.
Bardon, R.E., Countryman, D.W., Hall, R.B., 1999. Tree Shelters Reduced Growth and Survival of Underplanted Red Oak Seedlings in Southern Iowa. North. J. Appl. For. 16

(2), 103–107.
Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29 (6), 705–713.
Bauhus, J., Forrester, D.I., Pretzsch, H., 2017. From observations to evidence about effects of mixed-species stands. In: Pretzsch, H., Forrester, D.I., Bauhus, J. (Eds.), Mixedspecies Forests - Ecology and Management. Springer, Berlin, Germany, pp. 27–71.
Bonser, S.P., Aarssen, L.W., 1994. Plastic allometry in young sugar maple (Acer saccharum): adaptive responses to light availability. Am. J. Bot. 81 (4), 400–406.
Botero, C.A., Weissing, F.J., Wright, J., Rubenstein, D.R., 2015. Evolutionary tipping points in the capacity to adapt to environmental change. PNAS 112 (1), 184–189.
Bradford, J.B., Palik, B.J., 2009. A comparison of thinning methods in red pine: consequences for stand-level growth and tree diameter. Can. J. For. Res. 39 (3), 489–496.
Brandeis, T.J., Newton, M., Cole, E.C., 2001. Underplanted conifer seedling survival and growth in thinned Douglas-fir stands. Can. J. For. Res. 31 (2), 302–312.
Bravo-Oviedo, A., 2018. The role of mixed forests in a changing social-ecological world. In: Dynamics, Silviculture and Management of Mixed Forests. Springer, Cham, pp. 1–25.
Bullock, S.H., 2000. Developmental Patterns of Tree Dimensions in a Neotropical Deciduous Forest1. Biotropica 32 (1), 42–52.
Caldeira, M.C., Ibáñez, I., Nogueira, C., Bugalho, M.N., Lecomte, X., Moreira, A., Pereira, J.S., 2014. Direct and indirect effects of tree canopy facilitation in the recruitment of M editerranean oaks. J. Appl. Ecol. 51 (2), 349–358.
Cañellas, I., Rio, M.D., Roig, S., Montero, G., 2004. Growth response to thinning in Quercus pyrenaica Willd. coppice stands in Spanish central mountain. Annals of Forest Science 61 (3), 243–250.
Cavard, X., Macdonald, S.E., Bergeron, Y., Chen, H.Y., 2011. Importance of mixedwoods for biodiversity conservation: evidence for understory plants, songbirds, soil fauna, and ectomycorrhizae in northern forests. Environmental Reviews 19 (NA), 142–161.
Chauvat, M., Titsch, D., Zaytsev, A.S., Wolters, V., 2011. Changes in soil faunal assemblages during conversion from pure to mixed forest stands. For. Ecol. Manage. 262 (3), 317–324.
Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field? Photosynthesis and growth. Annals of botany 89 (7), 907–916.
Chirino, E., Bonet, A., Bellot, J., Sánchez, J.R., 2006. Effects of 30-year-old Aleppo pine plantations on runoff, soil erosion, and plant diversity in a semi-arid landscape in south eastern Spain. Catena 65 (1), 19–29.
Coll, L., Ameztegui, A., Collet, C., Löf, M., Mason, B., Pach, M., et al., 2018. Knowledge gaps about mixed forests: What do European forest managers want to know and what answers can science provide? For. Ecol. Manage. 407, 106–115.
Crouzeilles, R., Ferreira, M.S., Chazdon, R.L., Lindenmayer, D.B., Sansevero, J.B., Monteiro, L., et al., 2017. Ecological restoration success is higher for natural regeneration than for active restoration in tropical forests. Sci. Adv. 3, e1701345.
Cuesta, B., Villar-Salvador, P., Puértolas, J., Benayas, J.M.R., Michalet, R., 2010. Facilitation of Quercus ilex in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. J. Ecol. 98 (3), 687–696.
Cusack, D., Montagnini, F., 2004. The role of native species plantations in recovery of understory woody diversity in degraded pasturelands of Costa Rica. For. Ecol. Manage. 188 (1–3), 1–15.
David, H.C., Arce, J.E., Netto, S.P., Corte, A.P.D., Marinheski Filho, A., Araújo, E.J.G.D., 2016. Thinning and Site Quality Effects on the Height-diameter Relationship of Pinus taeda. Floresta e Ambiente 23 (2), 220–228.
Deng, C., Zhang, S., Lu, Y., Froese, R.E., Ming, A., Li, Q., 2019. Thinning effects on the tree height–diameter allometry of Masson pine (*Pinus massoniana* Lamb.). Forests 10 (12), 1129.
Erskine, P.D., Lamb, D., Bristow, M., 2006. Tree species diversity and ecosystem function: can tropical multi-species plantations generate greater productivity? For. Ecol. Manage. 233 (2–3), 205–210.
Evans, J., 1999. Planted forests of the wet and dry tropics: their variety, nature, and significance. New Forest. 17 (1–3), 25–36.
Evans, J., Turnbull, J., 2004. Plantation Forestry in the Tropics, 3rd ed. Oxford University Press, Oxford.
FAO, 2001. Global Forest Resources Assessment 2000. Food and Agriculture Organization of the United Nations, Rome.
Farjon, A., 2010. A Handbook of the World's Conifers: Leiden. Brill Academic Publishers, Netherlands, pp. 1112.
Fedorová, B., Kadavý, J., Adamec, Z., Kneifl, M., Knott, R., 2016. Response of diameter and height increment to thinning in oak-hornbeam coppice in the southeastern part of the Czech Republic. Journal of Forest Science 62, 229–235.
Ferraz, Filho, A.C., Mola-Yudego, B., Gonzalez-Olabarria, J.R., Scolforo, J.R.S., 2018. Thinning regimes and initial spacing for Eucalyptus plantations in Brazil. An Acad Bras Cienc, 90, 255-265.
Fleischbein, K., Wilcke, W., Goller, R., Boy, J., Valarezo, C., Zech, W., Knoblich, K., 2005. Rainfall interception in a lower montane forest in Ecuador: effects of canopy properties. Hydrol. Process. 19 (7), 1355–1371.
Frank, G., Rathfon, R., Saunders, M., 2018. Ten-Year Responses of Underplanted Northern Red Oak to Silvicultural Treatments, Herbivore Exclusion, and Fertilization. Forests 9 (9), 571.
Gavinet, J., Vilagrosa, A., Chirino, E., Granados, M.E., Vallejo, V.R., Prévosto, B., 2015. Hardwood seedling establishment below Aleppo pine depends on thinning intensity in two Mediterranean sites. Annals of forest science. 72 (8), 999–1008.
Ghorbani, M., Sohrabi, H., Sadati, S.E., Babaei, F., 2018. Productivity and dynamics of pure and mixed-species plantations of Populous deltoids Bartr. ex Marsh and Alnus subcordata CA Mey. For. Ecol. Manage. 409, 890–898.
Groll, A., Tutz, G., 2012. Regularization for generalized additive mixed models by likelihood-based boosting. Methods Inf. Med. 51 (02), 168–177.
Haynes, K.J., Allstadt, A.J., Klimetzek, D., 2014. Forest defoliator outbreaks under climate change: effects on the frequency and severity of outbreaks of five pine insect pests. Glob. Change Biol. 20 (6), 2004–2018.
Heine, P., Hausen, J., Ottermanns, R., Schäffer, A., Roß-Nickoll, M., 2019. Forest conversion from norway spruce to european beech increases species richness and functional structure of aboveground macrofungal communities. For. Ecol. Manage. 432, 522–533.
Heinrichs, S., Gossner, M., Schall, P., 2018. European beech controls biodiversity in mixed forests–Mixed versus pure forests of beech and conifers. In ECCB2018: 5th European Congress of Conservation Biology. 12th-15th of June 2018, Jyväskylä, Finland. Open Science Centre, University of Jyväskylä.
Hoffmann, W.A., Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. Ann. Bot. 90 (1), 37–42.
Hood, S.M., Baker, S., Sala, A., 2016. Fortifying the forest: thinning and burning increase resistance to a bark beetle outbreak and promote forest resilience. Ecol. Appl. 26 (7), 1984–2000.
Ishii, H.T., Maleque, M.A., Taniguchi, S., 2008. Line thinning promotes stand growth and understory diversity in Japanese cedar (Cryptomeria japonica D. Don) plantations. Journal of forest research 13 (1), 73–78.
Jactel, H., Brockerhoff, E.G., 2007. Tree diversity reduces herbivory by forest insects. Ecol. Lett. 10 (9), 835–848.
Jactel, H., Nicoll, B.C., Branco, M., Gonzalez-Olabarria, J.R., Grodzki, W., Långström, B., Moreira, F., Netherer, S., Orazio, C., Piou, D., Santos, H., Schelhaas, M.J., Tojic, K., Vodde, F., 2009. The influences of forest stand management on biotic and abiotic risks of damage. Annals of forest science 66 (7) 701-701.
Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., Gardiner, B., Gonzalez-Olabarria, J.R., Koricheva, J., Meurisse, N., Brockerhoff, E.G., 2017. Tree diversity drives forest stand resistance to natural disturbances. Current Forestry Reports 3, 223–243.
Juodvalkis, A., Kairiukstis, L., Vasiliauskas, R., 2005. Effects of thinning on growth of six tree species in north-temperate forests of Lithuania. Eur. J. Forest Res. 124 (3), 187–192.
Keenan, R.J., Lamb, D., Parrotta, J., Kikkawa, J., 1999. Ecosystem management in tropical timber plantations: satisfying economic, conservation, and social objectives. J. Sustainable For. 9 (1–2), 117–134.
Kenk, G., Guehne, S., 2001. Management of transformation in central Europe. For. Ecol. Manage. 151 (1–3), 107–119.
Kerr, G., 1996. The effect of heavy or 'free growth' thinning on oak (Quercus petraea and Q. robur). Forestry 69 (4), 303–317.
Keskitalo, E.C.H., 2011. How can forest management adapt to climate change? Possibilities in different forestry systems. Forests 2 (1), 415–430.
Knapp, B.O., Wang, G.G., Walker, J.L., Hu, H., 2016. Using silvicultural practices to regulate competition, resource availability, and growing conditions for Pinus palustris seedlings underplanted in Pinus taeda forests. Can. J. For. Res. 46 (7), 902–913.
Koutika, L.S., Epron, D., Bouillet, J.P., Mareschal, L., 2014. Changes in N and C concentrations, soil acidity and P availability in tropical mixed acacia and eucalypt plantations on a nutrient-poor sandy soil. Plant Soil 379, 205–216.
Lamb, D., Erskine, P.D., Parrotta, J.A., 2005. Restoration of degraded tropical forest landscapes. Science 310 (5754), 1628–1632.
Liancourt, P., Callaway, R.M., Michalet, R., 2005. Stress tolerance and competitive-response ability determine the outcome of biotic interactions. Ecology 86 (6), 1611–1618.
Lin, X., Zhang, D., Fryzlewicz, P., Keilegom, I.V., 2010. Inference in generalized additive mixed models by using smoothing splines. J. Roy. Stat. Soc. 61 (2), 381–400.
Lindroth, A., Holst, J., Heliasz, M., Vestin, P., Lagergren, F., Biermann, T., Cai, Z., Mölder, M., 2018. Effects of low thinning on carbon dioxide fluxes in a mixed hemiboreal forest. Agric. For. Meteorol. 262, 59–70.
Liu, C.L.C., Kuchma, O., Krutovsky, K.V., 2018. Mixed-species versus monocultures in plantation forestry: Development, benefits, ecosystem services and perspectives for the future. Global Ecol. Conserv. 15, e00419.
Löf, M., Karlsson, M., Sonesson, K., Welander, T.N., Collet, C., 2007. Growth and mortality in underplanted tree seedlings in response to variations in canopy closure of Norway spruce stands. Forestry 80 (4), 371–383.
Löf, M., Bergquist, J., Brunet, J., Karlsson, M., Welander, T., 2010. Conversion of norway spruce to broadleaves-regeneration systems, fencing and performance of planted seedlings. Ecological Bulletins 53, 165–173.
Lu, D., Wang, G.G., Zhang, J., Fang, Y., Zhu, C., Zhu, J., 2018. Converting larch plantations to mixed stands: Effects of canopy treatment on the survival and growth of planted seedlings with contrasting shade tolerance. For. Ecol. Manage. 409, 19–28.
Magurran, A.E., 1988. Ecological Diversity and Its Measurements. Princeton University Press, Princeton, NJ.
Mäkinen, H., Isomäki, A., 2004. Thinning intensity and growth of Norway spruce stands in Finland. Forestry 77 (4), 349–364.
Malkamäki, A., D'Amato, D., Hogarth, N., Kanninen, M., Pirard, R., Toppinen, A., Zhou, W., 2017. The socioeconomic impacts of large-scale tree plantations on local communities. A systematic review protocol, Center for International Forestry Research (CIFOR), Bogor, Indonesia.
Mason, W.L., Edwards, C., Hale, S.E., 2004. Survival and early seedling growth of conifers with different shade tolerance in a Sitka spruce spacing trial and relationship to understorey light climate. Silva Fennica 38 (4), 357–370.
Mason, W.L., Löf, M., Pach, M., Spathelf, P., 2018. The development of silvicultural guidelines for creating mixed forests. In: Dynamics, Silviculture and Management of Mixed Forests. Springer, Cham, pp. 255–270.
Mcmahon, T.A., 1973. Size and Shape in Biology. Science 179 (4079), 1201–1204.

Medeiros, R.A., Paiva, H.N.D., Soares, A.A.V., Cruz, J.D., Leite, H.G., 2017. Thinning from below: effects on height of dominant trees and diameter distribution in Eucalyptus stands. J. Trop. For. Sci. 29 (2), 238–247.
Miller, M., Emmingham, B., 2001. Can selection thinning convert even-age Douglas-fir stands to uneven-age structures? West. J. Appl. For. 16 (1), 35–43.
Missanjo, E., Kamanga-Thole, G., 2015. Effect of first thinning and pruning on the individual growth of pinus patulatree species. J. For. Res. 26 (4), 827–831.
Moles, A.T., Warton, D.I., Warman, L., Swenson, N.G., Laffan, S.W., Zanne, A.E., et al., 2009. Global patterns in plant height. J. Ecol. 97 (5), 923–932.
Montagnini, F., 2000. Accumulation in above-ground biomass and soil storage of mineral nutrients in pure and mixed plantations in a humid tropical lowland. For. Ecol. Manage. 134, 257–270.
Montagnini, F., González, E., Rheingans, R., Porras, C., 1995. Mixed and pure forest plantations in the humid neotropics: a comparison of early growth, pest damage and establishment costs. The Commonwealth Forestry Review 74 (4), 306–314.
Navarro-Cerrillo, R.M., Sánchez-Salguero, R., Rodriguez, C., Lazo, J.D., Moreno-Rojas, J.M., Palacios-Rodriguez, G., Camarero, J.J., 2019. Is thinning an alternative when trees could die in response to drought? The case of planted Pinus nigra and P. Sylvestris stands in southern Spain. For. Ecol. Manage. 433, 313–324.
Niklas, K.J., 1993. The Scaling of Plant Height: A Comparison Among Major Plant Clades and Anatomical Grades. Ann. Bot. 72 (2), 165–172.
Nishizono, T., 2010. Effects of thinning level and site productivity on age-related changes in stand volume growth can be explained by a single rescaled growth curve. For. Ecol. Manage. 259 (12), 2276–2291.
Padilla, F.M., Ortega, R., Sánchez, J., Pugnaire, F.I., 2009. Rethinking species selection for restoration of arid shrublands. Basic Appl. Ecol. 10 (7), 640–647.
Paquette, A., Bouchard, A., Cogliastro, A., 2006. Survival and growth of under-planted trees: a meta-analysis across four biomes. Ecol. Appl. 16 (4), 1575–1589.
Pausas, J.G., Bladé, C., Valdecantos, A., Seva, J.P., Fuentes, D., Alloza, J.A., Vilagrosa, A., Bautista, S., Cortina, J., Vallejo, R., 2004. Pines and oaks in the restoration of Mediterranean landscapes of Spain: new perspectives for an old practice—a review. Plant Ecol. 171 (1–2), 209–220.
Payn, T., Carnus, J.M., Freer-Smith, P., Kimberley, M., Kollert, W., Liu, S.R., Orazio, C., Rodriguez, L., Silva, L.N., Wingfield, M.J., 2015. Changes in planted forests and future global implications. For. Ecol. Manage. 352, 57–67.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team., 2019. nlme: Linear and Nonlinear Mixed Effects Models; R Package Version 3.1-142; Available online: https://CRAN.R-project.org/package=nlme (accessed on 20 November, 2019).
Pretzsch, H., Bielak, K., Block, J., Bruchwald, A., Dieler, J., Ehrhart, H.P., Kohnle, U., Nagel, J., Spellmann, H., Zasada, M., Zingg, A., 2013. Productivity of mixed versus pure stands of oak (Quercus petraea (Matt.) Liebl. and Quercus robur L.) and European beech (Fagus sylvatica L.) along an ecological gradient. Eur. J. Forest Res. 132, 263–280.
Prévosto, B., Monnier, Y., Ripert, C., Fernandez, C., 2011. Diversification of Pinus halepensis forests by sowing Quercus ilex and Quercus pubescens acorns: testing the effects of different vegetation and soil treatments. Eur. J. Forest Res. 130 (1), 67–76.
Rio, M.D., Bravooviedo, A., Pretzsch, H., Löf, M., Ruizpeinado, R., 2017. A review of thinning effects on Scots pine stands: From growth and yield to new challenges under global change. Forest Systems 26 (2), eR03S.
Russell, M.B., Amateis, R.L., Burkhart, H.E., 2010. Implementing regional locale and thinning response in the loblolly pine height-diameter relationship. South. J. Appl. For. 34 (1), 21–27.
Salamon, J.A., Scheu, S., Schaefer, M., 2008. The Collembola community of pure and mixed stands of beech (Fagus sylvatica) and spruce (Picea abies) of different age. Pedobiologia 51 (5–6), 385–396.
Sampson, D.A., Smith, F.W., 1993. Influence of canopy architecture on light penetration in lodgepole pine (Pinus contorta var. latifolia) forests. Agric. For. Meteorol. 64 (1–2), 63–79.
Schelhaas, M.J., Hengeveld, G., Moriondo, M., Reinds, G.J., Kundzewicz, Z.W., Ter Maat, H., Bindi, M., 2010. Assessing risk and adaptation options to fires and windstorms in European forestry. Mitig. Adapt. Strat. Glob. Change 15 (7), 681–701.
Sevillano, I., Short, I., Grant, J., O'Reilly, C., 2016. Effects of light availability on morphology, growth and biomass allocation of Fagus sylvatica and Quercus robur seedlings. For. Ecol. Manage. 374, 11–19.
Sharma, M., Smith, M., Burkhart, H.E., Amateis, R.L., 2006. Modeling the impact of thinning on height development of dominant and codominant loblolly pine trees. Annals of forest science 63 (4), 349–354.
Sohn, J.A., Saha, S., Bauhus, J., 2016. Potential of forest thinning to mitigate drought stress: A meta-analysis. For. Ecol. Manage. 380, 261–273.
Spake, R., Yanou, S., Yamaura, Y., Kawamura, K., Kitayama, K., Doncaster, C.P., 2019. Meta-analysis of management effects on biodiversity in plantation and secondary forests of Japan. Conservation Science and Practice 1 (3), e14.
Spiecker, H., Hansen, J., Klimo, E., Skovsgaard, J.P., Sterba, H., Teuffel, K., 2004. Norway Spruce Conversion-Options and Consequences. EFI Research Report 18, Brill Academic Publishers, Leiden, Boston. PP, 121-164.
Stanturf, J.A., Palik, B.J., Dumroese, R.K., 2014. Contemporary forest restoration: A review emphasizing function. For. Ecol. Manage. 331, 292–323.
Swift, D.E., Knight, W., Béland, M., Boureima, I., Bourque, C.P.A., Meng, F.R., 2016. Stand dynamics and tree quality response to precommercial thinning in a northern hardwood forest of the Acadian forest region: 23 years of intermediate results. Scand. J. For. Res. 32, 45–59.
Swinfield, T., Afriandi, R., Antoni, F., Harrison, R.D., 2016. Accelerating tropical forest restoration through the selective removal of pioneer species. For. Ecol. Manage. 381, 209–216.
Thomas, S.C., Halpern, C.B., Falk, D.A., Liguori, D.A., Austin, K.A., 1999. Plant diversity in managed forests: understory responses to thinning and fertilization. Ecol. Appl. 9 (3), 864–879.
Trentini, C.P., Campanello, P.I., Villagra, M., Ritter, L., Ares, A., Goldstein, G., 2017. Thinning of loblolly pine plantations in subtropical Argentina: Impact on microclimate and understory vegetation. For. Ecol. Manage. 384, 236–247.
Ulvcrona, K.A., Karlsson, K., Ulvcrona, T., 2014. Identifying the biological effects of precommercial thinning on diameter growth in young Scots pine stands. Scand. J. For. Res. 29, 427–435.
Valladares, F., Dobarro, I., Sánchez-Gomez, D., Pearcy, R.W., 2004. Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. J. Exp. Bot. 56 (411), 483–494.
Valladares, F., Laanisto, L., Niinemets, Ü., Zavala, M.A., 2016. Shedding light on shade: ecological perspectives of understorey plant life. Plant Ecolog. Divers. 9 (3), 237–251.
Vallejo, V.R., Smanis, A., Chirino, E., Fuentes, D., Valdecantos, A., Vilagrosa, A., 2012. Perspectives in dryland restoration: approaches for climate change adaptation. New Forest. 43 (5–6), 561–579.
Venäläinen, A., Lehtonen, I., Laapas, M., Ruosteenoja, K., Tikkanen, O., Viiri, H., Ikonen, V., Peltola, H., 2020. Climate change induces multiple risks to boreal foress and forestry in Finland: A literature review. Global change biology, 00, 1-19 (online).
Wormald, T.J., 1992. Mixed and pure forest plantations in the tropics and subtropics. FAO Forestry Paper 103, Rome.
Xie, H., Fawcett, J.E., Wang, G.G., 2020. Fuel dynamics and its implication to fire behavior in loblolly pine-dominated stands after southern pine beetle outbreak. Forest ecology and management, 466,118130 (online).
Wang, X., Song, Y., Wang, L., 2001. Study on Restoration of Evergreen Broad-leaved Forest from Pinus massoniana Forest. Chinese Journal of Ecology 20 (1), 30–32.
Yan, Q., Gang, Q., Zhu, J., Sun, Y., 2016. Variation in survival and growth strategies for seedlings of broadleaved tree species in response to thinning of larch plantations: implication for converting pure larch plantations into larch-broadleaved mixed forests. Environ. Exp. Bot. 129, 108–117.
Zhang, H., Zhou, G., Wang, Y., Bai, S., Sun, Z., Berninger, F., Bai, Y., Penttinen, P., 2019. Thinning and species mixing in Chinese fir monocultures improve carbon sequestration in subtropical China. Eur. J. Forest Res. 138 (3), 433–443.
Zhang, R., Tielbörger, K., 2019. Facilitation from an intraspecific perspective-stress tolerance determines facilitative effect and response in plants. New Phytol. 221 (4), 2203–2212.
Zhang, S., Burkhart, H.E., Amateis, R.L., 1997. The influence of thinning on tree height and diameter relationships in loblolly pine plantations. South. J. Appl. For. 21 (4), 199–205.

3.2 Development of improved and comprehensive growth and yield models for genetically improved stands

Annals of Forest Science (2020) 77:89
https://doi.org/10.1007/s13595-020-00995-5

REVIEW PAPER

Development of improved and comprehensive growth and yield models for genetically improved stands

Cheng Deng[1] · Robert E. Froese[2] · Shougong Zhang[3] · Yuanchang Lu[4] · Xiaojun Xu[1] · Qingfen Li[1]

Received: 5 February 2020 / Accepted: 27 August 2020

Abstract

• ***Key message*** **This synthesis of the literature on incorporation of genetic gain into growth and yield models reveals a fundamental challenge associated with the rapid progress in genetics and breeding and limited empirical data on improved stands. Model improvements depend on a better understanding of both the biological basis for gain and of interactions between genetic and non-genetic factors on gain.**

• ***Context*** Continued development of new genetic varieties of trees requires accurate stand growth and yield models to predict growth trajectories and genetic gain of the new varieties using early-age growth data.

• ***Aims*** To identify how the effects of genetic variety on growth and yield models could be analyzed and genetic information could be incorporated into these models for accurate growth simulation and improved yield prediction of genetically improved stands.

• ***Results*** Genetic variety may affect one or several of the asymptotic parameters, shape parameters, and rate parameters of growth and yield models, which can be assessed by testing the parameter differences of the models. After determination of the influence of genetic varieties on model parameters and considering the existing general stand growth equation, the genetic gain can be incorporated into growth and yield models by calculation of genetic gain multipliers, adjustment of the site index, and calibration of the new model parameters.

• ***Conclusion*** Accurate and effective growth and yield models for genetically improved stands require a better understanding of the effects of genetics, environment, and silviculture measures on tree and stand growth.

Keywords Genetically improved stands · Growth difference · Genetic gain · Growth simulation · Yield prediction

1 Introduction

Growth and yield models can simulate the natural growth processes of trees, stands, and forests and reflect the impact of management measures on development and condition, making these effective tools to consider dynamic changes of spatial and temporal stand structure and to accurately predict response to management interventions (Cao and Strub 2008; Pretzsch 2009; Weiskittel et al. 2011; Orellana et al. 2016; Soukhovolsky and Ivanova 2018). Forestry research and

Handling Editor: John M Lhotka

✉ Qingfen Li
li63757416@163.com

Cheng Deng
dengcheng6618@126.com

Robert E. Froese
froese@mtu.edu

Shougong Zhang
sgzhang@caf.ac.cn

Yuanchang Lu
ylu@ifrit.ac.cn

Xiaojun Xu
xuxiaojun6107@163.com

[1] College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China

[2] School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI, USA

[3] Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China

[4] Institute of Forest Resource Information Techniques, Chinese Academy of Forestry, Beijing, China

Published online: 07 September 2020

remarkable advances in data science, mathematical statistics, computing capacity, and modeling approaches have facilitated the development of many computer-based forest growth and yield models (Mustafaa et al. 2011; Cao 2014; Adamec 2015; Fortin et al. 2017). Models take many different forms, including individual equations such as those that describe attributes of trees, stands, or forests; systems of equations that comprise empirical simulators; and concept-driven representations of underlying community of physiological processes (Vanclay 1995; Robinson and Ek 2000; Froese and Robinson 2007). Many different forms of these models have been established and are used widely in forestry (Coops et al. 2012; Collalti et al. 2014; Seely et al. 2015; Njana et al. 2016).

With continued advances in forest genetics and tree breeding, many new genetic varieties have been developed and deployed for forest production (Koskela et al. 2014). Growth and yield models that can accurately reflect the growth characteristics of these genetically improved stands are necessary for several reasons. Accurate predictions of the future volume yield are required by tree breeders to quantify the potential financial payoffs from investment in tree breeding improvement programs, to compare different breeding strategies, and to assign economic weights to selection criteria (Chang et al. 2019). The precise planning of wood flows from a forest estate can greatly enhance the profitability of a forest management enterprise, both to precisely regulate yield and to plan processing facilities, and growth and yield is an essential input. Forest managers also use predictions of stand growth to determine optimum times for thinning, pruning, and felling specific stands and to analyze the economics of silviculture (Goulding 1994).

A critical limitation of most existing growth and yield models is that they are based on data collected in genetically unimproved stands. Although such models are often based on large sample sizes with juvenile and mature trees growing across a wide range of sites (Stoehr et al. 2010), they mainly focus on the effects of forest development (e.g., stand age), site occupancy or tree competition status (e.g., stand density), productive potential (e.g., site quality), and silviculture (e.g., land preparation, fertilization, thinning) on forest growth, without consideration of the genetic factors of the planting material (Sprinz 1987; Sun et al. 2004). Genetically improved stands may have many differences in growth from unimproved stands, such as genetic gain in diameter, tree height, volume, and other traits (Matziris 2005; Vergara et al. 2007). Different varieties may have different growth patterns (Gwaze et al. 2002; Andersson et al. 2006; Gould et al. 2008), stem shapes (profile, taper, etc.), allometry (e.g. tree height-diameter ratio) (Kroon et al. 2008; Weng et al. 2008; Sharma et al. 2013; Egbäck et al. 2014), and different wood properties (Missanjo and Matsumura 2016; Kimberley et al. 2016; Moore et al. 2017). The use of models based on unimproved stands to predict the future growth and yield of genetically improved stands has greatly limited prediction accuracy and model application, which can adversely affect management decision-making (Adams et al. 2006).

Thus, it is necessary to develop growth and yield models that consider the characteristics of genetically improved materials. These models are essential to guide the scientific management of plantation forests and would be effective tools for the selection and evaluation of genetically improved material, shortening the forest breeding cycle and improving the efficiency and benefit of tree breeding efforts (Wu 1999).

2 Need for growth and yield models for genetically improved stands

With the development of tree breeding, there have been parallel efforts to quantitatively study and predict genetic gain. The use of growth and yield models to quantify and predict selection gains during rotation according to early growth differences of various genetic materials can provide another effective strategy for traditional selection of fine seed varieties (genotype, family, and population) and prediction of genetic gains as an alternative to quantitative genetics theory. This approach is simpler and can be more accurate because growth and yield curves effectively average irregularities in the data caused by the measurement error or environmental fluctuation and allow for prediction at ages for which measurements are missing (Rehfeldt 1992; Hamilton and Rehfeldt 1994; Gwaze et al. 2002). Therefore, since the 1980s, forest scientists have begun to study the effects of genetic variety on forest growth and yield models and on the development of these models for genetically improved stands (Nance and Bey 1979; Buford 1989; Schmidtling and Froelich 1993; Danjon 1995).

An increasing number of genetically improved materials are widely used in operational forest management. In addition to the traditional selection of improved material and evaluation of genetic gain, accurate simulation of the growth process of an improved forest and prediction of its response to different treatments are essential for forest management planning and decision-making. For this reason, increased attention has been paid to the development of growth and yield models of genetically improved stands (Wang et al. 2004; Sabatia and Burkhart 2013; Kimberley et al. 2015; Zheng 2017).

3 Experimental data for growth and yield models of genetically improved stands

The development and validation or verification of growth and yield models generally require long-term and repeated measures data from permanent sample plots to obtain reliable inferences (Sun et al. 2004; Weiskittel et al. 2011). However, in many practical situations, because of the long rotation length in many forest types and the lag of cultivation behind breeding

efforts, there are insufficient data from sample plots to reflect the growth of genetically improved stands for an entire rotation (Sun et al. 2004). In order to assess how the offspring of selected parent genotypes perform in a mixture with other genotypes selected for growth, and to see if predicted growth gains persist through rotation, genetic testing has been carried out on a large scale. Many provenances, families, and clonal test plantations have been established for many species around the world since the 1960s (Magnussen and Yeatman 1990; Svensson et al. 1999; Nagamitsu et al. 2018), providing some experimental data to analyze the growth situation and genetic variation of genetically improved materials (Carson et al. 1999; Lambeth 2000; St. Clair et al. 2004).

Given the limited data from the existing provenance, family progeny, or clonal test plantations are not adequate to establish growth and yield models of genetically improved stands, it is often necessary to select good varieties according to the early growth performance to shorten the breeding cycle, and the early selection age is often less than half of the rotation period (Hallingbäck et al. 2018; Cornelius et al. 2018). Therefore, development of a growth and yield model for genetically improved stands may need to be based on the early growth data from test plantations, before maturity. However, it is a significant challenge to deduce the growth and yield of the whole rotation period based on these limited data (Talbert and Hyink 1988).

4 Establishment of growth and yield models for genetically improved stands

4.1 Basic or common strategy

Due to the insufficient long-term growth data on genetically improved stands, it is currently infeasible to develop entire new empirical models or refit growth equations for most improved forests. Additionally, tree improvement presents a moving target for forest modelers, as a new generation of test plantation is likely to be established before an improved test plantation completes its rotation (Gould and Marshall 2010). To solve these problems, growth survey data from test plantations are used with existing growth and yield equations or model systems based on unimproved stands for growth and yield prediction of genetically improved stands. The most common strategy for genetically improved materials (provenances, families, clones, etc.) has been to first use data from existing test plantations to compare growth differences and evaluate genetic variation and to determine if the relationships of mensurational characteristics (height-age relationship, height-diameter relationship, diameter distribution, etc.) are practically or statistically different. Next, the effects of genetic varieties on the growth and yield of unimproved stands are verified. Finally, specific genetic effect information is incorporated into established growth and yield equations or model systems for unimproved stands to correct the original models for simulation of genetically improved stands. Quantifying any similarities or differences in the effects of genetic factors on tree mensurational characteristics is the first step (Hamilton and Rehfeldt 1994; Nance and Bey 1979; Buford 1989; Schmidtling and Froelich 1993; Danjon 1995).

4.2 Illustrating essential concepts using the height-age relationship

The growth differences of genetically improved stands could manifest in changes to various relationships between tree mensurational characteristics. Some examples include the following: (i) the height-age relationship (and for top-height trees, the site index curve), which reflects the dynamic change process of tree height; (ii) the diameter-age relationship, which reflects the dynamic change process of stem basal area; (iii) the height-diameter relationship, which reflects the relationship between diameter classes and average tree height and also reflects the tree stem shape to some extent; (iv) the number-age relationship (survival curve), which reflects the dynamic change of stand density; and (v) the height or diameter distribution, which may indicate differential and nonlinear changes to stand demographics.

Because of the prominence of height modeling in the genetic gain literature and prominence of site index in growth and yield models, we focus on the height-age model in particular in this section, to illustrate some common issues and themes in modeling genetic improvement in trees and stands. There is a close correspondence between site index, volume production, and stand dynamics. As site index integrates many factors, and because height is usually understood to be independent of density (Weiskittel et al. 2011), the height-age relationship can most intuitively reflect the potential growth differences of genetically improved stands even if planted in varied spatial arrangements. For this reason, analysis of height growth difference was the basis for many studies on growth difference analysis of genetically improved stands. For example, Buford and Burkhart (1987) found that the most fundamental effect of genetic factors on forest growth was the change of tree height-age relationship (site index curve), and the most important challenge in predicting the growth of genetically improved stand was to accurately determine this relationship. Joo et al. (2020) compared approaches to model genetic gain in Douglas-fir and concluded that assuming genetic gain differences were represented by site index that produced similar estimates of realized yield gain to more complex modifications of model components involving height and diameter. In many efforts to predict the growth and yield of genetically improved stands, the site index model of a forest growth and yield model system was adjusted to consider genetic effect (provenance effect, family effect, etc.) based on

the measured effect of genetic factors on the tree height-age curve (Buford 1986; Buford 1989; Knowe and Foster 1989; Danjon 1995).

The Chapman-Richards equation (Richards 1959) is an extremely popular base function for modeling yield of forest attributes in the growth and yield literature. Zeide (1993) and others have explored the underlying biological basis for many growth equations; the utility of these equations has also been clearly demonstrated using empirical data from many published studies that compared alternative basic equation forms. The Chapman-Richards equation can reflect subtle changes in the growth process of genetically improved material due to its flexibility, accuracy, and attractive analytical properties (Pesonen et al. 2009). Furthermore, this equation accommodates a wide range of growth curves, which are typical of empirical data associated with forest research (Yang et al. 2005). The basic form is as follows:

$$Y = a \times \left(1\text{-}e^{-bA}\right)^{c} + \varepsilon \quad (1)$$

where Y is the yield of a characteristic of interest (e.g., height, diameter, volume, or basal area; see, e.g., Smith et al. 2014) at age A; a is the asymptotic parameter, which represents the asymptotic maximum size of the organism; b is the rate parameter, which represents the intrinsic growth rate; c is the shape parameter, which is related to the power exponent of assimilation; and ε is a normally distributed zero-expectation random error due to observation of the total growth at age A (Richards 1959). Models of unimproved stands simulated using Eq. (1) have often been used as foundation or core models for growth and yield. In practice, the parameters of the equation can be expanded to introduce more factors (site index, stand density, etc.) to better simulate the growth and yield of stands.

4.3 Growth and yield differences in genetically improved stands

The growth differences between improved and unimproved stock can be reflected in the growth equation curves. Taking tree height growth as an example, from the point of view of genetic gain, there are three main classes of differences. First, the genetic gain does not disappear over time, and the growth process (the shape of the growth curve) is the same as that of the unimproved stand (e.g., Gnc in Fig. 1a). In this case, the genetic gain is constant throughout the growth period. For example, if a genetically improved stand has a genetic gain of 10% for height, its average height at any given age in the rotation period is expected to be 10% greater than that of the unimproved stand growing in the same environment. Second, the genetic gain does not disappear over time, but the growth process is different from that of the unimproved stand, which indicates the genetic gain is not constant during the growth period, and there may be many variations (e.g., Gn1, Gn2, and Gn3 in Fig. 1a). Third, the genetic gain will disappear over time, which means that the genetically improved stand and the unimproved stand have the same average height at maturity and only differ in growth processes (Fig. 1b).

When the genetic gain does not disappear over time, the coefficients of Eq. (1) vary depending on the growth process. When the genetic gain is constant throughout the growth period, the genetic variety only affects the asymptotic coefficient a (Gnc in Fig. 1a). When the genetic gain is not constant during the growth period, the genetic variety may affect the asymptotic coefficient a and the rate parameter b (Gn1 in Fig. 1a), affect the asymptotic coefficient a and the shape parameter c (Gn2 in Fig. 1a), or affect all three parameters (Gn3 in Fig. 1a).

4.4 Testing genetic variety effects on a growth and yield model

If the difference in growth is significant between the genetically improved stand and the unimproved stand, it is necessary to test if the difference has an effect on components or parameters of growth and yield models. Indeed, growth model parameters may be better indicators of changes in growth patterns associated with improved stands than simple measures like age-specific height, and more likely to be related to mature growth (Gwaze et al. 2002). Genetic improvement may affect multiple parameters in the growth and yield models, resulting in different versions of these models. Two approaches have been commonly used to test if the genetic improvement causes a change in model parameters: the use of dummy variables and including random effects to create mixed-effects models (Callister et al. 2013).

In the dummy variable approach, genetic improvement factors (provenance effects, family effects, etc.) are introduced into the basic model as dummy variables through reparameterization, and then significance tests are performed to determine the effects on each parameter of the model. Equation (1) was expanded as follows to facilitate testing of the statistical hypotheses:

$$Y = (a_1p_1 + a_2p_2 + \cdots + a_mp_m) \times \left(1\text{-}e^{-bA}\right)^{c} + \varepsilon \quad (2)$$

$$Y = (a_1p_1 + a_2p_2 + \cdots + a_mp_m) \times \left(1\text{-}e^{-(b_1p_1+b_2p_2+\cdots+b_mp_m)A}\right)^{c} + \varepsilon \quad (3)$$

$$Y = (a_1p_1 + a_2p_2 + \cdots + a_mp_m) \times \left(1\text{-}e^{-(b_1p_1+b_2p_2+\cdots+b_mp_m)A}\right)^{(c_1p_1+c_2p_2+\cdots c_mp_m)} + \varepsilon \quad (4)$$

Where m is the number of genetically improved materials (provenance, family and clone etc.); a_i, b_i, and c_i are the asymptotic height parameter, growth rate parameter, and shape

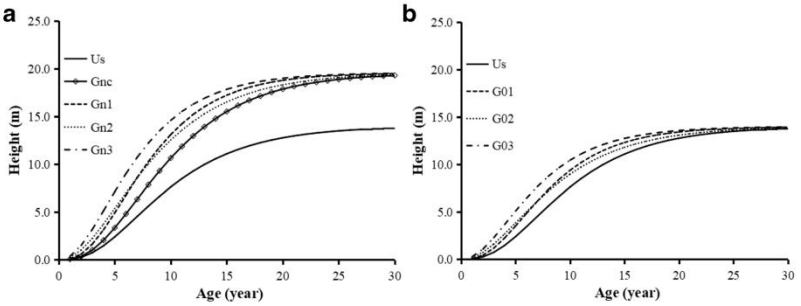


Fig. 1 Conceptual illustration of growth differences of genetically improved stands. **a** Different patterns where gain persists across time and **b** where gain diminishes with time. Us represents the growth process of unimproved stand; Gn means that the initial genetic gain did not disappear over time; Gnc represents the growth process of genetically improved stand which the genetic gain is constant throughout the growth period; Gn1, Gn2, and Gn3 represent the growth process of genetically improved stand whose genetic gain is varied throughout the growth period; 1, 2, and 3 represent different specific forms; G0 means that the initial genetic gain disappeared over time (equal to zero at maturity); G01, G02, and G03 represent the growth process of genetically improved stand which the initial genetic gain disappeared over time

parameter, respectively, of the genetically improved material i; P_i is the dummy variable, if genetically improved material i, $p_i = 1$, otherwise $p_i = 0$. For each genetically improved material, the F test statistic can be used to judge whether genetic improvement has a significant effect on each parameter, that is, to verify the following hypothesis by F-test:

$H_{01} : a_1 = a_2 = \ldots = a_m, H_{11}$: at least one a_i different;

$H_{02} : b_1 = b_2 = \ldots = b_m, H_{12}$: at least one b_i different;

$H_{03} : c_1 = c_2 = \ldots = c_m, H_{13}$: at least one c_i different.

The F-ratio was calculated as follows:

$$F = \left(\frac{SSE_1 - SSE_0}{SSE_0}\right) \times \left(\frac{df_1 - df_0}{df_1}\right) \quad (5)$$

where SSE_1 and df_1 are the residual sum of squares and the degrees of freedom, respectively, of the extended model modified to include genetic improvement effects and SSE_0 and df_0 are the residual sum of squares and degrees of freedom, respectively, of the basic model without addition of genetic improvement effect (Nance and Wells 1981; Buford and Burkhart 1987; Tang et al. 2001).

In the mixed-effects model approach, the effect of genetic improvement on the growth and yield model is modeled as a random effect on the parameters of the basic model. Equation (1) was expanded as follows to facilitate testing of the statistical hypotheses of interest:

$$Y = (a + r_{ai}) \times \left(1\text{-}e^{-bA}\right)^c + \varepsilon \quad (6)$$

$$Y = (a + r_{ai}) \times \left(1\text{-}e^{-(b+r_{bi})A}\right)^c + \varepsilon \quad (7)$$

$$Y = (a + r_{ai}) \times \left(1\text{-}e^{-(b+r_{bi})A}\right)^{(c+r_{ci})} + \varepsilon \quad (8)$$

where a, b, and c are the fixed-effect parameters and r_{ai}, r_{bi}, and r_{ci} are random-effect parameters due to the ith genetically improved material. The effect of the genetic improvement factors on model parameters was investigated by individually excluding the random-effect parameters from the model and evaluating the effect of the exclusion on the overall model fit using the likelihood ratio test (LRT) statistic. Thus, the following hypothesis is tested by the Chi-square test:

$H_{01} : r_{ai} = 0, H_{11} : r_{ai} \neq 0;$

$H_{02} : r_{bi} = 0, H_{12} : r_{bi} \neq 0;$

$H_{03} : r_{ci} = 0, H_{13} : r_{ci} \neq 0;$

The LRT was calculated as follows:

$$LRT = 2\log\left(\frac{L}{L_1}\right) = 2[\log(L_0)\text{-}\log(L_1)] \quad (9)$$

where L_0 and L_1 are the likelihoods of the basic model without random-effect parameters and the extended model with random-effect parameters, respectively (Fang and Bailey 2001; Sabatia and Burkhart 2013). The same methods can also be used to test the effect of genetic variety on the parameters of other theoretical growth equations.

4.5 Interpreting parameter differences

Studies have found complex and varied influences of genetic variety on growth and yield model parameters that were dependent on growth differences of specific tree species, the specific genetically improved materials, and site environmental conditions. For example, using early stand growth measurement data, genetic variants of loblolly pine (*Pinus taeda* L.) exhibited significantly different asymptotic height and

shape parameters of a height-diameter curve (Sabatia and Burkhart 2013). At the level of seed source of loblolly pine, the genetic variety was found to affect only the asymptotic height parameter (Nance and Wells 1981; Buford 1986; Buford and Burkhart 1987) or the shape parameter (Sprinz et al. 1989) of a height-age model. At the family levels of loblolly pine, the genetic variety has affected the asymptotic height parameter (Du 1990), as well as the asymptotic height and rate parameters (Knowe and Foster 1989). According to the observations of asymptotic height, Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) provenances exhibited changed asymptotic parameters of a height model and a basal area model (Tang et al. 2001). *Pinus armandi* Franch provenances have exhibited different asymptotic height parameters (Wang et al. 2016). At the provenance level of Japanese larch (*Larix kaempferi* (Lamb.) Carr.), the genetic variety affected the asymptotic height parameter, the rate parameter, and the shape parameter of a height model (Wang et al. 2015). At the family levels of Japanese larch, the genetic variety affected the asymptotic height and shape parameters of a height model (Sun et al. 2005).

Collectively, many studies have suggested that the genetic improvement has significantly affected the asymptotic height parameters of the growth and yield models, with no or little effect on shape and rate parameters. In contrast, a small number of studies have found that the genetic improvement affected both the asymptotic height parameters and shape parameters (or rate parameters) and fewer still found no effects of genetic improvement on the asymptotic height parameters of the growth and yield models, with only effects on the shape parameters (or rate parameters). Thus, it is not simple to predict the effects of genetic improvement to alter specific model parameters, though collectively the results favor the conclusion that the asymptote is most affected. A weakness in many studies is that they present analyses of genetic gain effects on asymptote parameters using datasets where the asymptote is not yet observed. For example, in the loblolly pine data presented by Sabatia and Burkhart (2013), the height is still increasing, though at a decreasing rate, within the range of the training data. Seeking the most faithful empirical representation likely enhances predictive utility for models of genetic improvement. However, in terms of advancing understanding, more rigor in establishing a theoretical model prior to conducting analyses would likely improve the veracity of research results and statistical tests.

4.6 Incorporation of genetic effects into models for genetically improved stands

Incorporating genetic effects into growth and yield models for genetically improved stands has most commonly adopted one of three approaches. These are the following: (1) the calculation of specific genetic gain multipliers, (2) manipulation of the site index, and (3) calibration of the model parameters.

In the first approach, the relative difference in growth between genetically improved and unimproved (natural) stands is quantified through genetic gain multipliers. Then, multipliers are used to modify the coefficients of a reference model equation (Rehfeldt et al. 1991; Carson et al. 1999; Gould et al. 2008; Stoehr et al. 2010; Hamilton and Rehfeldt 1994; Gould and Marshall 2010; Kimberley et al. 2015; Haapanen et al. 2016; Ahtikoski et al. 2018). This approach provides a means to extrapolate existing growth models that are a representative of unimproved or average stands using the results of progeny tests or deployment studies (Carson et al. 1999; Gould et al. 2008).

In the second approach, site index manipulation is performed to account for genetic gains based on the height increase at a given age, i.e., by adjustment of the underlying site index assigned to the stand to change the height-age curve of the unimproved growth and yield model system (Buford and Burkhart 1987; Sprinz et al. 1989; Knowe and Foster 1989; Danjon 1995; Gwaze et al. 2002; Xie and Yanchuk 2003). This approach is based on the premise that genetic variety affects only the asymptotic height parameter. In one method the height growth trajectory of a genetically improved stand is estimated from early-age height growth data by adjusting the reference height-age curve by a constant proportion, where height growth trajectories of different genetic varieties are a series of anamorphic height-age curves (Sabatia and Burkhart 2013). The simplest method to represent of genetic improvement may be to simply assume that genetic gain is implicit in site index estimates that obtained a mid-rotation in improved stands (e.g., Joo et al. 2020).

In the third approach to prediction of the growth and yield of a genetically improved stand, parameters that are not affected by genetic improvement are used as global parameters with consistent value across all genetically improved and unimproved stands, and parameters that are significantly affected by genetic improvement are considered additional variety-specific effects. The model with global parameters is used as a reference model, and this model can be used with early growth survey data of the genetically improved stand to obtain estimates of the parameters that are specific to each genetic variety (Adams et al. 2006; Sabatia and Burkhart 2013; Wang et al. 2015). Depending on the context, the variety-specific effects could be treated as "fixed" or "random" effects (Callister et al. 2013) both for estimating genetic gain and for use in generating model predictions.

These three methods have advantages and disadvantages. In general, the genetic gain multiplier is the simplest and allows model users to utilize incomplete information on the characteristics of trees grown from improved stands (Gould et al. 2008). Multipliers can be used to represent constant and variable genetic gains. For example, a multiplier to the

asymptote parameter of the yield model (e.g., parameter a in Eq. (1)) represents a constant gain, while a multiplier on the growth parameter (e.g., parameter c in Eq. (1)) can change the shape, as in Fig. 1b. The use of genetic gain multipliers is currently the most common approach when growth data are insufficient, and this is a viable method to estimate the amount of expected volume gain from tree improvement programs. Projecting stand development with genetic gain multipliers can also provide insight into how genetic gain may interact with other variables such as stand density and site index (Gould and Marshall 2010). However, this approach is still based on individual tree data and relies on accurate model prediction of the average stand (Carson et al. 1999).

Manipulation of site index is straightforward and may integrate effects associated with the adaptability of genetically improved stands to site conditions and interactions with other site or environmental factors. However, increasing the site index alone may not be sufficient if the total increase in volume production is affected by corresponding increases in diameter observed in selected genetically improved stands. Therefore, it may be essential to also consider the height-diameter relationship when predicting the volume yield of a genetically improved stand using this approach (Stoehr et al. 2010).

Theoretically, calibration of model parameters using empirical data collected from long-term trials has a wider adaptability than the other approaches and can obtain higher estimation accuracy when there is enough supporting data. Clearly, this approach is the most complicated, costly, and time-consuming. Moreover, this approach requires more data or prior information to achieve accuracy comparable with that of reference models from unimproved stands (Sabatia and Burkhart 2013).

In the literature, a compelling case for an optimal approach has yet to be made across a wide variety of studies. This is, at least in a large part, because of the diversity of studies that have had different and often narrowly focused objectives or involve modeling efforts that are constrained by prior decisions to adopt larger frameworks. For example, Kimberley et al. (2015) develop an approach for quantifying gain in radiata pine that was necessary in part because of a change in the accepted rating system for gain, but also in part because of the adoption of a new national-level growth and yield model that superseded prior model efforts and efforts to develop genetic gain multipliers. Joo et al. (2020) found that multipliers in an individual tree model compared favorably to the relatively simple approach of using realized plot-level site index expressed at the most recent re-measurement, which is encouraging. However, the authors also report "striking" changes in relative and absolute gain over 17–21-year measurement periods and 39–43-year simulation periods that suggest significant challenges remain in developing effective modeling and simulation approaches (Joo et al. 2020).

5 Other factors affecting growth and yield models of genetically improved stands

There can be a significant variation in the influence of genetic varieties on growth and yield model parameters, and previous studies have reported conflicting results. The conflicting data may be because the biological basis for genetic gain is not well understood, and there is not necessarily a single biological basis for genetic gain. For example, a breeding program may select genotypes with different growth rates but the same asymptotic heights, while another program, perhaps focused on a different species, selects genotypes with different asymptotic heights. Furthermore, the realization of genetic gain is affected not only by the genetic variety itself but also by other non-genetic factors, which can affect the growth and yield of the genetically improved stand (Joo et al. 2020).

In addition to genetic factors, the parameters of stand growth and yield models are influenced by environmental factors that may exhibit different spatial characteristics, such as soil attributes (including soil fertility, texture, moisture, and depth), climate, topography, and wind exposure (Smith et al. 2014). Phenotypic responses of genotypes in different environments are distinct, resulting in differences in stand growth patterns (Fu et al. 1999; Silva et al. 2001; Baltunis et al. 2010; Rohner et al. 2018). Studies have shown that the genetic gain is not consistent across sites; in many cases greater genetic gain is achieved with increased site productivity (Carson et al. 1999; Gould and Marshall 2010), but this is not universal (Martin and Shiver 2002). The maximum projected gains may also occur earlier in the simulation period with increasing site productivity (Gould and Marshall 2010). In some quite extreme sites (such as barren sites), the genetic differences may be poorly expressed (Carson et al. 1999).

Silviculture practices are an additional non-genetic factor affecting the growth and yield of genetically improved stands. Stand density may reflect the degree of space utilization of trees and competition between trees and is always considered an important variable in growth and yield models. Density is clearly affected by decisions about tree planting and by silviculture measures that reduce density, such as thinning. Stand density affects the growth rate and pattern of a stand, as well as the stem height-diameter allometry (Zhang et al. 1997; Cañellas et al. 2004; Adams et al. 2006; Russell et al. 2010; David et al. 2016; Jiang et al. 2016; Egbäck 2016). Further, the stem slenderness, profile, and relative survival of trees are affected by both stand density and genetic variation. Stand density also affects the expression of genetic variation and the realization of genetic gain. For example, some studies have showed that effects of genetic variety on the height-diameter relationship depended on stand density, with significant effects at higher stand density and no effect at lower density (Sabatia and Burkhart 2013). Thus, different silviculture practices may lead to differences in stand growth and

yield models (Westfall 1998), and determination of how stand density will affect the growth and survival of genetically improved stands is a critical part of predicting volume gain (Gould and Marshall 2010).

The carrying capacity may play a very important role in accurately modeling genetic gain across an entire commercial rotation. Maximum density may constrain the level of volume gain that can be achieved with improved stands because faster growth may cause stands to reach maximum density more quickly, and gains may be limited if mortality reduces volumes commensurate with gains (Long and Smith 1984). Alternatively, genetic gain may manifest in increased carrying capacity (Joo et al. 2020), which could suggest a reinforcement effect. In addition, fertilization, soil improvement, and drainage can improve the overall site quality and microenvironment, thus affecting the growth rate and process of stands to enhance forest productivity (Kytö et al. 2010).

In living systems, biological traits can confer the ability to alter their phenotypes to better respond to environmental change or developmental signals, during which there must exist a particular set of genes that regulate or reflect such alteration (Gilchrist and Nijhout 2001; Salazar-Ciudad and Jernvall 2010). With the development of molecular biology and bioinformatics, the opportunity may exist to identify the genes that control the height and diameter development of trees (Jiang et al. 2016). Therefore, in the future, non-genetic factors and genetic factors could be combined. Consideration should be given to the influence of non-genetic factors on the expression of these genes, so as to determine the influence of non-genetic factors on the height and diameter growth of genetically improved stands. Ideally, greater understanding of biological or genetic theory would allow mechanisms that drive expression of and change in genetic gain to be built into growth and yield models (Joo et al. 2020).

6 Improvable aspects of growth and yield models for genetically improved stands

In the past few decades, there have been many studies involving growth and yield models of genetically improved stands. These studies provided much useful information and tools for more accurate simulation of growth process and prediction of genetic gain. However, while this review found many promising results in recent work, much remains uncertain, and there is no consensus view on the best way forward for modeling genetic improvement. Still, due to various constraints, there are several aspects that can be improved for greater model accuracy.

First, the effect of genetic differences in height, diameter, taper, and other traits at the individual tree level and competitive ability on a stand level should be evaluated. The experimental data utilized in most studies were not obtained from the actual stands but from a separate test plantation (provenance trial, progeny trial, or clone trial stands) used in the genetic testing and selection programs (Kimberley et al. 2015). Thus, the competitive ability of improved strains has typically not been addressed because growth and yield models have instead focused mainly on the genetic contributions of individual tree traits (Adams et al. 2006). Test plantations typically use single-tree plots or small multiple-tree (generally 4–16) row plots. These designs allow high precision for the partitioning of additive genetic variance, for efficient rankings of genotypes, but do not provide adequate estimates of gain under operational conditions of inter-tree competition. A typical test plantation includes a large number of genotypes, where both high and low performers are grown together (Carson et al. 1999). In contrast, a typical genetically improved stand will contain a mix of offspring, but only from the very highest performers (Carson et al. 1999). Single-tree plot designs can magnify family differences due to the effects of competition among trees (Magnussen 1989), but in row plots, members of the same varieties (provenance, family, and clone) have increased probability of sampling the same microenvironment, thus reducing estimates of environmental variation (Magnussen 1993). This can cause overestimation of heritable improvements and gain in growth traits (Dhakal and White 1996; Vergara et al. 2004; Terrance et al. 2010). Stand-level competition has a profound effect on growth and final tree size, so predicting gain using only data from a test plantation in the selection program may not represent actual realized gains in yield, making less accurate predictions (Carson et al. 1999; Adams et al. 2006; Vergara et al. 2007; Kimberley et al. 2015). It is better to compare improved and unimproved stand planted as large block plots for more accurate prediction of changes in growth and yield, as these designs better represent actual stands and can largely eliminate potential competition effect from trees of differing genetics (Carson et al. 1999; Stoehr et al. 2010; Terrance et al. 2010; Kimberley et al. 2015, Joo et al. 2020).

The effects of genetic gain are sometimes subtle and multivariate, affecting several mensurational characteristics simultaneously, nonlinearly, at different scales, and in interaction with site and environmental factors. Yet many growth and yield models focus on a few focal tree mensurational characteristics and lack sophistication to capture interactions. Most simulation models have focused mainly on genetic gain selection indexes such as tree height and diameter, because these metrics are easy to measure and important to forest yield and, therefore, they are the most frequently used indicators for early selection. In turn, these measurements are often the only data, other than survival, available from tree breeding programs that can be used for modeling. Overall, more attention has focused on the effects of different genetic materials on the height-age relationship (site index), height-diameter relationship, and the modification of existing general stand models to reflect the genetic gain of improved stands, with less attention paid to tree height and diameter distributions, basal area growth, crown width, and

biomass models, or the relationships between various models (Sun et al. 2004). Genetics obviously has significant effects on height, diameter, and survival rate (Sharma et al. 2013; Ye et al. 2010), but genetic factors may affect diameter and height development disproportionally, thus affecting the stem shape and profile (Sabatia and Burkhart 2013). Genetics also can have a significant effect on height distributions and tree size distributions (Weng et al. 2010; Sabatia and Burkhart 2013). Stand volume yield is affected by all these factors simultaneously. This implies that height growth, diameter growth, the height-diameter relationship, and mortality models must be considered wholistically to obtain accurate prediction of gain in stem volume (Carson et al. 1999). In addition, consideration of the effect of genetic varieties on biomass allocation is also needed to accurately reflect any differences in ecosystem services such as carbon sequestration in genetically improved stands (Aspinwall et al. 2012). Many references have emphasized the need for such work, but thus far no comprehensive study has been reported (Adams et al. 2006).

Genetic improvement factors, site conditions, silviculture measures, and the interaction of these factors must also be considered simultaneously as factors affecting growth and yield. In practical application, genetically improved stands would be planted at different sites, and the planting density and management measures would differ depending on the purpose of cultivation. In contrast, test plantations have limited scope and lack an operational objective, so simulations using data from a test plantation do not fully reflect potential impacts of site quality, silviculture measures, and genetic improvement factors on operational realization of genetic gain (Talbert and Hyink 1988; Carson et al. 1999; Stoehr et al. 2010; Joo et al. 2020). Gene expression and realized genetic gain are affected not only by the characteristics of the genetic material itself but also by the site conditions and management measures (Hamilton and Rehfeldt 1994; Fu et al. 1999; Silva et al. 2001; Egbäck 2016). Importantly, these factors do not work alone but work together to influence the overall growth and development of the stand, with potential interactive effects (Wu and Matheson 2005; Rubilar et al. 2018; Resende et al. 2018). Without consideration of all these effects on stand growth, the application scope and prediction accuracy of growth and yield models for actual production will be limited. Therefore, future work to develop growth and yield models for genetically improved stands will require greater understanding of genetic factors, site environment, cultivation measures, and their interactions for more rational assessment of costly genetically improved stocks for different sites (Sabatia and Burkhart 2013; Kimberley et al. 2015).

7 Conclusion

Growth and yield models are essential tools for operational stand and forest management. Where genetically improved trees are deployed, models clearly need to be adapted to capture the effect of genetic improvement. Moreover, models themselves have been effective frameworks for quantifying genetic gain, by allowing for tests of different parameters to reveal differences that are isolated from noise where there is sufficient data. Despite decades of research and many successes, no clear consensus has emerged in the literature on the optimal strategy for refining models to represent genetic gain. However, from the literature, several insights can help guide model development in the future.

Due to the lack of long-term data for improved stands, and the rapid pace of breeding programs, development of new empirical models and the refitting of growth equations for improved forests may never be an effective strategy. Still, the importance of long-term data from improved and unimproved stands planted as large block plots is required, as this simulates actual stands in operational forestry. Past modeling efforts based on early observations should be re-evaluated as growth data are accumulated to guide model evolution and efforts with new genetic varieties that have limited data. Long-term data are also essential so that asymptotes (as in height-age) can be observed instead of approximated.

As research proceeds in genetics and on the biological basis for genetic gain, it seems likely that this will provide new insights into how to adapt models to capture more complexity and thus better simulate genetic gain. Some literature has suggested that modelers pay more attention to theory in rationalizing modeling efforts. Future growth and yield simulation systems should be able to predict most tree mensurational characteristics, including complex factors such as volume, which is affected by tree height, but also diameter, taper, vigor, and survival and interactions among trees within stands that capture both competition and environmental influences. Overall, the development of improved and robust growth and yield models for genetically improved stands may simply require more research in operational contexts, to develop a more comprehensive understanding of the effects of genetic, environment, and silviculture measures' interactions on tree and stand growth.

Acknowledgments We appreciate the editors and anonymous reviewers for their constructive comments.

Authors' contributions Conceptualization: CD, SZ, and YL. Writing (original draft): CD. Literature searching: QL and XX. Writing (review and editing): QL and REF. Funding acquisition: YL. All authors have read and approved the content of the manuscript.

Funding This research was funded by the Central Public-Interest Scientific Institution Basal Research Fund for Chinese Academy of Forestry (grant number IFRIT201501).

Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adamec Z (2015) Comparison of linear mixed effects model and generalized model of the tree height-diameter relationship. J For Sci 61(10):439–447

Adams JP, Matney TG, Land SB Jr, Belli KL, Duzan HW Jr (2006) Incorporating genetic parameters into a loblolly pine growth-and-yield model. Can J For Res 36(8):1959–1967

Ahtikoski A, Haapanen M, Hynynen J, Karhu J, Kärkkäinen K (2018) Genetically improved reforestation stock provides simultaneous benefits for growers and a sawmill, a case study in Finland. Scand J For Res 33(5):484–492

Andersson B, Elfving B, Persson T, Ericsson T, Kroon J (2006) Characteristics and development of improved Pinus sylvestris in northern Sweden. Can J For Res 37(1):84–92

Aspinwall MJ, Mckeand SE, King JS (2012) Carbon sequestration from 40 years of planting genetically improved loblolly pine across the Southeast United States. For Sci 58(5):446–456

Baltunis BS, Gapare WJ, Wu HX (2010) Genetic parameters and genotype by environment interaction in Radiata pine for growth and wood quality traits in Australia. Silvae Genetica 59(1–6):113–124

Buford MA (1986) Notes: height-diameter relationships at age 15 in loblolly pine seed sources. For Sci 32(3):812–818

Buford MA (1989) Mean stem size and total volume development of various loblolly pine seed sources planted at one location. Can J For Res 19(3):396–400

Buford MA, Burkhart HE (1987) Genetic improvement effects on growth and yield of loblolly pine plantations. For Sci 33(3):707–724

Callister AN, England N, Collins S (2013) Predicted genetic gain and realised gain in stand volume of *Eucalyptus globulus*. Tree Genet Genomes 9:361–375

Cañellas I, Del Río M, Roig S, Montero G (2004) Growth response to thinning in Quercus pyrenaica Willd. coppice stands in Spanish central mountain. Ann For Sci 61(3):243–250

Cao QV (2014) Linking individual-tree and whole-stand models for forest growth and yield prediction. The For Ecosyst 1(3):1–8

Cao Q, Strub M (2008) Evaluation of four methods to estimate parameters of an annual tree survival and diameter growth model. For Sci 54(6):617–624

Carson SD, Garcia O, Hayes JD (1999) Realized gain and prediction of yield with genetically improved *Pinus radiata* in New Zealand. For Sci 45(2):186–200

Chang WY, Wang S, Gaston C, Cool J, An H, Thomas B (2019) Economic evaluations of tree improvement for planted forests: a systemic review. BioProducts business, pp 1-14

Collalti A, Perugini L, Santini M, Chiti T, Nolè A, Matteucci G, Valentini R (2014) A process-based model to simulate growth in forests with complex structure: evaluation and use of 3D-CMCC forest ecosystem model in a deciduous forest in Central Italy. Ecol Model 272: 362–378

Coops NC, Waring RH, Hilker T (2012) Prediction of soil properties using a process-based forest growth model to match satellite-derived estimates of leaf area index. Remote Sens Environ 126: 160–173

Cornelius JP, Pinedoramírez R, Montes CS, Ugarte-Guerra J, Weber JC (2018) Efficiency of early selection in *Calycophyllum spruceanum* and *Guazuma, two fast-growing timber species of the Peruvian Amazon*. Can J For Res 48(4):1–7

Danjon F (1995) Observed selection effects on height growth, diameter and stem form in maritime pine. Silv Genet 44:10–19

David HC, Arce JE, Netto SP, Corte APD, Marinheski Filho A, Araújo EJGD (2016) Thinning and site quality effects on the height-diameter relationship of Pinus taeda. Floresta e Ambiente 23(2): 220–228

Dhakal LP, White TL (1996) Hodge GR. Realized genetic gains from slash pine tree improvement. Silvae Genetica 45(4):190–196

Du W (1990) Incorporating genetic effects into a forest growth and yield model. University of Idaho, Idaho

Egbäck S (2016) Growth of genetically improved stands of Norway spruce, scots pine and loblolly pine. Swedish University of Agricultural Sciences, Alnarp

Egbäck S, Bullock BP, Isik F, McKeand SE (2014) Height-diameter relationships for different genetic planting stock of loblolly pine at age 6. For Sci 61(3):424–428

Fang Z, Bailey RL (2001) Nonlinear mixed effects modeling for slash pine dominant height growth following intensive silvicultural treatments. For Sci 47(3):287–300

Fortin M, Robert N, Manso R (2017) Uncertainty assessment of large-scale forest growth predictions based on a transition-matrix model in Catalonia. Ann For Sci 73(4):1–13

Froese RE, Robinson AP (2007) A validation and evaluation of the prognosis individual-tree basal area increment model. Can J For Res 37: 1438–1449

Fu YB, Yanchuk AD, Namkoong G (1999) Spatial patterns of tree height variations in a series of Douglas-fir progeny trials: implications for genetic testing. Can J For Res 29(6):714–723

Gilchrist MA, Nijhout HF (2001) Nonlinear developmental processes as sources of dominance. Genetics 159:423–432

Gould PJ, Marshall DD (2010) Incorporation of genetic gain into growth projections of Douglas-fir using ORGANON and the forest vegetation simulator. West J Appl For 25(2):391–401

Gould P, Johnson R, Marshall D, Johnson G (2008) Estimation of genetic-gain multipliers for modeling Douglas-fir height and diameter growth. For Sci 54(6):588–596

Goulding CJ (1994) Development of growth models for *Pinus radiata* in New Zealand - experience with management and process models. For Ecol Manag 69(1–3):331–343

Gwaze D, Bridgwater F, Williams C (2002) Genetic analysis of growth curves for a woody perennial species, *Pinus taeda L*. Theor Appl Genet 105(4):526–531

Haapanen M, Hynynen J, Ruotsalainen S, Siipilehto J, Kilpeläinen M (2016) Realised and projected gains in growth, quality and simulated yield of genetically improved scots pine in southern Finland. Eur J For Res 135(6):997–1009

Hallingbäck HR, Högberg KA, Säll H, Lindeberg J, Johansson M, Jansson G (2018) Optimal timing of early genetic selection for sawn timber traits in *Picea abies*. Eur J For Res 137(4):1–12

Hamilton DA, Rehfeldt GE (1994) Using individual tree growth projection models to estimate stand-level gains attributable to genetically improved stock. For Ecol Manag 68(2–3):189–207

Jiang L, Ye M, Zhu S, Zhai Y, Xu M, Huang M, Wu R (2016) Computational identification of genes modulating stem height–diameter allometry. Plant Biotechnol J 14(12):2254–2264

Joo S, Maguire DA, Jayawickrama KJS, Ye TZ, St. Clair JB (2020) Estimation of yield gains at rotation-age from genetic tree improvement in coast Douglas-fir. For Ecol Manag 466:117390 (available online)

Kimberley MO, Moore JR, Dungey HS (2015) Quantification of realised genetic gain in radiata pine and its incorporation into growth and yield modelling systems. Can J For Res 104(2):1–40

Kimberley MO, Moore JR, Dungey HS (2016) Modelling the effects of genetic improvement on radiata pine wood density. N Z J For Sci 46(1):8

Knowe SA, Foster GS (1989) Application of growth models for simulating genetic gain of loblolly pine. For Sci 35(1):211–228
Koskela J, Vinceti B, Dvorak W, Bush D, Dawson IK, Loo J, Kjaer ED, Navarro C, Padolina C, Bordács S, Jamnadass R, Graudal L, Ramamonjisoa L (2014) Utilization and transfer of forest genetic resources: a global review. For Ecol Manag 333:22–34
Kroon J, Andersson B, Mullin TJ (2008) Genetic variation in the diameter-height relationship in Scots pine (*Pinus sylvestris*). Can J For Res 38:1493–1503
Kytö M, Niemelä P, Annila E, Varama M (2010) Effects of forest fertilization on the radial growth and resin exudation of insect-defoliated Scots pines. J Appl Ecol 36(5):763–769
Lambeth C (2000) Realized genetic gains for first generation improved loblolly pine in 45 tests in coastal North Carolina. South J Appl For 24(3):140–144
Long JN, Smith FW (1984) Relation between size and density in developing stands: a description and possible mechanisms. For Ecol Manag 7(3):191–206
Magnussen S (1989) Effects and adjustments of competition bias in progeny trials with single-tree plots. For Sci 35(2):532–547
Magnussen S (1993) Bias in genetic variance estimates due to spatial autocorrelation. Theor Appl Genet 86(2–3):349–355
Magnussen S, Yeatman CW (1990) Predictions of genetic gain from various selection methods in open pollinated *Pinus banksiana* progeny trials. Silvae Genetica 39(324):140–153
Martin SW, Shiver BD (2002) Impacts of vegetation control, genetic improvement and their interaction on loblolly pine growth in the southern United States – age 12 results. For Sci 26(1):37–42
Matziris D (2005) Genetic variation and realized genetic gain from black pine tree improvement. Silvae Genetica 54(3):96–104
Missanjo E, Matsumura J (2016) Genetic improvement of wood properties in Pinus kesiya Royle ex Gordon for sawn timber production in Malawi. Forests 7(11):253
Moore JR, Dash JP, Lee JR, McKinley RB, Dungey HS (2017) Quantifying the influence of seedlot and stand density on growth, wood properties and the economics of growing radiata pine. Forestry 91(3):327–340
Mustafaa YT, Tolpekin V, Stein A (2011) Application of the EM-algorithm for Bayesian network modelling to improve forest growth estimates. Procedia Environ Sci 7(7):74–79
Nagamitsu T, Matsuzaki T, Nagasaka K (2018) Provenance variations in stem productivity of 30-year-old Japanese larch trees planted in northern and central Japan are associated with climatic conditions in the provenances. J For Res 23(5):270–278
Nance WL, Bey CF (1979) Incorporating genetic information in growth and yield models. In: Proc south for. Tree Improvement Conference, Starkville, pp. 140–148
Nance WL, Wells OO (1981) Site index models for height growth of planted loblolly pine (Pinus taeda L.) seed sources. Proceedings of the 16th southern forest tree improvement conference. Southern Forest tree improvement committee, Blacksburg, VA, pp 86-96
Njana MA, Bollandsås OM, Eid T, Zahabu E, Malimbwi RE (2016) Above-and belowground tree biomass models for three mangrove species in Tanzania: a nonlinear mixed effects modelling approach. Ann For Sci 73(2):353–369
Orellana E, Filho AF, Netto SP, Vanclay JK (2016) Predicting the dynamics of a native Araucaria forest using a distance-independent individual tree-growth model. Forest Ecosystems 3(1):12
Pesonen A, Eerikäinen K, Maltamo M, Tahvanainen T (2009) Models for predicting tree and stand development on larch plantations in Hallormsstaeur, Iceland. New For (Dordr) 37(1):63–83
Pretzsch H (2009) Forest growth models. In: Forest Dynamics, Growth and Yield. Springer, Berlin
Rehfeldt GE (1992) Early selection in *Pinus ponderosa*: compromises between growth potential and growth rhythm in developing breeding strategies. For Sci 38(3):661–677
Rehfeldt GE, Wykoff WR, Hoff RJ, Steinhoff RJ (1991) Genetic gains in growth and simulated yield of *Pinus monticola*. For Sci 37:326–342
Resende RT, Soares AAV, Forrester DI, Marcatti GE, Santos ARD, Takahashi EK, Silva FFE, Grattapaglia D, Resende MDV, Leite HG (2018) Environmental uniformity, site quality and tree competition interact to determine stand productivity of clonal Eucalyptus. For Ecol Manag 410(76):76–83
Richards FJ (1959) A flexible growth function for empirical use. J Exp Bot 10(2):290–301
Robinson AP, Ek AR (2000) The consequences of hierarchy for modeling in forest ecosystems. Can J Forest Res 30:1837–1846
Rohner B, Waldner P, Lischke H, Ferretti M, Thürig E (2018) Predicting individual-tree growth of central European tree species as a function of site, stand, management, nutrient, and climate effects. Eur J For Res 137:29–44
Rubilar RA, Allen HL, Fox TR, Cook RL, Albaugh TJ, Campoe OC (2018) Advances in silviculture of intensively managed plantations. Curr For Rep 4(1):23–34
Russell MB, Amateis RL, Burkhart HE (2010) Implementing regional locale and thinning response in the loblolly pine height-diameter relationship. South J Appl For 34(1):21–27
Sabatia CO, Burkhart HE (2013) Height and diameter relationships and distributions in loblolly pine stands of enhanced genetic material. For Sci 59(3):278–289
Salazar-Ciudad I, Jernvall J (2010) A computational model of teeth and the developmental origins of morphological variation. Nature 464:583–U138
Schmidtling RC, Froelich RC (1993) Thirty-seven year performance of loblolly pine seed sources in eastern Maryland. For Sci 39(4):706–721
Seely B, Welham C, Scoullar K (2015) Application of a hybrid forest growth model to evaluate climate change impacts on productivity, nutrient cycling and mortality in a montane forest ecosystem. PLoS One 10(8):e0135034
Sharma S, Adams JP, Schuler JL, Bragg DC, Ficklin RL (2013) Genetic effects on early stand development of improved loblolly pine (Pinus taeda L.) seedlings. In: Cunningham MW (ed) Proceedings of the 32nd southern forest tree improvement conference; 2013 June 10–13; Clemson, SC, Sponsored publication no, vol 54. Southern Forest Tree Improvement Committee, Clemson, pp 30–35
Silva JCE, Dutkowski GW, Gilmour AR (2001) Analysis of early tree height in forest genetic trials is enhanced by including a spatially correlated residual. Can J For Res 31(11):1887–1893
Smith BC, Bullock BP, Isik F, McKeand SE (2014) Modeling genetic effects on growth of diverse provenances and families of loblolly pine across optimum and deficient nutrient regimes. Can J For Res 44:1453–1461
Soukhovolsky V, Ivanova Y (2018) Modeling production processes in forest stands: an adaptation of the Solow growth model. Forests 9(7):391
Sprinz PT (1987) Effects of genetically improved stands on growth and yield principles. Opt Commun 233(s 4-6):425–430
Sprinz PT, Talbert CB, Strub MR (1989) Height-age trends from an Arkansas seed source study. For Sci 35(3):677–691
St. Clair JB, Mandel NL, Jayawickrama KJS (2004) Early realized genetic gains for coastal Douglas-fir in the northern Oregon cascades. West J Appl For 19(3):195–201
Stoehr M, Bird K, Nigh G, Woods J, Yanchuk A (2010) Realized genetic gains in coastal Douglas-fir in British Columbia: implications for growth and yield projections. Silvae Genetica 59(1–6):223–233
Sun X, Zhang S, Li F, Tan X (2004) An overview on growth and yield projection models for genetically improved stands. For Res 17(4):525–532
Sun X, Zhang S, Kong F, Sun X (2005) Analyzing parameters of height–age models for open-pollinated Japanese larch families. Sci Silvae Sin 41(1):78–84

Svensson JC, Mckeand SE, Allen HL, Campbell RG (1999) Genetic variation in height and volume of loblolly pine open-pollinated families during canopy closure. Silvae Genetica 48(3):204–208

Talbert CB, Hyink DM (1988) Implications of genetic improvement for the growth and yield of trees and stands. In: Ek AR, Shifley SR, Burk TE. Forest growth modeling and prediction. Proc IUFRO Conf, USDA For Serv Gen Tech, p 562–571

Tang S, Meng FR, Bourque CP (2001) Analyzing parameters of growth and yield models for Chinese fir provenances with a linear mixed model approach. Silvae Genetica 50(324):140–145

Terrance ZY, Jayawickrama KJ, Clair JS (2010) Realized gains from block-plot coastal Douglas-fir trials in the northern Oregon Cascades. Silvae Genetica 59(1–6): 29–39

Vanclay JK (1995) Models for tropical forests: a synthesis of models and methods. For Sci 41:7–42

Vergara R, White TL, Huber DA, Shiver BD, Rockwood DL (2004) Estimated realized gains for first-generation slash pine (*Pinus elliottii* var. elliottii) tree improvement in the southeastern United States. Can J For Res 34(12):2587–2600

Vergara R, White TL, Huber DA, Schmidt RA (2007) Realized genetic gains of rust resistant selections of slash pine (*Pinus elliottii* var. elliottii) planted in high rust hazard sites. Silvae Genetica 56(1–6): 231–242

Wang T, Aitken SN, Woods JH, Polsson K, Magnussen S (2004) Effects of inbreeding on coastal *Douglas fir* growth and yield in operational plantations: a model-based approach. Theor Appl Genet 108(6): 1162–1171

Wang Y, Wang J, Qi W, Gao X, Wang Q, Zhang P (2015) Effects of Pinus armandi Franch provenances on parameters of height-age models. Journal of Northwest A & F University (Nat Sci Ed) 43(7):74–81

Wang Y, Wang J, Zhang S, Ma W (2016) Parameters of height-age models for Larix kaempferi provenances. Journal of Northeast Forestry University 44(9):1–4

Weiskittel AR, Kershaw JA, Vanclay JK, Hann DW (2011) Forest growth and yield modeling. Chichester, Wiley-Blackwell, p 415

Weng YH, Kershaw J, Tosh K, Adams G, Fullarton MS (2008) Height-diameter relationships for jack pine seedlots of different genetic improvement levels. Silvae Genetica 57(1–6):276–282

Weng Y, Park Y, Simpson D, Tosh K, Fullarton M (2010) Tree improvement effects on tree size distributions for *Picea glauca* and *Picea mariana* in New Brunswick, Canada. Scand J For Res 25(1):10–20

Westfall JA (1998) Growth models and mortality functions for unthinned and thinned loblolly pine plantations. Virginia Polytechnic Institute and State University

Wu HX (1999) Study of early selection in tree breeding. 2. Advantage of early selection through shortening the breeding cycle. Silvae Genetica 48(2):78–83

Wu HX, Matheson AC (2005) Genotype by environment interactions in an Australia-wide radiata pine diallel mating experiment: implications for regionalized breeding. For Sci 51(1):29–40

Xie C, Yanchuk AD (2003) Breeding values of parental trees, genetic worth of seed orchard seedlots, and yields of improved stocks in British Columbia. West J Appl For 18(2):88–100

Yang Z, Cohen WB, Harmon ME (2005) Modeling early forest succession following clear-cutting in western Oregon. Can J For Res 35(8): 1889–1900

Ye TZ, Jayawickrama K, Clair JBST (2010) Realized gains from block-plot coastal Douglas-fir trials in the northern Oregon cascades. Silvae Genetica 59(1–6):29–39

Zeide B (1993) Analysis of growth equations. For Sci 39:594–616

Zhang S, Burkhart HE, Amateis RL (1997) The influence of thinning on tree height and diameter relationships in loblolly pine plantations. South J Appl For 21(4):199–205

Zheng ZK (2017) Establishment of height-diameter relationship model of Pinus massoniana provenances. Journal of Green Science and Technology 1:5–8

3.3 Allocation Patterns and Temporal Dynamics of Chinese Fir Biomass in Hunan Province, China

forests

MDPI

Article

Allocation Patterns and Temporal Dynamics of Chinese Fir Biomass in Hunan Province, China

Cheng Deng [1,†], Fengfeng Ma [2,3,†], Xiaojun Xu [1,†], Baoqi Zhu [1], Ji Tao [4] and Qingfen Li [1,*]

[1] College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China
[2] Ministry of Education Key Laboratory of Silviculture and Conservation, Beijing Forestry University, Beijing 100083, China
[3] Hunan Academy of Forestry, Changsha 410018, China
[4] Hunan Prospecting Designing and Research General Institute for Agriculture Forestry and Industry, Changsha 410007, China
* Correspondence: qfli@scau.edu.cn
† These authors contributed equally to this work.

Abstract: How trees allocate their biomass among different components has important implications for their survival and growth and ecosystem carbon cycling. Data on the distribution pattern and dynamics of tree biomass are essential for fully exploiting forest carbon sequestration potential and achieving the goal of carbon neutralization. However, there has not been enough research to-date on tree biomass spatial allocation and temporal dynamics in different site qualities at specific tree species scales. This study aimed to evaluate the biomass allocation patterns within tree components of Chinese fir and to examine how they are affected by tree age and site quality. A total of 87 trees were destructively sampled and measured for stem, branch, leaf, bark and root biomass. The biomass proportion difference of tree components in different age stages (8-40 years) was analysed, and the influence process of tree age and site quality on biomass allocation was examined. Our results indicate that the biomass allocation varied with tree age and was also affected by site quality. Stem biomass accounted for the largest proportion of total tree biomass, followed by leaf, root, branch and bark biomass in young forests, and it was followed by root, bark, branch and leaf biomass in other age groups. The biomass proportion of each component all nonlinearly changed with tree age. The proportion of stem biomass increased with increasing tree age, and the biomass proportion of branches and leaves decreased with increasing tree age. The proportion of root biomass first increased and then decreased with tree age, while the bark biomass proportion first decreased and then increased with increasing tree age. Site quality had a positive effect on the biomass proportion of stems but a negative effect on the biomass proportion of branches and bark. The interaction of tree age and site quality also had a significant effect on the proportion of stem biomass as well as root biomass. Therefore, to obtain accurate estimates of Chinese fir forest biomass and carbon stocks, age-specific changes and the influence of site conditions on it need to be considered.

Keywords: Chinese fir plantation; biomass; allocation pattern; temporal and spatial dynamics; change rule

Citation: Deng, C.; Ma, F.; Xu, X.; Zhu, B.; Tao, J.; Li, Q. Allocation Patterns and Temporal Dynamics of Chinese Fir Biomass in Hunan Province, China. *Forests* **2023**, *14*, 286. https://doi.org/10.3390/f14020286

Academic Editor: Brian Tobin

Received: 8 November 2022
Revised: 28 January 2023
Accepted: 31 January 2023
Published: 2 February 2023

1. Introduction

As a crucial indicator of forest growth and quality, estimating biomass plays a key role in monitoring the global carbon cycle and forest health assessments, and it is an important task in ensuring the long-term viability and sustainability of forest resources on regional to national and international scales, particularly in regard to carbon accounting [1]. The monitoring and estimation of forest biomass can also help to understand the implications of policy actions and how climate change may affect sustainability [2]. It is an important part of modern forest ecosystem research, an important basis for revealing the law of

mutual restriction and interaction between forest and environmental components, and is of great significance for studying the fixation, consumption, distribution, accumulation and transformation of material and energy in the ecosystem [3]. Additionally, measuring the status of and change in forest biomass is also critical for formulating national forest resource management policies [4].

Currently, there are three main inventory data types for estimating forest biomass: ground-based measurement methods, remote-sensing-based estimates, and a combination of both methods [5]. When the sample sizes are sufficiently large, field-based measurement methods are generally considered to be more accurate than remote-sensing-based estimates, but the latter can be more cost-effective, especially for large scales and inaccessible terrain [6]. Generally, there are three major methods for estimating tree biomass: direct estimation of biomass from predictor variables; indirect estimation of biomass from tree volume; or simultaneous estimation of both biomass and volume [7]. The direct prediction method is probably the most accurate but involves the costly process of felling and weighing sample trees to acquire the data. In addition, it assumes that the various properties of the sample trees selected are representative of the larger predicted stand or forest [6]. The indirect method is translating stem volume to biomass through some form of biomass expansion factor, assuming a constant or variable density within trees and between species [8], and the biomass of other organs (branch, leaf, bark, root) is calculated according to the stem biomass and the corresponding proportion coefficients. The indirect method is cost-effective but may lead to errors if the conversion coefficients are inaccurate. The third method attempts to avoid these errors by accounting for the difference in both wood density and tree volume in prediction models [7].

Regardless of the data types and methods, accurate individual tree or stand-level biomass estimation models are necessary to translate field measurements or remotely sensed data into estimates of forest biomass. These models commonly rely on traditional forest inventory factors such as tree diameter and total height as independent variables in equations that estimate tree biomass [6]. Tree-based estimates are then summed on sample plots and applied to larger forest areas using probability or area-based expansion factors. Improving the estimation accuracy of forest biomass is essential for determining the change in global carbon balance [9], predicting forest growth [10], modelling the carbon budget [11], and developing sustainable forestry strategies [12]. Much effort has been invested in improving the accuracy of existing forest biomass estimation models, such as by further adding tree height [13], wood density [14] and crown structure factors [15] into existing estimation models, by acquiring more accurate stem form and developing stem taper models [16], or by amending the structures and forms of the allometric models [17].

There is an obvious vertical distribution of tree biomass on various organs [18]. Trees allocate biomass among different organs in response to resource limitation, and this physiological activity is considered to be evolutionary strategies to help them better adapt to different habitats [19,20]. Because of this, biomass allocation has a significant impact on plant productivity, thus affecting the spatial distribution of overall biomass and carbon storage of the forest communities and forest ecosystem [21]. Therefore, comprehensively understanding the spatial allocation pattern and change in forest biomass in different organs is essential for establishing accurate estimation models of forest biomass [22]. At present, many studies on the spatial distribution of forest biomass at local [23], national [24], regional [25], international [26] and global [27] scales have been carried out. Additionally, more detailed information on the spatial allocation patterns of biomass in different organs has been continuously provided [28]. Previous studies have shown that the allocation pattern of forest biomass in different organs is affected by tree species [18], stand age [29,30], stand density [31], light environments [32], precipitation [33] and site conditions [34]. However, different researchers have obtained varied conclusions, which may be related to specific tree species and site conditions, but the specific tree traits that drive this variation remain poorly understood [35].

It is a costly process to collect tree biomass data because it requires felling trees and must collect, weigh and analyse sample tissues of stems, branches, leaves, bark, roots and other organs [6]. Therefore, more than 75% of the biomass research sample trees were less than 50, and only approximately 8% provided the prediction of the root biomass [36], which also hindered the accurate and comprehensive understanding of the distribution pattern of tree biomass on various organs. With the proposal of the goal of carbon peak and carbon neutrality in the world, it is necessary and urgent to more accurately master the spatial allocation pattern and change dynamics of biomass of various organs on the specific tree species scale to establish a more accurate forest biomass estimation model.

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is a fast-growing planted coniferous tree species, with height up to 30 m and diameter at breast height up to 2.5 m. As a high-quality timber (i.e., excellent material, straight and full stem forms) species, Chinese fir has been widely planted in 18 provinces and regions in southern China for more than one thousand years. In recent decades, the annual timber production of Chinese fir has accounted for 20%–25% of the national commercial timber output, which has provided considerable economic benefits to local farmers. Simultaneously, Chinese fir forests provide considerable ecological benefits to the region and nation through their ecosystem services, such as carbon sequestration, increasing groundwater resources and conserving soil erosion [37]. In recent years, with the construction of ecological environment in China, more attention has been given to the ecological services of forests. After the goal of carbon neutrality is proposed, the carbon fixation function of forest ecological services will be further emphasized [38]. The area and volume of Chinese fir plantations have reached 9.90 million hectares and 755 million cubic metres, and they account for 27.23% and 32.57% of the main dominant tree species of planted forest in China, respectively [39]. It is of great significance to study the temporal and spatial allocation pattern and change in Chinese fir biomass to give full play to its carbon sequestration potential and achieve the goal of carbon neutralization

This study focused on the spatial allocation patterns of biomass in various organs and its change dynamics with the stand age of Chinese fir plantations. In addition, we also examined the influence of site quality on the allocation pattern and change in biomass of Chinese fir. We expect the results to provide in-depth insights for a better understanding of the formation and change mechanism of forest biomass, therefore providing a basis and support for more accurate biomass estimation model establishment and carbon stock determination.

2. Materials and Methods

2.1. Study Area

The study was carried out in Chinese fir plantations of Hunan Province, China. Hunan is the main production area of Chinese fir; it is located in the central and southern parts of China (24°38′~30°08′ N, 108°47′~114°15′ E), belongs to the subtropical zone and has a continental monsoon humid climate. Its mean annual rainfall is 1200–1800 mm, relative humidity is 79%, annual sunshine hours are 1300–1800 h, and annual average temperature is 16.0–18.5 °C. The landform type is mainly mountainous and hilly with an elevation of 100–800 m above sea level. The soils are mainly red soil, yellow soil, purple soil, and paddy soil developed from slate and shale with a clay–loam texture, stoniness in the range of 4%–15%, medium fertility, and a mean depth of 80 cm.

The native vegetation in Hunan Province is dominated by evergreen broad-leaved forest, mixed evergreen and deciduous broad-leaved forest, deciduous broad-leaved forest and mountain top moss copse. However, due to the great disturbance by human activities, native vegetation has been seriously damaged. After several decades of vegetation restoration and artificial afforestation, the current vegetation is mainly composed of Chinese fir (*Cunninghamia lanceolata*) plantations, Masson pine (*Pinus massoniana*) plantations, swamp pine *(Pinus elliottii*) plantations, poplar (*Populus*) plantations, bamboo groves (*Phyllostachys heterocycla*) and so on. According to the results of the Ninth National Forest

Resources Continuous Inventory, the forest area and stand volume of Hunan Province are 10.53×10^4 km^2 and 4.07×10^8 m^3, respectively, of which the area and volume of Chinese fir plantation accounted for 38.54% and 41.25%, respectively.

2.2. Sample Plot Setting and Investigation

According to the distribution of Chinese fir plantations in Hunan Province, temporary observation sample plots were installed for sample tree mensuration and biomass sample collection in five state-owned forest farms in Hunan, namely, the Jindong forest farm, Paiyashan forest farm, Huangfengqiao forest farm, Shichangxi forest farm, and Xishan forest farm (Figure 1). The stands selected for sample plots installation are all plantations with the same or similar initial planting density, and the same management measures were adopted during the whole rotation period, such as weeding in the first three years after planting and thinning with roughly the same intensity at the age of eight years. Therefore, these stands should have roughly the same density at the same age in the growth process if their site condition is roughly the same. Each sample plot had an area of 400 m^2 (20 m × 20 m), totaling 29 plots established in stands of different ages and at least 3 plots for each age group of young forest, middle age forest, near mature forest, mature forest and over mature forest. At the same time, the sample plots within the same age group were set up in stands with different site conditions as much as possible.

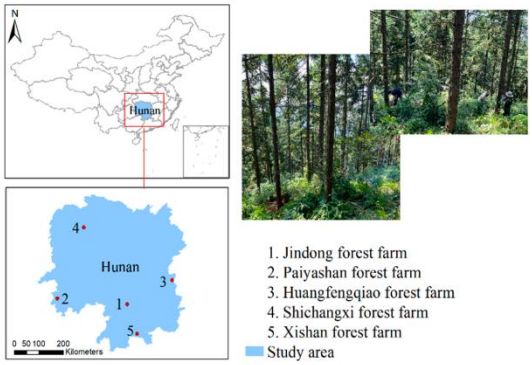


Figure 1. Locations of the temporary observation sample plots.

All trees with a diameter at breast height greater than 5 cm in the sample plots were numbered. The total height, diameter at breast height and crown width of these trees, as well as the site environment and growth status of stands, were inventoried in August 2021, prior to sample collection. Then, the mean diameter at breast height, average total tree height, stand density and other factors were calculated according to the measurement results. The stand characteristics for each temporary plot are shown in Table 1.

2.3. Sample Tree Selection and Sample Collection

In each observation sample plot, three mean trees were selected as sample trees according to the mean diameter at breast height, average total tree height and average stem form, then they were felled for biomass sample collection; altogether, 87 sample trees were harvested. The breast height position and the north-south direction of these sample trees were marked before they were cut down. After the sampled trees were cut down, the height of the first living branch and the first dead branch were measured, and then all branches were cut off and counted. The total height of the stem and the diameter of the bark and peeling at one quarter, one half and three-quarters of the tree height were measured. The stem was divided into upper, middle and lower segments of equal length and then cut

into several sections at heights of 1 m intervals (stem length $\leq$ 10 m) or 2 m intervals (stem length > 10 m) up to the treetop. The fresh weight of each section was weighed, and then the fresh weight of the upper, middle and lower parts and the total weight of the stem were calculated. At the ground level, breast height, and middle position of each section, one cross-sectional stem disc (approximately 5 cm thick) was cut for stem analysis, as well as one disc at the middle position of the upper, middle and lower segments as biomass samples. If there was a branch at the height of the disc, a replacement disc was collected 5 cm above or below. The north direction, section height, plot and tree number and other information were recorded on each disc, and the fresh weight (with and without bark) of stem and bark sample discs were weighed. All the roots with a diameter greater than 0.2 cm of the sample tree were dug out and weighed, and then 500 g of root samples were collected. All branches and leaves were also weighed separately, and then samples of 500 g each were collected. All samples were taken back to the laboratory for measurement and analysis of the biomass. The information of the felled sample trees is presented in Table 2.

Table 1. Stand characteristics from temporary plots established in 5 Chinese fir plantations in Hunan province.

Sampling Forest Farm	Plot No.	Stand Age (a)	Age Group	Site Index	Mean Diameter at Breast Height (cm)	Average Total Tree Height (m)	Stand Density (Stems/ha)
Jingdong	JD-1	8	I	14	8.3	6.4	3250
	JD-2	17	II	14	12.1	10.8	2550
	JD-3	25	III	16	19	15	1200
	JD-4	27	IV	18	20.4	17.4	1175
	JD-5	31	IV	12	14.9	12.2	1675
	JD-6	40	V	14	19.9	16.5	950
Paiyashan	PYS-1	18	II	20	18.4	17.2	1875
	PYS-2	21	III	12	11.9	9.9	2025
	PYS-3	24	III	12	17	11.8	1350
	PYS-4	28	IV	14	15.8	12.9	1475
	PYS-5	29	IV	20	27.7	20.4	800
	PYS-6	36	V	16	24	19	1150
	PYS-7	39	V	20	25.2	23	975
Huangfengqiao	HFQ-1	14	II	14	11.4	9	2650
	HFQ-2	18	II	16	16.2	12.5	2125
	HFQ-3	24	III	18	22.5	17.9	1200
	HFQ-4	24	III	18	22.2	16	1325
	HFQ-5	33	IV	16	22.3	18.7	900
Shichangxi	SCX-1	8	I	12	7.9	6.1	3350
	SCX-2	13	II	16	14.8	10.3	2700
	SCX-3	17	II	16	17.2	11.8	2175
	SCX-4	23	III	16	19.8	13.3	1325
	SCX-5	27	IV	18	25	16.7	925
	SCX-6	31	IV	18	27.2	17.9	1075
Xishan	XS-1	9	I	12	8.5	6.4	2850
	XS-2	13	II	12	11	8	2750
	XS-3	21	III	18	22	14.7	1150
	XS-4	31	IV	14	21.8	14.6	950
	XS-5	39	V	14	22	14.7	825

I represents young stage ($\leq$10 years); II, middle stage (11–20 years); III, near mature (21–25 years); IV, mature (26–35 years); V, over mature ($\geq$36 years). Site index was represented by stand dominant height at a given base age of 20 years.

Table 2. Sample tree characteristics of temporary plots of Chinese fir plantation.

Age Group	Number of Sample Trees	Diameter Classes Range (cm)	Quadratic Mean Diameter (cm)	Average Total Tree Height (m)	Average Biomass (kg)					
					Total Tree	Stem	Branch	Leaf	Bark	Root
I	9	8–12	8.1	6.1	11.88	5.10	1.46	2.29	1.27	1.77
II	21	8–20	14.4	11.6	55.45	29.25	5.26	4.42	5.81	10.71
III	21	12–26	19.2	14.2	107.27	60.30	8.87	6.74	9.38	21.97
IV	24	14–32	21.9	16.8	180.90	103.37	13.24	9.80	17.62	36.87
V	12	18–26	22.7	17.6	171.29	104.11	9.88	6.47	16.37	34.46
Total	87	6–32	18.4	13.9	114.04	65.02	8.58	6.53	10.92	23.00

I represents young stage; II, middle stage; III, near mature; IV, mature; V, over mature.

2.4. Data Statistics and Analysis

The proportion (P_j) of the biomass of various organs (the upper, middle and lower segments of the stem and total stem, branch, leaf, bark, and root) to the total biomass of the sample tree was calculated.

$$P_j = \frac{W_j}{W_T} \times 100\% \quad (1)$$

where W_j and W_T represent the biomasses of the jth organ and the total sample tree, respectively.

Two-way analysis of variance (ANOVA) and Tukey's multiple comparisons were used to separate the significance differences ($p < 0.05$) among tree age and site index after the homogeneity of variance and normal distribution tests were passed (if the test of homogeneity of variance and normal distribution were not passed, the data were treated with inverse sine transformation, logarithmic transformation, etc.).

The influence process of tree age and site quality on biomass allocation was analysed using a generalized additive mixed model (GAMM) from the "gamm4" package in R software [40]. From the perspective of forest growth and yield, the site index, which is the mean height of dominant and codominant or top height trees at a given base age, is often used to quantify site quality [41]. The measurement of the site index was represented by the stand dominant height in this study. The GAMM is a semiparametric model with a linear predictor involving a sum of smooth functions of covariates, which allows flexible functional dependence of an outcome variable on covariates via nonparametric regression while accounting for correlation among observations using random effects [42]. GAMM is increasingly applied in ecological and environmental research [43] as follows:

$$E_{ijk} = K_0 + f_{ijk}(A_i) + f_{ijk}(S_i) + R_{ij} + \varepsilon_{ijk} \quad (2)$$

where E_{ijk} is the dependent variable (proportion of the kth organ biomass to the total biomass of the sample tree I in plot j), K_0 is the overall intercept, f_{ijk} (A_i) is a smooth function of tree age (A) corresponding to the kth organ, f_{ijk} (S_i) is a smooth function of site index (S) corresponding to the kth organ of sample tree i in plot j, R_{ij} is the random effect of the sample tree i in plot j which is assumed to be distributed as $N(0, \sigma^2)$ with a variance component σ^2, and ε_{ij} is an error vector.

3. Results

3.1. Allocation Pattern of Biomass in Different Organs

As shown in Table 3, in general, among the various organs of Chinese fir, the biomass of stems accounted for the largest proportion, ranging from 43.61% to 59.94% in different age groups, with an average of 54.61%, followed by roots, bark and branches, ranging from 15.12% to 20.46%, 9.21% to 10.87%, and 6.06% to 11.95% in different age groups, with averages of 19.45%, 10.20% and 8.34%, respectively. The biomass of leaves accounted for the smallest proportion, ranging from 3.88% to 18.45% in the different age groups, with an average of 7.41%. The proportion of biomass of each organ to the total biomass of trees in Chinese fir varies in different age groups. In other age groups, except for young-stage

forests, the proportion of biomass of various organs followed was stem, root, bark, branch and leaf, while in young-stage forests, the permutation order followed was stem, leaf, root, branch and bark.

Table 3. Proportion (%) of biomass of various organs in different age groups of Chinese fir.

Age Group	Total Tree	Stem	Lower Part of Stem	Middle Part of Stem	Upper Part of Stem	Branch	Leaf	Bark	Root
I	100.00	43.61	28.77	11.88	2.96	11.95	18.45	10.87	15.12
II	100.00	52.47	31.77	16.47	4.24	9.50	8.18	10.89	18.96
III	100.00	55.59	32.59	18.33	4.67	8.37	6.37	9.21	20.46
IV	100.00	57.08	33.37	18.93	4.77	7.09	5.26	10.17	20.41
V	100.00	59.94	34.30	20.24	5.40	6.06	3.88	10.26	19.85
Total	100.00	54.61	32.44	17.65	4.52	8.34	7.41	10.20	19.45

I represents young stage; II, middle stage; III, near mature; IV, mature; V, over mature.

For tree stems, the biomass of the lower part accounts for the largest proportion, ranging from 57.45% to 65.96% in different age groups, with an average of 59.60%, followed by the middle part, ranging from 27.26% to 33.78% in different age groups, with an average of 32.15%. The biomass of the upper part accounts for the smallest proportion, ranging from 6.78% to 8.77% in different age groups, with an average of 8.25% (Figure 2).

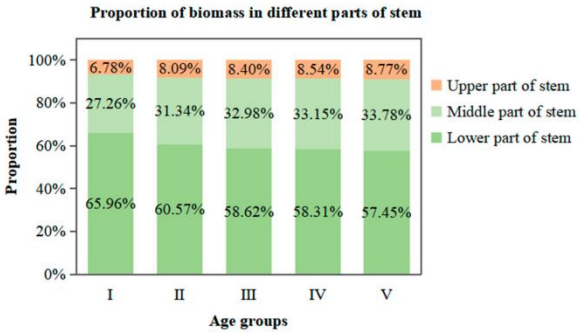


Figure 2. The proportion of biomass of different parts of stems in different age groups of Chinese fir.

3.2. Dynamic Changes in Biomass Allocation Patterns

The biomass of each organ and total tree all increased with time and gradually reached a stable level after maturity, but the proportion of biomass of different organs changed differently with time. The results of variance analysis showed that tree age had a significant effect on the proportion of biomass of tree stems ($p < 0.001$). The proportion of stem biomass nonlinearly increased with increasing tree age, showing an "S-shaped" curve similar to the growth process of tree diameter (Figure 3a). In addition, for tree stems, the proportion of biomass in the lower part decreased gradually with increasing tree age, while the proportion of biomass in the middle and upper parts increased gradually with increasing tree age. Tree age also had a significant effect on the proportion of branch, leaf and root biomass ($p < 0.001$). The proportion of biomass of branches and leaves decreased with increasing tree age, but their change process was different (Figure 3b,c). For roots, the proportion of biomass first increased as the tree age increased before the trees matured and then decreased with the increase in tree age after the trees reached maturity (Figure 3d). For bark, the biomass proportion was also affected by tree age ($p < 0.05$), and it first decreased and then increased with increasing tree age, showing a slightly concave curve (Figure 3e).

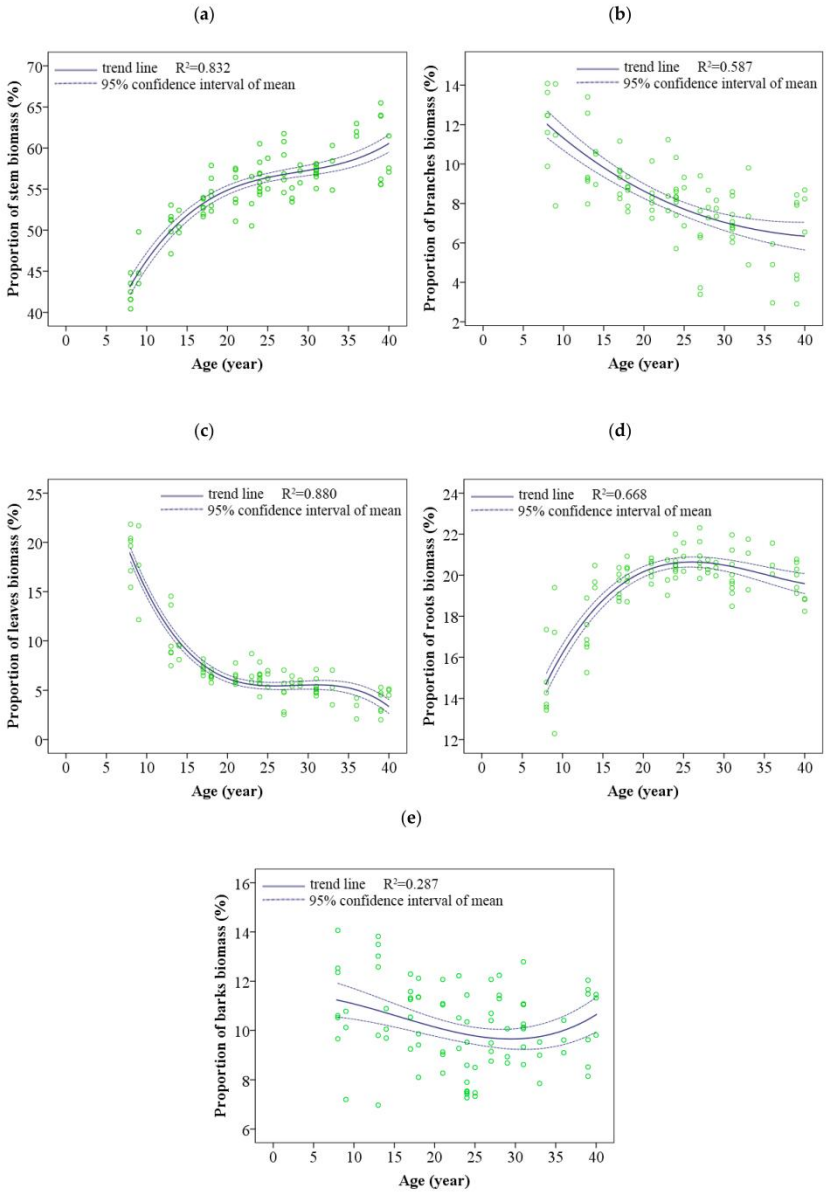


Figure 3. The change trend of the proportion of biomass for various organs with tree age in Chinese fir. (**a**) Stem, (**b**) branches, (**c**) leaves, (**d**) roots, and (**e**) bark.

3.3. Effects of Site Quality on Biomass Allocation Pattern

The proportion of stem biomass was influenced by site quality ($p < 0.001$). The fitting results of the generalized additive mixed models showed that the proportion of stem biomass was nonlinearly correlated with site quality and, to be more precise, the proportion of stem biomass decreased slightly with the increase in the site index when the site index was less than 16 and increased with the increase in site index when the site index was greater than 16 (Figure 4a). Site quality also had a significant effect on the proportion of bark biomass ($p < 0.001$) and branch biomass ($p < 0.001$). Significant linearities were observed among the proportion of bark biomass and site index (Figure 4b), and the proportion of bark biomass decreased with increasing site index. The proportion of branch biomass was nonlinearly correlated with the site index; it increased slightly with the increase in the site index when the site index was less than 16 and decreased with the increase in the site index when the site index was greater than 16 (Figure 4c).

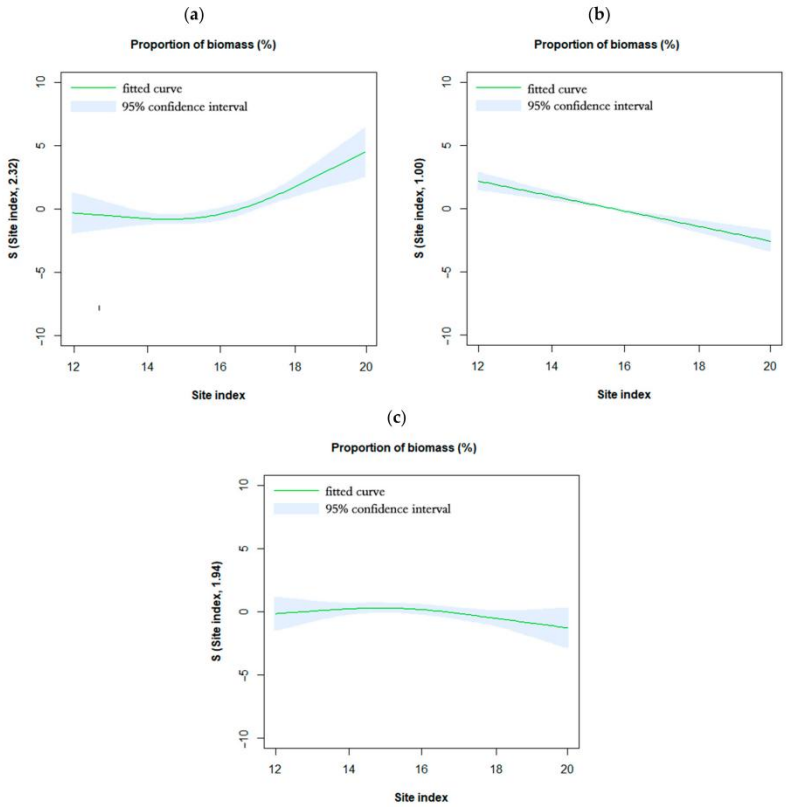


Figure 4. Observed effects of site quality on the proportion of biomass of different tree organs in GAMM. (**a**) Stem, (**b**) bark, and (**c**) branches. The S () of the vertical axis represents the smooth spline functions; The numbers in brackets represent the estimated degrees of freedom with smooth spline functions, 1 represents linearity, greater than 1 represents nonlinearity, and the larger the number, the stronger the nonlinearity.

Although the proportion of leaf biomass decreased gradually with increasing site index, there was no significant difference in the proportion of biomass of leaves between the different site indices ($p > 0.1$). In addition, although the proportion of root biomass increased gradually with increasing site index, there was also no significant difference in the proportion of biomass of roots between the different site indices ($p > 0.1$).

3.4. Interaction of Site Quality and Tree Age on Biomass Allocation

The results of variance analysis showed that the interaction of tree age and site quality had a significant effect on the proportion of stem biomass ($p < 0.05$) as well as on the proportion of root biomass ($p < 0.05$). As shown in Figure 5a, both tree age and site condition affected the proportion of stem biomass, but the influence of tree age was greater. The proportion of stem biomass increased with increasing tree age, and better site conditions could accelerate this trend. Although the proportion of root biomass was not significantly affected by site quality ($p > 0.1$), it was still affected by the interaction of site quality and tree age (Figure 5b). Before the trees mature, the influence of site quality and tree age on the proportion of root biomass was in the same direction; that is, better site conditions and greater age would increase the proportion of root biomass. After the trees mature, their influence on the proportion of root biomass was in the opposite direction; that is, better site conditions would increase the proportion of root biomass, while older age would reduce the proportion of root biomass.

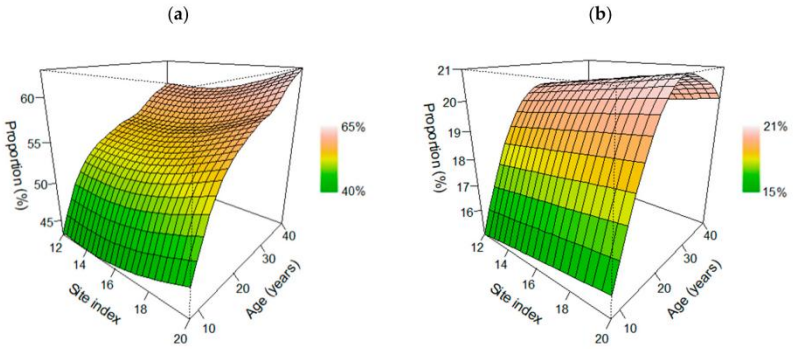


Figure 5. Observed proportion of biomass of stems and roots with site index and time interactions by GAMM. (**a**) Represents stems, (**b**) represents roots.

4. Discussion

How trees allocate their biomass among stems, branches, leaves, barks and roots has important implications not only for their survival and growth but also for ecosystem carbon cycling [35]. Measuring the status of and change in forest biomass is critical to the establishment of forest management policies [4].

The share of biomass components varies across tree species [44]. The results of this study showed that stem biomass had the largest contribution to total tree biomass in all age groups of Chinese fir, which is consistent with some published studies related to Chinese fir [45,46] and other species [47,48]. The biomass proportion of other organs varied with forest age. In the young-stage forests, the permutation order of the biomass proportion of other organs was as follows: leaves, roots, branches and bark, whereas in the forests of other age groups, the sequence was as follows: roots, bark, branches and leaves. As far as the stem, its biomass was mainly concentrated in the lower part, accounting for approximately 60%, followed by the middle and upper parts, accounting for approximately 30% and 10%, respectively.

The biomass of total trees and each organ in Chinese fir all increased with forest growth and gradually reached a stable level after maturity, but the biomass proportion of different organs varied with time. Some existing studies indicated that stem biomass had constant relationships with total biomass [49]. However, this study showed that as the trees grew larger, the relative contribution of the stem to the total biomass increased, which agreed with many previous studies [29,45,47,48,50]. We found that the proportion of stem biomass increased nonlinearly with increasing tree age in an "S" curve similar to the growth process of tree diameter, which indicated that it was feasible to use diameter as an independent variable to predict stem biomass. When the stem was divided into upper, middle and lower parts, we found that the biomass proportion of the lower part decreased gradually, whereas the biomass proportion of the middle and upper parts increased gradually, which could be explained by the fact that the trees shifted their stem increment upwards and became more cylindrical and less tapered over time [51,52].

For other organs, the dynamics of their biomass proportions were relatively complex. For branches, it is generally believed that the biomass proportion decreases with stand age [29,47,48,53], but some researchers have shown the reverse conclusion [47,54]. Our study results support the former; additionally, we further revealed that the proportion of branch biomass decreases not linearly but nonlinearly with age. Stem biomass usually accumulates at the expense of leaf biomass [44,55], and most studies have indicated that leaf biomass proportion decreases with stand age or tree size [29,47,48,50,54,56]. We have observed the same conclusion. The leaf biomass proportion decreased with increasing tree age, which could be explained by the fact that leaves are grown on younger branches rather than on older branches, which implies that the leaf mass per unit branch mass decreases as trees grow larger [57]. However, some studies claimed that tree age and size had no significant effect on leaf biomass proportion [49,53]. For bark, some studies have shown that the biomass proportion decreases with increasing tree age [47], but others have observed that the proportion of its biomass decreases first, then increases, and then decreases with age [45]. In this study, the biomass proportion first decreased and then increased with increasing tree age, showing a slightly concave curve. These results indicate that the changes in the proportion of bark biomass are more complex, and the factors that cause these changes need to be further studied.

Belowground components are not often evaluated because it is very difficult, expensive and time-consuming to collect root biomass data, especially for large trees [36,58]. However, roots may account for a significant proportion of the whole tree biomass and carbon storage [59,60], which has essential effects on the biomass proportion of various organs of trees. Previous studies have shown that the biomass proportions of roots to total tree biomass decreased with stand age [34,48,53]. However, some studies have observed that its biomass proportion exhibited a slight increase with tree growth [50]. However, our research found that the proportion of root biomass increased with increasing age and then decreased gradually after the trees matured. Generally, the part below the ground from the cutting place is treated as roots, so the root usually contains part of the stem, and the root has more knots and higher density [61], which may be the reason why the proportion of root biomass increases with age.

In addition to tree age, the allocation pattern of forest biomass in different organs is also affected by the origin of forest [62], stand density [31,63], site conditions [34] and management activities [64]. Because the selected forests in this study were all plantations with the same or close initial planting density and the same management measures are used throughout the rotation period, this study only considered the influence of site quality on biomass distribution but did not consider the influence of stand density on biomass distribution. The results showed that site quality had significant effects on the biomass proportion of stems, branches and bark but had no significant effects on the biomass proportion of leaves and roots. According to the “optimal partitioning theory”, plants preferentially allocate biomass to organs that harvest the most limiting resource [65,66]. Some studies suggested that trees allocated more biomass to the stems and leaves in the

fertile sites, while allocation to belowground components became more important in the barren site [58]. Our study showed that the proportion of stem biomass increased with an increasing site index when the site index was greater than 16, which is partly consistent with the optimal partitioning theory. However, the proportion of stem biomass decreased slightly with the increase in the site index when the site index was less than 16, which may be due to the fight result of light limiting and nutrients or water limiting [32,58]. The proportion of bark biomass linearly decreased with an increasing site index, which seems completely consistent with the optimal partitioning theory. This may indicate that water and nutrients are more abundant with the improvement of site quality, and trees can invest fewer resources for the carrier of water and nutrient transportation, namely barks. Contrary to the change in stem biomass proportion, the biomass proportion increased slightly with the increase in site index when the site index was less than 16 and decreased with the increase in site index when the site index was greater than 16.

Differences in biomass allocation may be explained by both the tree age and strategies of trees to maximize light, nutrient and water capture for survival in different site conditions [19,20,67]. Our results also showed that the interaction of tree age and site quality had a significant effect on the proportion of stem biomass as well as root biomass, which made the change dynamics of biomass allocation more complex. Site quality is defined by many factors, including altitude and temperature and the availability of water and nutrients [58], while biomass allocation involves the coordination among different organs, and their interrelationships and dynamics are complex. Therefore, more detailed research on the biomass allocation and temporal dynamics of specific tree species in different habitats is necessary.

Biomass is the basis of carbon storage. Since the collection of biomass data is time-consuming and laborious, the usual method is to estimate the biomass of the tree stem through some factors that are easily available such as diameter and tree height, and the biomass of other organs is calculated according to the stem biomass and the corresponding proportion coefficients [6]. For a given tree species, these proportional coefficients are often considered to be fixed [8]. However, our research showed that the ratio of the biomass of various organs to the total tree biomass was varied, and the ratio of the biomass of each organ to the biomass of the stem would also change accordingly, which was affected by tree age and site quality. Therefore, accurate estimation of forest biomass needs to consider the effect of forest age and site on the allocation patterns of tree biomass. In addition, the same coefficient is often used for the carbon content rate of various organs when estimating tree carbon storage by biomass, but in fact, the carbon content rates of different organs are different [68,69]. More accurate estimation of forest carbon storage should be the accumulation of carbon storage of each organ, which comes from the multiplication of their corresponding biomass and carbon content, and this study can provide a better insight into this method.

5. Conclusions

Comprehensively understanding the spatial allocation pattern and change dynamics of biomass in specific tree species is essential for establishing accurate estimation models of forest biomass and carbon storage, particularly against the vision of carbon peak and carbon neutralization. This study revealed the biomass allocation and its temporal dynamics of Chinese fir plantations, as well as the effect of site quality on them. The biomass proportion of various organs of Chinese fir varied nonlinearly with tree age. Therefore, the previous method of calculating the biomass of each organ through stem biomass and the constant conversion coefficient may increase the uncertainty and error for the estimation of forest biomass and carbon storage. Site quality also had a positive effect on the biomass proportion of stems, and a negative effect on the biomass proportion of branches and bark but had no significant effects on the biomass proportion of leaves and roots. Moreover, the interaction of tree age and site quality had a significant effect on the proportion of stem biomass as well as root biomass. Therefore, to obtain accurate estimates of Chinese fir forest biomass

and carbon stocks, age-specific changes and the influence of site on it need to be considered. Our results are helpful to better understand the formation and change mechanism of forest biomass, therefore providing in-depth insights for more accurate biomass estimation model establishment and carbon stock determination.

Author Contributions: Conceptualization, C.D., Q.L. and F.M.; methodology, C.D. and Q.L.; software, C.D. and J.T.; validation, B.Z. and J.T.; formal analysis, X.X.and B.Z.; investigation, C.D., X.X. and B.Z.; resources, F.M. and J.T.; data curation, X.X.; writing—original draft preparation, C.D.; writing—review and editing, Q.L.; visualization, C.D. and X.X.; supervision, Q.L.; project administration, Q.L. and F.M.; funding acquisition, F.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Comprehensive monitoring of forest and grass ecology-growth rate of main tree species (xczhz2022-09), the Forestry Science and Technology Innovation Fund Project of Hunan province (XLK202104-1), and the Hunan forestry engineering science and technology support project (ly2021-02).

Data Availability Statement: Not applicable.

Acknowledgments: We appreciate the staff of Five forest farm for their help during the field survey.

Conflicts of Interest: The authors declare that there are no conflict of interest.

References

1. Miner, R.; Abt, R.; Bowyer, J.; Buford, M.; Malmsheimer, R.; O'Laughlin, J.; Oneil, E.; Sedjo, R.; Skog, K. Forest carbon accounting considerations in US bioenergy policy. *J. For.* **2014**, *112*, 591–606.
2. Birdsey, R.; Pregitzer, K.; Lucier, A. Forest carbon management in the United States: 1600–2100. *J. Environ. Qual.* **2006**, *35*, 1461–1469. [CrossRef]
3. Fang, J.; Chen, A.; Peng, C.; Zhao, S.; Ci, L. Changes in forest biomass carbon storage in China between 1949 and 1998. *Science* **2001**, *292*, 2320–2322. [CrossRef] [PubMed]
4. McKinley, D.; Ryan, M.; Birdsey, R.; Giardina, C.; Harmon, M.; Heath, L.; Houghton, R.; Jackson, R.; Morrison, J.; Murray, B.; et al. A synthesis of current knowledge on forests and carbon storage in the United States. *Ecol. Appl.* **2011**, *21*, 1902–1924. [CrossRef] [PubMed]
5. Lu, D. The potential and challenge of remote sensing-based biomass estimation. *Int. J. Remote Sens.* **2006**, *27*, 1297–1328. [CrossRef]
6. Weiskittel, A.; MacFarlane, D.; Radtke, P.; Affleck, D.; Temesgen, H.; Woodall, C.; Westfall, J.; Coulston, J. A call to improve methods for estimating tree biomass for regional and national assessments. *J. For.* **2015**, *113*, 414–424. [CrossRef]
7. Planck, N.; MacFarlane, D. A vertically integrated whole-tree biomass model. *Trees-Struct. Funct.* **2015**, *29*, 449–460. [CrossRef]
8. Domke, G.; Woodall, C.; Smith, J.; Westfall, J.; McRoberts, R. Consequences of alternative tree-level biomass estimation procedures on U.S. forest carbon stock estimates. *For. Ecol. Manag.* **2012**, *270*, 108–116. [CrossRef]
9. Le Toan, T.; Quegan, S.; Davidson, M.; Balzter, H.; Paillou, P.; Papathanassiou, K.; Plummer, S.; Rocca, F.; Saatchi, S.; Shugart, H.; et al. The biomass mission: Mapping global forest biomass to better understand the terrestrial carbon cycle. *Remote Sens. Environ.* **2011**, *115*, 2850–2860. [CrossRef]
10. Fang, J.; Guo, Z.; Hu, H.; Kato, T.; Muraoka, H.; Son, Y. Forest biomass carbon sinks in East Asia, with special reference to the relative contributions of forest expansion and forest growth. *Glob. Chang. Biol.* **2014**, *20*, 2019–2030. [CrossRef]
11. Kurz, W.A.; Beukema, S.J.; Apps, M.J. Estimation of root biomass and dynamics for the carbon budget model of the Canadian forest sector. *Can. J. For. Res.* **1996**, *26*, 1973–1979. [CrossRef]
12. van Breugel, M.; Ransijn, J.; Craven, D.; Bongers, F.; Hall, J. Estimating carbon stock in secondary forests: Decisions and uncertainties associated with allometric biomass models. *For. Ecol. Manag.* **2011**, *262*, 1648–1657. [CrossRef]
13. Rutishauser, E.; Noor'an, F.; Laumonier, Y.; Halperin, J.; Hergoualc'h, K.; Verchot, L. Generic allometric models including height best estimate forest biomass and carbon stocks in Indonesia. *For. Ecol. Manag.* **2013**, *307*, 219–225. [CrossRef]
14. Ketterings, Q.M.; Coe, R.; van Noordwijk, M.; Ambagau', Y.; Palm, C.A. Reducing uncertainty in the use of allometric biomass equations for predicting above-ground tree biomass in mixed secondary forests. *For. Ecol. Manag.* **2001**, *146*, 199–209. [CrossRef]
15. Goodman, R.; Phillips, O.; Baker, T. The importance of crown dimensions to improve tropical tree biomass estimates. *Ecol. Appl.* **2014**, *24*, 680–698. [CrossRef] [PubMed]
16. McTague, J.; Weiskittel, A. Evolution, history, and use of stem taper equations: A review of their development, application, and implementation. *Can. J. For. Res.* **2021**, *51*, 210–235. [CrossRef]
17. Hame, T.; Salli, A.; Andersson, K.; Lohi, A. A new methodology for the estimation of biomass of coniferdominated boreal forest using NOAA AVHRR data. *Int. J. Remote Sens.* **1997**, *18*, 3211–3243. [CrossRef]
18. Powell, S.; Cohen, W.; Kennedy, R.; Healey, S.; Huang, C. Observation of trends in biomass loss as a result of disturbance in the conterminous US: 1986–2004. *Ecosystems* **2014**, *17*, 142–157. [CrossRef]

19. Liao, R.Y.; Wu, X.Q.; Jin, C.; Huang, L.; Qian, S.H.; Yang, Y.C. Effect of canopy condition on *Machilus nanmu* seedling configuration and biomass allocation. *Chin. J. Appl. Ecol.* **2021**, *32*, 2061–2069. (In Chinese)
20. Potkay, A.; Trugman, A.; Wang, Y.; Venturas, M.; Anderegg, W.; Mattos, C.; Fan, Y. Coupled whole-tree optimality and xylem hydraulics explain dynamic biomass partitioning. *New Phytol.* **2021**, *230*, 2226–2245. [CrossRef]
21. Agathokleous, E.; Belz, R.; Kitao, M.; Koike, T.; Calabrese, E. Does the root to shoot ratio show a hormetic response to stress? An ecological and environmental perspective. *J. For. Res.* **2019**, *30*, 1569–1580. [CrossRef]
22. Poorter, H.; Niklas, K.; Reich, P.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytol.* **2012**, *193*, 30–50. [CrossRef] [PubMed]
23. Brown, S.L.; Schroeder, P.; Kern, J.S. Spatial distribution of biomass in forests of the eastern USA. *For. Ecol. Manag.* **1999**, *123*, 81–90. [CrossRef]
24. Rodriguez-Veiga, P.; Saatchi, S.; Tansey, K.; Balzter, H. Magnitude, spatial distribution and uncertainty of forest biomass stocks in Mexico. *Remote Sens. Environ.* **2016**, *183*, 265–281. [CrossRef]
25. Mitchard, E.T.; Saatchi, S.S.; Baccini, A.; Asner, G.P.; Goetz, S.J.; Harris, N.L.; Brown, S. Uncertainty in the spatial distribution of tropical forest biomass: A comparison of pan-tropical maps. *Carbon Balanc. Manag.* **2013**, *8*, 10. [CrossRef]
26. Verkerk, P.; Fitzgerald, J.; Datta, P.; Dees, M.; Hengeveld, G.; Lindner, M.; Zudin, S. Spatial distribution of the potential forest biomass availability in Europe. *For. Ecosyst.* **2019**, *6*, 5. [CrossRef]
27. Pan, Y.; Birdsey, R.; Phillips, O.; Jackson, R. The structure, distribution, and biomass of the world's forests. *Annu. Rev. Ecol. Evol. Syst.* **2013**, *44*, 593–622. [CrossRef]
28. Poorter, H.; Jagodzinski, A.; Ruiz-Peinado, R.; Kuyah, S.; Luo, Y.; Oleksyn, J.; Usoltsev, V.; Buckley, T.; Reich, P.; Sack, L. How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. *New Phytol.* **2015**, *208*, 736–749. [CrossRef]
29. Hu, M.; Lehtonen, A.; Minunno, F.; Makela, A. Age effect on tree structure and biomass allocation in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.). *Ann. For. Sci.* **2020**, *77*, 90. [CrossRef]
30. Xiang, W.; Li, L.; Ouyang, S.; Xiao, W.; Zeng, L.; Chen, L.; Lei, P.; Deng, X.; Zeng, Y.; Fang, J.; et al. Effects of stand age on tree biomass partitioning and allometric equations in Chinese fir (*Cunninghamia lanceolata*) plantations. *Eur. J. For. Res.* **2021**, *140*, 317–332. [CrossRef]
31. Wertz, B.; Bembenek, M.; Karaszewski, Z.; Ochal, W.; Skorupski, M.; Strzelinski, P.; Wegiel, A.; Mederski, P. Impact of stand density and tree social status on aboveground biomass allocation of Scots pine *Pinus sylvestris* L. *Forests* **2020**, *11*, 765. [CrossRef]
32. Lu, D.; Wang, G.; Yan, Q.; Gao, T.; Zhu, J. Effects of gap size and within-gap position on seedling growth and biomass allocation: Is the gap partitioning hypothesis applicable to the temperate secondary forest ecosystems in Northeast China? *For. Ecol. Manag.* **2018**, *429*, 351–362. [CrossRef]
33. Lie, Z.; Xue, L.; Jacobs, D. Allocation of forest biomass across broad precipitation gradients in China's forests. *Sci. Rep.* **2018**, *8*, 10536. [CrossRef]
34. Wang, X.; Huang, X.; Wang, Y.; Yu, P.; Guo, J. Impacts of site conditions and stand structure on the biomass allocation of single trees in Larch Plantations of Liupan Mountains of Northwest China. *Forests* **2022**, *13*, 177. [CrossRef]
35. Jevon, F.; Lang, A. Tree biomass allocation differs by mycorrhizal association. *Ecology* **2022**, *103*, 6. [CrossRef] [PubMed]
36. Zianis, D.; Muukkonen, P.; Mäkipää, R.; Mencuccini, M. *Biomass and Stem Volume Equations for Tree Species in Europe*; Tammer-Paino Oy: Tampere, Finland, 2005.
37. Deng, C.; Zhang, S.; Lu, Y.; Li, Q. Determining the ecological compensation standard based on forest multifunction evaluation and financial net present value analysis: A case study in Southwestern Guangxi, China. *J. Sustain. For.* **2020**, *39*, 730–749. [CrossRef]
38. Wang, Y.; Guo, C.; Chen, X.; Jia, L.; Guo, X.; Chen, R.; Zhang, M.; Chen, Z.; Wang, H. Carbon peak and carbon neutrality in China: Goals, implementation path and prospects. *China Geol.* **2021**, *4*, 720–746. [CrossRef]
39. National Forestry and Grassland Administration. *China Forest Resources Report*; China Forestry Publishing House: Beijing, China, 2019.
40. Wood, S.; Scheipl, F. gamm4: Generalized Additive Mixed Models Using "mgcv" and "lme4". R Package Version 0.2-6. Available online: https://cran.r-project.org/web/packages/gamm4/index.html (accessed on 31 December 2022).
41. Yang, Y.; Huang, S.; Vassov, R.; Pinno, B.; Chhin, S. Climate-sensitive height-age models for top height trees in natural and reclaimed oil sands stands in Alberta, Canada. *Can. J. For. Res.* **2020**, *50*, 297–307. [CrossRef]
42. Lin, X.; Zhang, D. Inference in generalized additive mixed modelsby using smoothing splines. *J. R. Stat. Soc. Ser. B-Stat. Methodol.* **1999**, *61*, 381–400. [CrossRef]
43. Groll, A.; Tutz, G. Regularization for generalized additive mixed models by likelihood-based boosting. *Methods Inf. Med.* **2012**, *51*, 168–177.
44. Mensah, S.; Kakai, R.; Seifert, T. Patterns of biomass allocation between foliage and woody structure: The effects of tree size and specific functional traits. *Ann. For. Res.* **2016**, *59*, 49–60. [CrossRef]
45. Tian, D.; Xiang, W.; Chen, X.; Yan, W.; Fang, X.; Kang, W.; Dan, X.; Peng, C.; Peng, Y. A long-term evaluation of biomass production in first and second rotations of Chinese fir plantations at the same site. *Forests* **2011**, *84*, 411–418. [CrossRef]
46. Zhou, L.; Shalom, A.; Wu, P.; He, Z.; Liu, C.; Ma, X. Biomass production, nutrient cycling and distribution in age-sequence Chinese fir (*Cunninghamia lanceolate*) plantations in subtropical China. *J. For. Res.* **2016**, *27*, 357–368. [CrossRef]

47. Meng, S.; Jia, Q.; Liu, Q.; Zhou, G.; Wang, H.; Yu, J. Aboveground biomass allocation and additive allometric models for natural *Larix gmelinii* in the western Daxing'anling Mountains, Northeastern China. *Forests* **2019**, *10*, 150. [CrossRef]
48. Yang, B.; Xue, W.; Yu, S.; Zhou, J.; Zhang, W. Effects of stand age on biomass allocation and allometry of *Quercus acutissima* in the Central Loess Plateau of China. *Forests* **2019**, *10*, 41. [CrossRef]
49. Peng, C.; Ai, W.; Qi, L.; Tu, J.; Meng, Y.; Yang, M.; Li, M. Cunninghamia lanceolata sprout population structure, dynamics and biomass allocation changes during *Phyllostachys edulis* expansion in middleo-southern China. *Trees-Struct. Funct.* **2022**, *36*, 1207–1218. [CrossRef]
50. Xiang, W.; Liu, S.; Deng, X.; Shen, A.; Lei, X.; Tian, D.; Zhao, M.; Peng, C. General allometric equations and biomass allocation of *Pinus massoniana* trees on a regional scale in southern China. *Ecol. Res.* **2011**, *26*, 697–711. [CrossRef]
51. Larson, P.R. Stem form development of forest trees. *For. Sci.* **1963**, *9*, 1–42. [CrossRef]
52. Kohler, S.; Koehler, H.; Figueiredo, A.; Arce, J.; Machado, S. Evolution of tree stem taper in *Pinus taeda* stands. *Cienc. Rural* **2016**, *46*, 1185–1191. [CrossRef]
53. Rather, S.A.; Qaisar, K.; Nabi, S.; Banyal, R.; Khan, P.; Islam, M. Biomass allocation and carbon stock in Elm (Ulmus Wallichiana Planch) plantation. *Curr. World Environ.* **2017**, *12*, 339–344. [CrossRef]
54. Xu, Z.; Du, W.; Zhou, G.; Qin, L.; Meng, S.; Yu, J.; Sun, Z.; SiQing, B.; Liu, Q. Aboveground biomass allocation and additive allometric models of fifteen tree species in northeast China based on improved investigation methods. *For. Ecol. Manag.* **2022**, *505*, 119918. [CrossRef]
55. Pajtik, J.; Konopka, B.; Lukac, M. Individual biomass factors for beech, oak and pine in Slovakia: A comparative study in young naturally regenerated stands. *Trees-Struct. Funct.* **2011**, *25*, 277–288. [CrossRef]
56. Konopka, B.; Pajtik, J.; Seben, V.; Surovy, P.; Merganicova, K. Biomass allocation into woody parts and foliage in young common aspen (*Populus tremula* L.)-trees and a stand-level study in the Western Carpathians. *Forests* **2020**, *11*, 464. [CrossRef]
57. Dimobe, K.; Mensah, S.; Goetze, D.; Ouedraogo, A.; Kuyahf, S.; Porembski, S.; Thiombiano, A. Aboveground biomass partitioning and additive models for *Combretum glutinosum* and *Terminalia laxiflora* in West Africa. *Biomass Bioenerg.* **2018**, *115*, 151–159. [CrossRef]
58. Veronica, G.; Luis, P.; Gerardo, R. Allometric relations for biomass partitioning of *Nothofagus antarctica* trees of different crown classes over a site quality gradient. *For. Ecol. Manag.* **2010**, *259*, 1118–1126.
59. Cairns, M.A.; Brown, S.; Helmer, E.H.; Baumgardner, G.A. Root biomass allocation in the world's upland forests. *Oecologia* **1997**, *111*, 1–11. [CrossRef]
60. Peichl, M.; Arain, M. Allometry and partitioning of above- and belowground tree biomass in an age-sequence of white pine forests. *For. Ecol. Manag.* **2007**, *253*, 68–80. [CrossRef]
61. Billard, A.; Bauer, R.; Mothe, F.; Jonard, M.; Colin, F.; Longuetaud, F. Improving aboveground biomass estimates by taking into account density variations between tree components. *Ann. For. Sci.* **2020**, *77*, 103. [CrossRef]
62. Baishya, R.; Barik, S.; Upadhaya, K. Distribution pattern of aboveground biomass in natural and plantation forests of humid tropics in northeast India. *Trop. Ecol.* **2009**, *50*, 295–304.
63. Dicus, C.; Dean, T.J. Stand density effects on biomass allocation patterns and subsequent soil nitrogen demand. In Proceedings of the Ninth Biennial Southern Silvilcultural Research Conference, Clemson, SC, USA, 25–27 February 1997; p. 564.
64. He, Y.; Xi, B.; Bloomberg, M.; Jia, L.; Zhao, D. Effects of drip irrigation and nitrogen fertigation on stand growth and biomass allocation in young triploid *Populus tomentosa* plantations. *For. Ecol. Manag.* **2020**, *461*, 1–8. [CrossRef]
65. Thornley, J.H.M. A balanced quantitative model for root: Shoot ratios in vegetative plants. *Ann. Bot.* **1972**, *36*, 431–441. [CrossRef]
66. Bloom, A.J.; Chapin, F.S.; Mooney, H.A. Resource limitation in plants—An economic analogy. *Annu. Rev. Ecol. Syst.* **1985**, *16*, 363–392. [CrossRef]
67. Morote, F.; Serrano, F.; Andres, M.; Rubio, E.; Jimenez, J.; de las Heras, J. Allometries, biomass stocks and biomass allocation in the thermophilic Spanish juniper woodlands of Southern Spain. *For. Ecol. Manag.* **2012**, *270*, 85–93. [CrossRef]
68. Thomas, S.C.; Martin, A.R. Carbon Content of Tree Tissues: A Synthesis. *Forests* **2012**, *3*, 332–352. [CrossRef]
69. Ma, S.; He, F.; Tian, D.; Zou, D.; Yan, Z.; Yang, Y.; Zhou, T.; Huang, K.; Shen, H.; Fang, J. Variations and determinants of carbon content in plants: A global synthesis. *Biogeosciences* **2018**, *15*, 693–702. [CrossRef]

3.4 Thinning Effects on the Tree Height-Diameter Allometry of Masson Pine (*Pimus massoniana* Lamb.)

forests

MDPI

Article

Thinning Effects on the Tree Height–Diameter Allometry of Masson Pine (*Pinus massoniana* Lamb.)

Cheng Deng [1], Shougong Zhang [2], Yuanchang Lu [3], Robert E. Froese [4], Angang Ming [5,6] and Qingfen Li [1,*]

1 College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China; cdeng@scau.edu.cn
2 Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China; sgzhang@caf.ac.cn
3 Research Institute of Forest Resource Information Techniques, Chinese Academy of Forestry, Beijing 100091, China; ylu@caf.ac.cn
4 College of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA; froese@mtu.edu
5 Experimental Center of Tropical Forestry, Chinese Academy of Forestry, Pingxiang 532600, China; mingangang0111@163.com
6 Guangxi Youyiguan Forest Ecosystem Research Station, Pingxiang 532600, China
* Correspondence: qfli@scau.edu.cn; Tel.: +86-189-9882-5286

Received: 2 November 2019; Accepted: 7 December 2019; Published: 10 December 2019

Abstract: The stem height–diameter allometric relationship is fundamental in determining forest and ecosystem structures as well as in estimating tree volume, biomass, and carbon stocks. Understanding the effects of silvicultural practices on tree height–diameter allometry is necessary for sustainable forest management, though the impact of measures such as thinning on the allometric relationship remain understudied. In the present study, the effects of thinning on tree height–diameter allometry were evaluated using Masson pine height and diameter growth data from a plantation experiment that included unthinned and thinned treatments with different intensities. To determine whether thinning altered the height–diameter allometry rhythm, the optimal height–diameter model was identified and dummy variable methods were used to investigate the differences among model parameters for different thinning treatments. Periodic (annual) allometric coefficients were calculated based on height and diameter increment data and were modeled using the generalized additive mixed model (GAMM) to further illustrate the response of tree height–diameter allometry to different thinning treatments over time. Significant differences were detected among the parameters of the optimal height–diameter model (power function) for different thinning treatments, which indicated that the pattern of the height–diameter allometry relationship of Masson pine was indeed altered by thinning treatments. Results also indicated a nonlinear trend in the allometric relationship through time which was significantly affected by thinning. The height–diameter allometric coefficient exhibited a unimodal convex bell curve with time in unthinned plots, and thinning significantly interfered with the original trend of the height–diameter allometric coefficient. Thinning caused trees to increase diameter growth at the expense of height growth, resulting in a decrease of the ratio of tree height to diameter, and this trend was more obvious as the thinning intensity increased.

Keywords: thinning intensity; allometry responses; height–diameter relationship; allometric equation; Masson pine

1. Introduction

Height and diameter are two basic dimensions of tree size that are fundamentally related to processes ranging from individual stem to whole-ecosystem scales [1]. The allocation pattern of tree

growth to height and stem diameter is an important structural trait of a tree that reflects its capacity to adapt to different environmental conditions and evolutionary competition [2–6]. For example, pioneer species typically have a larger height–diameter ratio, allowing them to quickly attain or maintain a position in the canopy [7,8]. Species that frequently encounter strong winds might evolve stouter stems to withstand the extreme weather [9].

As a key factor of stem form, the allometric relationship between tree height and diameter is thought to reflect the balance between growth and survival due to allocation strategies related to biomechanical and hydraulic constraints [10–13]. Tree height growth determines carbon gain via light capture [14], while stem diameter growth plays an important role in ensuring mechanical support and capacity for water absorption and transport [15–18]. Trees that invest less in mechanical support can grow faster and reach the canopy more quickly [19] but less structural support reduces the ability to resist elastic deformation and avoid buckling [20,21].

Many natural and biological factors affecting the tree height–diameter allometric relationship have been identified through research. For example, precipitation, temperature, geographic location, and site conditions have been shown to have significant effects on height–diameter allometry, and forest structure, tree species, and genetic variability within a species have also been shown to play a role [1,4,18,21–26]. However, our understanding is limited with regard to how anthropogenous measures, such as thinning, affect the tree height–diameter allometry in residual stems.

Thinning of a forest stand is a fundamental silvicultural tool used to achieve a variety of management objectives and is an integral part of even- and uneven-aged management for efficient and profitable production of timber products [27–29]. Traditionally, thinning has been used to increase production and improve the quality of residual trees by removing damaged, slow-growing, or unhealthy trees to promote more growing space and resource availability for healthy trees [30–33]. Thinning can also be used to promote forest understories and vertical structural characteristics [34–36] as well as alter the microenvironment, including altering light availability, temperature, evaporative demand, and soil properties [37,38].

Many investigators have indicated that thinning immediately stimulates diameter growth of residual stems [39–44], and it is generally believed that individual tree height growth of residual stems is less influenced by thinning than it is by diameter growth. However, the dynamics and relationship between periodic annual height growth and stand density following thinning are complex [45,46]. Some studies have suggested that height growth and total height are relatively unaffected by thinning [29,47–50]. On the other hand, other studies have observed thinning to have an obvious effect on tree height growth, especially at very high thinning intensities [27,51–55]. Some evidence has shown short-term decreases [56] followed by long-term increases [45,46] in height growth following thinning.

The height–diameter allometric relationship is a key factor determining tree volume, biomass, carbon storage, and wood structure [4,57]. In general, no matter how thinning affects diameter and height growth, it may be reasonable to expect that the allometric relationship of tree height and diameter will be different in thinned and unthinned stands. However, most existing studies on the influence of thinning on tree growth have often focused on how thinning affects tree height and diameter increments and have analyzed these two parameters separately [27,29,43,49,52]. We can only approximately deduce the influence of thinning on the tree height–diameter ratio from increment measurements, and we cannot obtain a detailed and accurate dynamic process of tree height–diameter allometry.

Masson pine is an important tree species of *Pinus* (Pinaceae) with a height of up to 45 m and a diameter at breast height of up to 1.5 m. The Pinaceae is the most diverse and widespread family of conifers, comprising 11 genera and about 230 species [58]. It dominates large areas of the Northern Hemisphere [59] and is one of the most important tree species for timber supply in the world, accounting for 42% of the world's industrial forest plantations [60]. As a native species, Masson pine has been widely planted for more than one thousand years due to its high-quality timber and occupies 13.2 percent of all forested land in China, covering 14.2 million ha. Masson pine and most other tree

species of Pinaceae are light-demanding pioneer species, so thinning is one important silvicultural practice used to manage the species. Studying the thinning effects of Masson pine is of great reference value for other Pinaceae species.

This study focuses on thinning effects on stem height–diameter allometry in Masson pine plantations, which are planted across a vast geographic area in China. We tested the hypothesis that thinning would affect the height–diameter allometric relationship of Masson pine. After the hypothesis was confirmed, an analysis of thinning effects on tree height and diameter allometry was conducted over a long-term scale. We expect the results to provide insight into the application of thinning so that it can produce the optimal tree height–diameter allometric relationship.

2. Materials and Methods

2.1. Data Description

We used data from a thinning experiment in Masson pine plantations which was established in 1993 at the Experimental Center of Tropical Forestry, which is located in Pingxiang City of the Guangxi Zhuang Autonomous Region, southwestern China (coordinates 21°57′ N, 106°39′ E to 22°19′ N, 106°59′ E). The experiment was installed with uniform environment and site conditions (site index 16 m and base age 20) and implemented in a randomized block design with five blocks and four treatments per block, totaling 20 experimental units. The landform of the area is low hills with an elevation of 400–450 m above sea level. The soils are latosolic red loams developed from granite with a clay–loam texture, a stoniness of about 6%, and a mean depth of 90 cm. In each block four permanent plots were installed. Each unit had an area of 2500 m^2 and was randomly assigned to one of four treatment categories: (1) T_0, unthinned control plot, (2) T_1, lightly thinned plot (approximately 30% of the planted Masson pine basal area removed), (3) T_2, moderately thinned plot (approximately 50% of the basal area removed), and (4) T_3, heavily thinned plot (approximately 70% of the basal area removed). In each plot, a fixed subplot of 400 m^2 was set up for tree growth measurements. Thinning and plot establishment were performed during the dormant season of 2007 at the age of 15 years. All thinning was performed from below and the height (H) and diameter at breast height (DBH) of all trees ≥5.0 cm were measured using Blume-Leiss hypsometer and diameter tape, respectively, before thinning. During thinning, inferior trees were removed, i.e., the smallest trees in height and/or diameter or trees that were crooked, forked, and/or broken were removed. When choosing which trees to remove emphasis was also given to achieving the most uniform spacing between residual trees as possible. Subplots have been measured every two years since 2008. To date, five remeasurements have been completed, a summary of which is given in Table 1. The growth data of tree height and stem diameter before 2007 were obtained through stem analysis of fifteen mean trees from thinned plots. In all analyses, trees that died (mainly in unthinned plots; very few died in thinned plots) during the observation period were ignored.

Table 1. Average diameter and height data of sample trees from different thinning treatments.

Treatment	Stand Density (Stems/ha)	Number of Sample Trees	DBH			H		
			2007b	2007a	2016	2007b	2007a	2016
T_0	1400	224	17.3	17.3	25.2	12.2	12.2	16.0
T_1	1375	117	17.5	18.3	29.1	12.3	12.7	17.5
T_2	1425	71	17.2	18.5	31.4	12.2	12.9	18.1
T_3	1325	49	17.7	19.9	34.1	12.5	13.4	19.1

Legend: T_0, unthinned; T_1, lightly thinned; T_2, moderately thinned; T_3, heavily thinned; DBH, mean diameter at breast height (cm); H, average total tree height (m); b, before thinning; a, after thinning for the residual stand. Stand density represents the number of trees per hectare before thinning. The number of sample trees represents the number of trees in the fixed subplots after thinning.

2.2. Testing the Effects of Thinning on the Height–Diameter Allometry Rhythm

2.2.1. Mathematical Modeling of Height–Diameter Allometry

In this study, the five most frequently used function forms in published research (Table 2) were tested and compared for their reliability and predictive ability [61,62].

Table 2. Models used to explore the relationship of height–diameter allometry.

Model	Function	References
Gompertz	$H = \alpha \cdot \exp(-\beta \cdot \exp(-\gamma \cdot D)$	Winsor, 1932 [63]
Logistic	$H = \alpha / (1 + \beta \cdot \exp(-\gamma \cdot D)$	Pearl and Reed, 1920 [64]
Power	$H = \alpha \cdot D^{\beta}$	Huxley, 1932 [65]
Richards	$H = 1.3 + \alpha \cdot (1 - \exp(-\beta \cdot D))^{\gamma}$	Richards, 1959 [66]
Wykoff	$H = 1.3 + \exp(\alpha + \beta / (1 + D))$	Wykoff et al., 1982 [67]

Legend: H, total tree height (m); D, diameter at breast height (cm); α, β, and γ, parameters to be estimated; exp, the exponential function. 1.3 is a constant used to account for the fact that D is measured at 1.3 m above the ground.

Model parameters were estimated using the "nls" function of the "nlme" package in the statistical environment R (version 3.1-137) [68]. The best model was selected using Akaike information criterion (AIC) [69,70], residual standard error (RSE), and coefficient of determination (R^2). Overall, models with higher R^2, lower AIC, and lower RSE were preferred [71,72].

2.2.2. Dummy Variable Models and Standard F-tests

To evaluate the effects of thinning on height–diameter allometry, the dummy variable method and nested model *F*-tests [73] were used to determine whether the thinning treatments altered height–diameter allometry. Dummy variables were created: (1) $p_1 = 1$ denotes the thinning treatment T_1 and 0 the rest of the cases, (2) $p_2 = 1$ denotes the thinning treatment T_2 and 0 the rest of the cases, and (3) $p_3 = 1$ denotes the thinning treatment T_3 and 0 the rest of the cases. The thinning treatment T_0 is represented by $p_1 = p_2 = p_3 = p_4 = 0$. Thus, the thinning treatments were introduced into the basic model Equation (1) and the extended models Equations (2) and (3) were obtained, i.e.,

$$H = \alpha_0 \times D^{\beta_0} \tag{1}$$

$$H = (\alpha_0 + \alpha_1 p_1 + \alpha_2 p_2 + \alpha_3 p_3) \times D^{\beta_0} \tag{2}$$

$$H = (\alpha_0 + \alpha_1 p_1 + \alpha_2 p_2 + a_3 p_3) \times D^{(\beta_0 + \beta_1 p_1 + \beta_2 p_2 + \beta_3 p_3)} \tag{3}$$

where H and D are the total tree height and diameter at breast height, respectively, and α_i and β_i are the model parameters to be estimated. The F test statistic was used to determine whether thinning treatments had a significant effect on each parameter, i.e.,

$$F = \left(\frac{RSS_1 - RSS_0}{RSS_1} \right) \times \left(\frac{df_1}{df_1 - df_0} \right) \tag{4}$$

where RSS_1 and df_1 are the residual sum of squares and degrees of freedom, respectively, of the extended model in which the thinning treatment was introduced, and RSS_0 and df_0 are the residual sum of squares and degrees of freedom, respectively, of the basic model in which the thinning treatment was not introduced.

2.3. Analysis of the Temporal Dynamics of Thinning Effects on Height–Diameter Allometry

2.3.1. Calculation of Relative Increments and Allometric Coefficient

To examine the temporal dynamics of the height–diameter allometry relationship more subtly, relative increments were used as a prerequisite for quantification of the allometric relationship [74] in this study. The periodic (annual) height–diameter allometric coefficient, which is widely used as distribution coefficient for growth resources between tree height and diameter, was calculated for the time series data of long-term observation plots [24,75], i.e.,

$$m_{h,d} = \frac{\ln(h_t) - \ln(h_{t-\Delta t})}{\ln(d_t) - \ln(d_{t-\Delta t})} \quad (5)$$

where $m_{h,d}$ is the allometric coefficient, and h_t, $h_{t-\Delta t}$ and d_t, $d_{t-\Delta t}$ are the tree height and stem diameter at breast height, respectively, at two subsequent points in the time of measurement, t and t-Δt.

Equation (5) reflects how the relative growth of one growth quantity, h, is correlated with the relative growth of another, d, by the periodic allometric coefficient $m_{h,d}$. In the case of the allometric coefficient, $m_{h,d} = 1$ indicates isometric growth and that relative increment values of both tree height and diameter at breast height are equal. If $m_{h,d} < 1$, the relative height increment is lower than the relative diameter increment and a negative allometric relationship is observed. If $m_{h,d} > 1$, the relative height increment is greater than the relative diameter increment, indicating a positive allometric relationship [76].

2.3.2. Generalized Additive Mixed Model Analysis

The allometric relationship of tree height versus diameter at breast height may vary during tree ontogeny [20,24], so the influence of time on allometric coefficient was considered when analyzing the effect of thinning on the height–diameter allometric relationship. To scrutinize the dynamic process of thinning effects on height–diameter allometry over time, the height–diameter allometric relationships of different thinning treatments were modeled using the generalized additive mixed model (GAMM) (version 0.2-5) from the "gamm4" package in R [68].

GAMM is a semiparametric model with a linear predictor involving a sum of smooth functions of covariates which allows flexible functional dependence of an outcome variable on covariates by using nonparametric regression while accounting for correlation between observations by using random effects [77]. GAMM has been increasingly applied in ecological and environmental research [78], as, i.e.,

$$m_{ijk} = K_0 + T_i + f_i(t_{ijk}) + R_{ij} + \varepsilon_{ijk} \quad (6)$$

where m_{ijk} is the dependent variable (periodic height–diameter allometric coefficient corresponding to the kth measurement of the jth tree of thinning treatment i), K_0 is the model intercept, T_i is the fixed effect of thinning treatment i, $f_i(t_{ijk})$ is a smooth function of tree age (t) corresponding to the kth measurement of the jth tree in thinning treatment i, R_{ij} is the random effect of the jth tree which is assumed to be distributed as $N(0, \sigma^2)$ with a variance component σ^2, and ε_{ijk} is a vector of errors.

Time series data may be auto-correlated; using models which do not consider this autocorrelation can cause inaccurate parameter estimation or inadequate quantification of uncertainty [79]. Since several height–diameter allometric coefficients were calculated for an individual tree, the allometric coefficients of individuals tended to be more similar than those computed for other trees [24]. Among the variety of correlation patterns in this context, first order auto-regressive structure (*AR1*) is often utilized [80]. Hence, the existence of any auto-correlation at the tree level (between the calculated height–diameter allometric coefficients of the same tree) was considered by the (*AR1*) of the GAMM [24,68,81–83].

3. Results

3.1. Overall Trends of Thinning Effects on Height–Diameter Allometry

3.1.1. The Best Height–Diameter Allometric Model

By fitting all thinned and unthinned survey data and comparing the series of commonly used height–diameter equations given above according to AIC, RSE, and R^2 values, we concluded that the power function optimizes the best fit of height–diameter allometric relationships for Masson pine (Table 3).

Table 3. Fitting results of height–diameter allometry relationship models.

Model	α	β	γ	AIC	RSE	R^2
Gompertz	21.949	1.990	0.073	11,764.952	0.863	0.896
Logistic	21.490	4.186	0.107	12,329.021	0.918	0.879
Power	2.186	0.612	-	10,960.653	0.791	0.906
Richards	32.966	0.019	0.851	11,055.019	0.801	0.903
Wykoff	3.258	−15.083	-	12,082.396	0.893	0.892

Legend: AIC, Akaike information criterion; RSE, residual standard error; R^2, coefficient of determination. α, β, and γ are model parameters.

3.1.2. Overall Effects of Thinning on Height–Diameter Allometry

As shown in Table 4, the parameters of height–diameter allometry models under different thinning treatments were different. The *F*-test results (Table 5) of the dummy variable model detected a significant difference between the coefficients of the basic model Equation (1) and extended models Equations (2) and (3). This difference indicated that the pattern of the height–diameter allometry relationship was indeed altered by thinning treatments.

Table 4. Parameter estimates of dummy variable models for height–diameter allometry relationship.

Parameters	Estimate	Standard Error	95% Confidence Interval	
			Lower Limit	Upper Limit
α_0	1.830	0.036	1.760	1.899
β_0	0.665	0.006	0.653	0.677
α_1	−0.011	0.057	−0.123	0.101
α_2	0.162	0.062	0.040	0.284
α_3	0.009	0.064	−0.117	0.135
β_1	0.006	0.010	−0.014	0.025
β_2	−0.029	0.010	−0.049	−0.009
β_3	−0.007	0.011	−0.028	0.014

Table 5. *F*-test results from dummy variable models of height–diameter allometry relationship.

Equation	RSS	DF	Calculated F Value	Critical F Value	*p*
(1)	3673.68	4625	-	-	-
(2)	3591.62	4622	F_{2-1} = 35.29	2.61	<0.05
(3)	3582.38	4619	F_{3-2} = 4.02	2.61	<0.05

Legend: RSS, residual sum of squares; DF, degrees of freedom. F_{2-1} and F_{3-2} represent *F* values calculated based on Equation (2) and Equation (1), and Equation (3) and Equation (2), respectively. *p* values for the *F*-tests in which values were less than 0.05 were considered significant.

From the height–diameter allometry relationship fit to the power function (Figure 1) it can be seen that with the increase in thinning intensity, the number of trees with larger diameters significantly increased. Similarly, trees at the same diameter are shown to have lower heights under higher thinning

intensities (for a given diameter of 20 cm, the tree heights of T_1, T_2, and T_3 thinning treatments were found to be 0.64 m, 0.90 m, and 0.99 m smaller, respectively). This trend suggests that thinning results in trees allocating more growth resources to diameter growth.

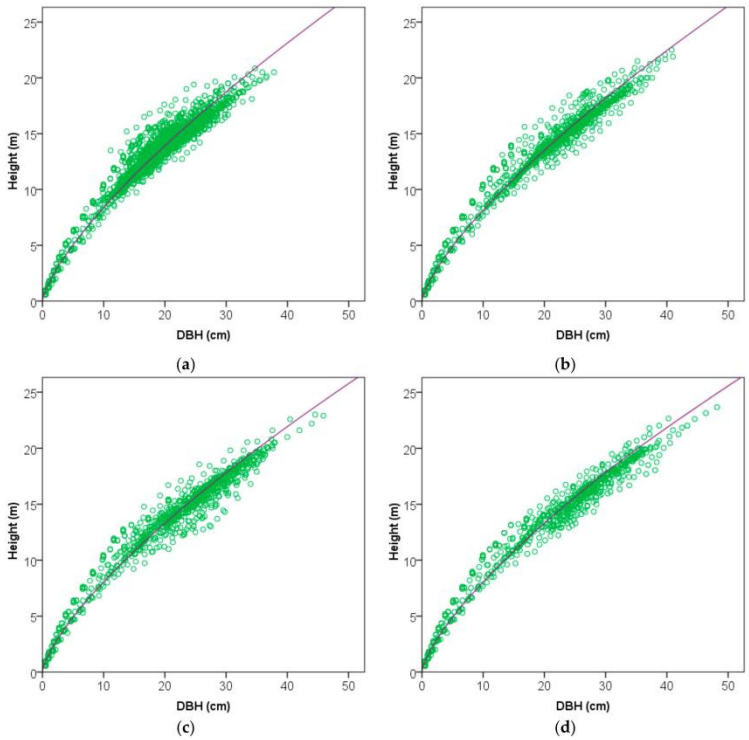


Figure 1. Height–diameter relationship performance for different thinning treatments. (**a**) T_0 treatment (unthinned control plot), (**b**) T_1 treatment (lightly thinned), (**c**) T_2 treatment (moderately thinned), and (**d**) T_3 treatment (heavily thinned).

3.2. The Temporal Dynamics of Thinning Effects on Height–Diameter Allometry

3.2.1. The Natural Allometric Trend of Height–Diameter

Fitting results of the generalized additive mixed models show that the height–diameter allometric relationship of Masson pine in unthinned control plots was not invariant over time. As shown in Figure 2a, the estimated degrees of freedom (4.41) was found to be greater than 1.0, suggesting that significant and strong nonlinearities exist among height–diameter allometric coefficient and tree age.

The height–diameter allometric coefficient exhibited a unimodal convex bell curve over time. A gradual increase was observed in the height–diameter allometric coefficient with increasing tree age in the early stage, indicating that growth resources were increasingly being used for height growth. The observed values reached a peak at approximately 10 years to 15 years and then continued to gradually decrease toward the later period of observation, indicating that growth resources were again used more for gradual diameter growth after the 15th year. Additionally, the height–diameter

allometric coefficients were greater than 1.0 from approximately the 6th year to the 20th year, while the coefficients for the remaining years were less than 1.0, indicating that the relative height increment was greater than the relative diameter increment during years 6 to 20, and that this relationship was reversed in the remaining years.

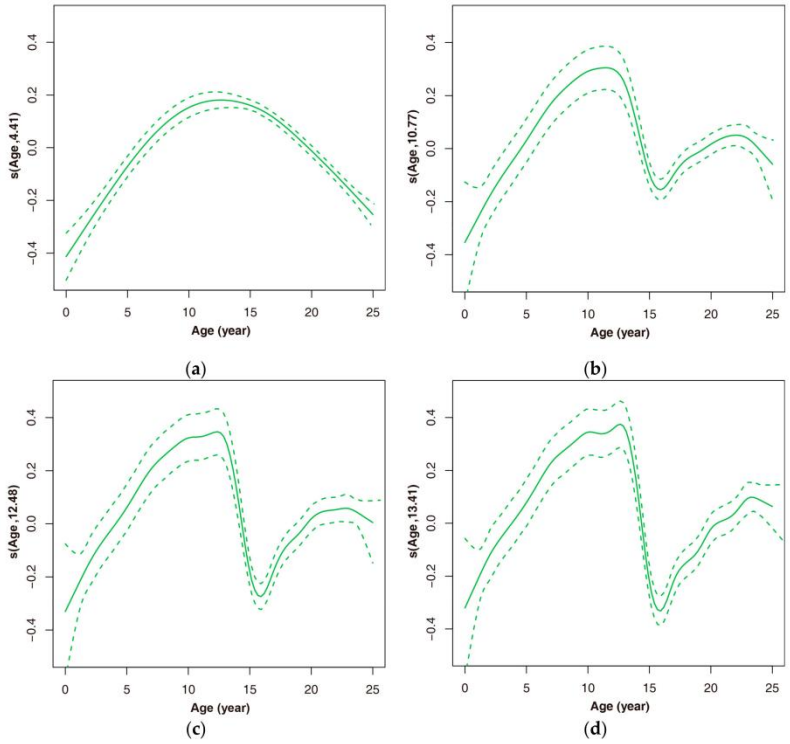


Figure 2. Estimated degrees of freedom with smooth spline functions on the observed height–diameter allometric coefficient ($m_{h,d}$) for different thinning treatments. (**a**) T_0 treatment (unthinned control plot), (**b**) T_1 treatment (lightly thinned), (**c**) T_2 treatment (moderately thinned), and (**d**) T_3 treatment (heavily thinned).

3.2.2. Effects of Thinning on Height–Diameter Allometry

As shown in Figure 2b–d, the estimated degrees of freedom (10.77, 12.48, and 13.41 for T_1, T_2, and T_3 treatment, respectively) were found to be all greater than 1.0 and the nonlinearity modeled by the smooth spline increased with the increase in thinning intensity. This result demonstrates that thinning significantly affected the original trend of the height–diameter allometric coefficient. After thinning, the coefficients had an immediate and sharp decrease from greater than 1.0 to less than 1.0. From the second year after thinning the coefficient increased gradually and from around the fifth year after thinning it decreased gradually again. This suggests that thinning caused trees to allocate more resources toward diameter growth and that the growth status of trees was reversed from the original case where the relative height increment was greater than the relative diameter increment. Later, about

5 years after thinning, the trend can be seen to have returned to the original pattern, in which more growth resources were allocated first toward height growth and then gradually towards diameter growth again.

Hence, the interaction of thinning and tree age ultimately determined the process of change in the height–diameter allometry (Figure 3). A higher intensity of thinning resulted in a greater magnitude in the reduction of the coefficients and a longer duration of time required for it to recover from less than 1.0 to greater than 1.0, which meant that trees in plots undergoing high intensity thinning were more likely to use growth resources for diameter growth than trees in plots undergoing low intensity thinning.

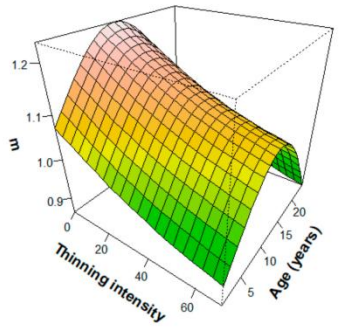


Figure 3. Visualization of height–diameter allometric coefficient ($m_{h,d}$) fit with time and thinning interactions.

4. Discussions

Mathematical equations are widely used to quantify the allometric relationship between tree height and stem diameter [1,61,84]. Many studies have shown that different regions and tree species have different optimum height–diameter allometry models [4,25,62]. To more accurately analyze the effects of thinning on Masson pine plantations, we first compared the ability of five commonly used height–diameter allometric models to estimate heights from diameters. The results showed that the power function had the best goodness of fit for our data. This function has several desirable properties, such as its flexible integrated and logarithmic representation [74,76,85–87], and it has also been widely used to described allometric relationships for pine and other species in America, Europe, Asia, and South Africa [1,18,25,88].

When comparing the curves of the height–diameter allometry relationship (Figure 1) fitted by the power function for different thinning treatments, we found that thinning significantly changed the ratio of height to diameter. The dummy variable methods and nested *F*-tests showed that there were significant differences in the parameters between thinned and unthinned treatments, demonstrating that thinning had changed the height–diameter allometry. This, combined with the nature of the change, suggests that thinning promoted diameter growth and loss of height growth. Some studies have shown that thinning promotes tree diameter growth at the cost of height growth [27,39,41,52] and our result appears to be consistent with these studies.

The main objective of height–diameter allometric research, from its beginning, has been mainly to find a universal allometric coefficient [89,90]. A periodic height–diameter allometric coefficient based on relative increments [24,75] was used in this study because it can make simple and unbiased comparisons of plant performance among plants of different sizes growing under different environmental conditions [91–93]. The means of the observed height–diameter allometric coefficients of different thinning treatments showed that tree height–diameter allometry was significantly affected by thinning. Compared with the height–diameter allometric growth model, which can only illustrate a general trend of change in height–diameter allometry under different thinning treatments, the

periodic height–diameter allometric coefficient can give a more precise description of how the tree height–diameter relationship changes over time and in response to thinning.

The exponent of the power function (β in this study) is itself a height–diameter allometric coefficient [74,76,85,87,88,94]. When describing tree height–diameter allometry with the power function, it is frequently assumed that the height–diameter allometric coefficient is constant throughout the growth period of the trees. However, the dynamic changes observed in this study in the height–diameter allometric relationship over time were diverse. Some studies have found that the allometric relationship of tree height versus diameter can change only slightly or even be invariant [95,96], while others have found that the relationship is not invariant over time [20,24,97]. Such ontogenetic strategies are often considered to be genetically determined [98], but for some species, allometric growth is not an invariant character of a genotype and environmental condition, and relieving competition by thinning can change how trees allocate their growth resources [99,100].

To scrutinize the temporal dynamics of the effects of thinning on height–diameter allometry, the GAMM was used in this study. The results showed that the allometric relationship of Masson pine varied over time and was also significantly affected by thinning. We believe that the effects of thinning on height–diameter allometry may be mainly achieved by affecting competition (growth space or stand canopy status). In living systems, biological traits can confer the ability to alter their phenotypes to better respond to environmental change [101]. Better height growth for a given diameter endows a tree with an advantage when competing for sunlight, while more rapid diameter growth for a given tree height can promote the maintenance of this competitive advantage [14]. After obtaining a spatial advantage, trees maintain this advantage through strengthening their mechanical support [15,16] and water transport capacity [17] in order to maximize fitness.

Masson pine is a typical light-demanding species, and during the early stage of stand growth, trees do not need to compete for above-ground growing space because the canopy has not yet closed. Thus, trees use more resources for diameter growth, causing the relative diameter increment to be greater than the relative height increment. As the canopy closes over time, trees begin to compete for space to capture sunlight, and, thus, they use more growth resources for height growth, causing the relative height increment to be greater than the relative diameter increment. This trend occurs until the initiation of self-thinning, during which growing space is released and the magnitudes of the relative increments are reversed.

Silvicultural thinning changes the canopy status of residual trees rapidly, which causes the remaining trees to suddenly have more growing space [102]. Consequently, these trees change their original growth pattern and use more growth resources for diameter growth to maintain their spatial advantage until the canopy again begins to close, after which the trees begin the next stage of space competition [103]. The sudden increase in diameter growth of retained trees after thinning supports this explanation. Further evidence is provided by the demonstrated nonlinearities among height–diameter allometric coefficients and tree age as well as the larger reduction in the magnitude of height–diameter allometric coefficients and the longer duration for the allometric coefficient to recover from less than 1.0 to greater than 1.0 with greater thinning intensity.

5. Conclusions

In this work, stem height–diameter allometry of Masson pine plantations was found to be not invariant over time and to vary nonlinearly with stand age. Thinning had a significant influence on the tree height–diameter allometry of Masson pine plantations, which altered the height–diameter allometry rhythm. Thinning caused trees to increase diameter growth at the expense of height growth, resulting in a decrease of the ratio of tree height to diameter, and this trend was more obvious as the thinning intensity increased. The change in stem height–diameter allometry of Masson pine was mainly related to competition (growth space) among the trees. When trees acquired a spatial advantage, they allocated more growth resources toward diameter growth, resulting in greater diameter growth relative to tree height growth; however, to consolidate the gained spatial advantage in order to facilitate

competition at the next stage, they were required to allocate more growth resources toward increasing height growth. Thinning affected the competition of light and water by changing the growth space of trees, thereby affecting the height–diameter allometric relationship. As thinning intensity increased, a greater spatial advantage was acquired by the remaining trees, and these trees subsequently allocated more growth resources to diameter growth, resulting in a longer period of time in which the relative diameter increment was greater than the relative height increment.

Author Contributions: Conceptualization, S.Z. and Y.L.; Methodology, S.Z., Y.L., C.D., and R.E.F.; Software, C.D.; Validation, A.M.; Formal analysis, C.D.; Investigation, C.D. and A.M.; Resources, S.Z. and Y.L.; Data curation, Q.L.; Writing—original draft preparation, C.D.; Writing—review and editing, Q.L. and R.E.F.; Project administration, Q.L.; Funding acquisition, Y.L.

Funding: This research was funded by the Central Public-Interest Scientific Institution Basal Research Fund (grant number IFRIT201501).

Acknowledgments: We appreciate the staff of the Experimental Center of Tropical Forestry for their help during the field survey. We also appreciate the editors and anonymous reviewers for their constructive comments.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hulshof, C.M.; Swenson, N.G.; Weiser, M.D. Tree height-diameter allometry across the United States. *Ecol. Evol.* **2015**, *5*, 1193–1204. [CrossRef] [PubMed]
2. Niklas, K.J.; Spatz, H.C. Growth and hydraulic (not mechanical) constraints govern the scaling of tree height and mass. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15661–15663. [CrossRef] [PubMed]
3. Price, C.A.; Enquist, B.J.; Savage, V.M. A general model for allometric covariation in botanical form and function. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13204–13209. [CrossRef] [PubMed]
4. Feldpausch, T.R.; Banin, L.; Phillips, O.L.; Baker, T.R.; Lewis, S.L.; Quesada, C.A.; Affum-Baffoe, K.; Arets, E.J.; Berry, N.J.; Bird, M.; et al. Height-diameter allometry of tropical forest trees. *Biogeosciences* **2011**, *8*, 1081–1106. [CrossRef]
5. Poorter, L.; Lianes, E. Architecture of Iberian canopy tree species in relation to wood density, shade tolerance and climate. *Plant Ecol.* **2012**, *213*, 707–722. [CrossRef]
6. Vizcaíno-Palomar, N.; Ibáñez, I.; Benito-Garzón, M.; González-Martínez, S.C.; Zavala, M.A.; Alía, R. Climate and population origin shape pine tree height-diameter allometry. *New For.* **2017**, *48*, 363–379. [CrossRef]
7. King, D.A. Allometry of saplings and understorey trees of a Panamanian forest. *Funct. Ecol.* **1990**, *4*, 27–32. [CrossRef]
8. Harrington, C.A.; DeBell, D.S. Above-and below-ground characteristics associated with wind toppling in a young Populus plantation. *Trees* **1996**, *11*, 109–118. [CrossRef]
9. Thomas, S.C.; Martin, A.R.; Mycroft, E.E.; Bellingham, P. Tropical trees in a wind-exposed island ecosystem: height-diameter allometry and size at onset of maturity. *J. Ecol.* **2015**, *103*, 594–605. [CrossRef]
10. Tilman, D. Plant strategies and the dynamics and structure of plant communities. *Bull. Math. Biol.* **1989**, *4*, 28–29.
11. Chave, J.; Andalo, C.; Brown, S.; Cairns, M.A.; Chambers, J.Q.; Eamus, D.; Fölster, H.; Fromard, F.; Higuchi, N.; Kira, T.; et al. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. *Oecologia* **2005**, *145*, 87–99. [CrossRef] [PubMed]
12. Ryan, M.G.; Phillips, N.; Bond, B.J. The hydraulic limitation hypothesis revisited. *Plant Cell Environ.* **2006**, *29*, 367–381. [CrossRef] [PubMed]
13. Henry, H.A.L.; Aarssen, L.W. The interpretation of stem diameter–height allometry in trees: biomechanical constraints, neighbour effects, or biased regressions? *Ecol. Lett.* **2010**, *2*, 89–97. [CrossRef]
14. Moles, A.T.; Warton, D.I.; Warman, L.; Swenson, N.G.; Laffan, S.W.; Zanne, A.E.; Pitman, A.; Hemmings, F.A.; Leishman, M. Global patterns in plant height. *J. Ecol.* **2009**, *97*, 923–932. [CrossRef]
15. McMahon, T. Size and shape in biology. *Science* **1973**, *179*, 1201–1204. [CrossRef]
16. Niklas, K.J. The scaling of plant height: A comparison among major plant clades and anatomical grades. *Annals of Botany (Lond.)* **1993**, *72*, 165–172. [CrossRef]

17. Bullock, S.H. Developmental patterns of tree dimensions in a neotropical deciduous forest. *Biotropica* **2000**, *32*, 42–52. [CrossRef]
18. Vizcaíno-Palomar, N.; Ibáñez, I.; González-Martínez, S.C.; Zavala, M.A.; Alía, R. Adaptation and plasticity in aboveground allometry variation of four pine species along environmental gradients. *Ecol. Evol.* **2016**, *6*, 7561–7573. [CrossRef]
19. Kobe, R.K. Light gradient partitioning among tropical tree species through differential seedling mortality and growth. *Ecology* **1999**, *80*, 187–201. [CrossRef]
20. Niklas, K.J. Size-dependent allometry of tree height, diameter and trunk-taper. *Ann. Bot.* **1995**, *75*, 217–227. [CrossRef]
21. Banin, L.; Feldpausch, T.R.; Phillips, O.L.; Baker, T.R.; Lloyd, J.; Affum-Baffoe, K.; Arets, E.J.; Berry, N.J.; Bradford, M.; Brienen, R.J.; et al. What controls tropical forest architecture? Testing environmental, structural and floristic drivers. *Glob. Ecol. Biogeogr.* **2012**, *21*, 1179–1190. [CrossRef]
22. Fayolle, A.; Panzou, G.J.L.; Drouet, T.; Swaine, M.D.; Bauwens, S.; Vleminckx, J.; Biwole, A.; Lejeune, P.; Doucet, J.L. Taller trees, denser stands and greater biomass in semi-deciduous than in evergreen lowland central African forests. *For. Ecol. Manag.* **2016**, *374*, 42–50. [CrossRef]
23. Imani, G.; Boyemba, F.; Lewis, S.; Nabahungu, N.L.; Calders, K.; Zapfack, L.; Riera, B.; Balegamire, C.; Cuni-Sanchez, A. Height-diameter allometry and above ground biomass in tropical montane forests: Insights from the Albertine Rift in Africa. *PLoS ONE* **2017**, *12*, e0179653. [CrossRef] [PubMed]
24. Motallebi, A.; Kangur, A. Are allometric relationships between tree height and diameter dependent on environmental conditions and management? *Trees* **2016**, *30*, 1429–1443. [CrossRef]
25. Mensah, S.; Pienaar, O.L.; Kunneke, A.; Du Toit, B.; Seydack, A.; Uhl, E.; Pretzsch, H.; Seifert, T. Height-Diameter allometry in South Africa's indigenous high forests: Assessing generic models performance and function forms. *For. Ecol. Manag.* **2018**, *410*, 1–11. [CrossRef]
26. Khamyong, N.; Wangpakapattanawong, P.; Chairuangsri, S.; Inta, A.; Tiansawat, P. Tree species composition and height-diameter allometry of three forest types in Northern Thailand. *CMU J. Nat. Sci.* **2018**, *17*, 289–306. [CrossRef]
27. Allen, R.H.; Marquis, D.A. *Effect of Thinning on Height and Diameter Growth of Oak and Yellow-Poplar Saplings*; Northeastern Forest Experiment Station, Forest Service, USDA For. Serv.: Upper Darby, PA, USA, 1970; Res. Pap. NE-173; pp. 1–11.
28. David, H.C.; Arce, J.E.; Netto, S.P.; Corte, A.P.D.; Marinheski Filho, A.; Araújo, E.J.G.D. Thinning and site quality effects on the height-diameter relationship of *Pinus taeda*. *Floresta E Ambiente* **2016**, *23*, 220–228. [CrossRef]
29. Medeiros, R.A.; Paiva, H.N.D.; Soares, A.A.V.; Cruz, J.D.; Leite, H.G. Thinning from below: effects on height of dominant trees and diameter distribution in Eucalyptus stands. *J. Trop. For. Sci.* **2017**, *29*, 238–247.
30. Drew, T.J.; Flewelling, J.W. Stand density management: An alternative approach and its application to Douglas-fir plantations. *For. Sci.* **1979**, *25*, 518–532.
31. Smith, D.M.; Larson, B.C.; Kelty, M.J.; Ashton, P.M.S. *The Practice of Silviculture: Applied Forest Ecology*; John Wiley and Sons, Inc.: New York, NY, USA, 1997.
32. Zeide, B. Thinning and growth: A full turnaround. *J. For.* **2001**, *99*, 20–25.
33. Nishizono, T. Effects of thinning level and site productivity on age-related changes in stand volume growth can be explained by a single rescaled growth curve. *For. Ecol. Manag.* **2010**, *259*, 2276–2291. [CrossRef]
34. Hayes, J.P.; Chan, S.S.; Emmingham, W.H.; Tappeiner, J.C.; Kellogg, L.D.; Bailey, J.D. Wildlife response to thinning young forests in the Pacific Northwest. *J. For.* **1997**, *95*, 28–33.
35. Miller, M.; Emmingham, B. Can selection thinning convert even-age Douglas-fir stands to uneven-age structures? *West. J. Appl. For.* **2001**, *16*, 35–43. [CrossRef]
36. Bradford, J.B.; Palik, B.J. A comparison of thinning methods in red pine: Consequences for stand-level growth and tree diameter. *Can. J. For. Res.* **2009**, *39*, 489–496. [CrossRef]
37. Gavinet, J.; Vilagrosa, A.; Chirino, E.; Granados, M.E.; Vallejo, V.R.; Prévosto, B. Hardwood seedling establishment below Aleppo pine depends on thinning intensity in two Mediterranean sites. *Ann. For. Sci.* **2015**, *72*, 999–1008. [CrossRef]
38. Navarro-Cerrillo, R.M.; Sánchez-Salguero, R.; Rodriguez, C.; Lazo, J.D.; Moreno-Rojas, J.M.; Palacios-Rodriguez, G.; Camarero, J.J. Is thinning an alternative when trees could die in response to

drought? The case of planted *Pinus nigra* and *P. Sylvestris* stands in southern Spain. *For. Ecol. Manag.* **2019**, *433*, 313–324. [CrossRef]
39. Kerr, G. The effect of heavy or 'free growth' thinning on oak (*Quercus petraea* and *Q. robur*). *Forestry* **1996**, *69*, 303–317. [CrossRef]
40. Peltola, H.; Miina, J.; Rouvinen, I.; Kellomäki, S. Effect of early thinning on the diameter growth distribution along the stem of Scots pine. *Silva Fenn.* **2002**, *36*, 813–825. [CrossRef]
41. Mäkinen, H.; Isomäki, A. Thinning intensity and growth of Norway spruce stands in Finland. *Forestry* **2004**, *77*, 349–364. [CrossRef]
42. Juodvalkis, A.; Kairiukstis, L.; Vasiliauskas, R. Effects of thinning on growth of six tree species in north-temperate forests of Lithuania. *Eur. J. For. Res.* **2005**, *124*, 187–192. [CrossRef]
43. Ulvcrona, K.A.; Karlsson, K.; Ulvcrona, T. Identifying the biological effects of pre-commercial thinning on diameter growth in young Scots pine stands. *Scand. J. For. Res.* **2014**, *29*, 427–435. [CrossRef]
44. Ferraz, Filho, A.C.; Mola-Yudego, B.; Gonzalez-Olabarria, J.R.; Scolforo, J.R.S. Thinning regimes and initial spacing for Eucalyptus plantations in Brazil. *An Acad Bras Cienc.* **2018**, *90*, 255–265. [CrossRef] [PubMed]
45. Oliver, W.W. *Fifteen-Year Growth Patterns after Thinning a Ponderosa-Jeffrey Pine Plantation in Northeastern California. Res. Pap. PSW-RP-141*; U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station: Berkeley, CA, USA, 1979; Volume 10, p. 141.
46. Harrington, C.A.; Reukema, D.L. Initial shock and long-term stand development following thinning in a Douglas-fir plantation. *For. Sci.* **1983**, *29*, 33–46.
47. Schubert, G.H. Growth response of even-aged ponderosa pine related to stand density levels in Arizona. *J. For.* **1971**, *69*, 857–860.
48. Weber, V.P.; Finger, C.A.G.; Fleig, F.D.; Souza, C.A.M.D.; Munareto, F.F.; Silva, L.T.D.M.D. Guidelines for thinning of *Pinus taeda* L. because of dominant height. *Ciência Florest.* **2013**, *23*, 193–201.
49. Fedorová, B.; Kadavý, J.; Adamec, Z.; Kneifl, M.; Knott, R. Response of diameter and height increment to thinning in oak-hornbeam coppice in the southeastern part of the Czech Republic. *J. For. Sci.* **2016**, *62*, 229–235. [CrossRef]
50. Swift, D.E.; Knight, W.; Béland, M.; Boureima, I.; Bourque, C.P.A.; Meng, F.R. Stand dynamics and tree quality response to precommercial thinning in a northern hardwood forest of the Acadian forest region: 23 years of intermediate results. *Scand. J. For. Res.* **2016**, *32*, 45–59. [CrossRef]
51. Larson, P.R. Stem form development of forest trees. *For. Sci.* **1963**, *9*, a0001–a0042. [CrossRef]
52. Zhang, S.; Burkhart, H.E.; Amateis, R.L. The influence of thinning on tree height and diameter relationships in loblolly pine plantations. *South. J. Appl. For.* **1997**, *21*, 199–205. [CrossRef]
53. Sharma, M.; Smith, M.; Burkhart, H.E.; Amateis, R.L. Modeling the impact of thinning on height development of dominant and codominant loblolly pine trees. *Ann. For. Sci.* **2006**, *63*, 349–354. [CrossRef]
54. Russell, M.B.; Amateis, R.L.; Burkhart, H.E. Implementing regional locale and thinning response in the loblolly pine height-diameter relationship. *South. J. Appl. For.* **2010**, *34*, 21–27. [CrossRef]
55. Missanjo, E.; Kamanga-Thole, G. Effect of first thinning and pruning on the individual growth of pinus patulatree species. *J. For. Res.* **2015**, *26*, 827–831. [CrossRef]
56. Ginn, S.E.; Seiler, J.R.; Cazell, B.H.; Kreh, R.E. Physiological and growth responses of eight-year-old loblolly pine stands to thinning. *For. Sci.* **1991**, *37*, 1030–1040.
57. Voelker, S.L.; Lachenbruch, B.; Meinzer, F.C.; Strauss, S.H. Reduced wood stiffness and strength, and altered stem form, in young antisense 4CL transgenic poplars with reduced lignin contents. *New Phytol.* **2011**, *189*, 1096–1109. [CrossRef] [PubMed]
58. Eckenwalder, J.E. *Conifers of the World*; Timber Press, Inc.: Portland, OR, USA, 2009; p. 720.
59. Farjon, A. *A Handbook of the World's Conifers*; Brill Academic Publishers: Leiden, The Netherlands, 2010; p. 1112.
60. Payn, T.; Carnus, J.M.; Freer-Smith, P.; Kimberley, M.; Kollert, W.; Liu, S.R.; Orazio, C.; Rodriguez, L.; Silva, L.N.; Wingfield, M.J. Changes in planted forests and future global implications. *For. Ecol. Manag.* **2015**, *352*, 57–67. [CrossRef]
61. Huang, S.; Titus, S.J.; Wiens, D.P. Comparison of nonlinear height-diameter functions for major alberta tree species. *Can. J. For. Res.* **1992**, *22*, 1297–1304. [CrossRef]

62. Ledo, A.; Cornulier, T.; Illian, J.B.; Iida, Y.; Kassim, A.R.; Burslem, D.F. Re-evaluation of individual diameter: Height allometric models to improve biomass estimation of tropical trees. *Ecol. Appl.* **2016**, *26*, 2376–2382. [CrossRef]
63. Winsor, C.P. The Gompertz Curve as a Growth Curve. *Proc. Natl. Acad. Sci. USA* **1932**, *18*, 1–8. [CrossRef]
64. Pearl, R.; Reed, L.J. On the rate of growth of the population of the United States since 1790 and its mathematical representation. *Proc. Natl. Acad. Sci. USA* **1920**, *6*, 275–288. [CrossRef]
65. Huxley, A. *Problems of Relative Growth*; The Dial Press: New York, NY, USA, 1932.
66. Richards, F.J. A flexible growth function for empirical use. *J. Exp. Bot.* **1959**, *10*, 290–301. [CrossRef]
67. Wykoff, W.R.; Crookston, N.L.; Stage, A.R. *User's Guide to the Stand Prognosis Model. Gen. Tech. Rep. INT-133*; US Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station: Ogden, UT, USA, 1982; Volume 112, p. 133.
68. Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D.; R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-137. 2018. Available online: https://CRAN.R-project.org/package=nlme (accessed on 20 September 2019).
69. Akaike, H. A new look at the statistical model identification. *Autom. Control IEEE Trans.* **1974**, *19*, 716–723. [CrossRef]
70. Burnham, K.P.; Anderson, D.R. *Model Selection and Inference: A Practical Information-Theoretic Approach*, 2nd ed.; Springer: New York, NY, USA, 2002.
71. Mugasha, W.A.; Bollandsås, O.M.; Eid, T. Relationships between diameter and height of trees in natural tropical forest in Tanzania. *South. For. A J. For. Sci.* **2013**, *75*, 221–237. [CrossRef]
72. Kearsley, E.; Moonen, P.C.; Hufkens, K.; Doetterl, S.; Lisingo, J.; Bosela, F.B.; Boeckx, P.; Beeckman, H.; Verbeeck, H. Model performance of tree height-diameter relationships in the central Congo basin. *Ann. For. Sci.* **2017**, *74*, 7. [CrossRef]
73. Buford, M.A.; Burkhart, H.E. Genetic improvement effects on growth and yield of loblolly pine plantations. *For. Sci.* **1987**, *33*, 707–724.
74. Gayon, J. History of the concept of allometry. *Am. Zool.* **2000**, *40*, 748–758. [CrossRef]
75. Murphy, S.T.; Pommerening, A. Modelling the growth of sitka spruce (*Picea sitchensis* (bong.) carr.) in wales using wenk's model approach. *Allg. Forst Und Jagdztg.* **2010**, *181*, 35–43.
76. Pommerening, A.; Muszta, A. Relative plant growth revisited: Towards a mathematical standardisation of separate approaches. *Ecol. Model.* **2016**, *320*, 383–392. [CrossRef]
77. Lin, X.; Zhang, D.; Fryzlewicz, P.; Keilegom, I.V. Inference in generalized additive mixed modelsby using smoothing splines. *J. R. Stat. Soc.* **2010**, *61*, 381–400. [CrossRef]
78. Groll, A.; Tutz, G. Regularization for generalized additive mixed models by likelihood-based boosting. *Methods Inf. Med.* **2012**, *51*, 168–177.
79. Latimer, A.M.; Wu, S.; Gelfand, A.E.; Silander, J.A. Building statistical models to analyze species distributions. *Ecol. Appl.* **2006**, *16*, 33–50. [CrossRef]
80. Simpson, S.L.; Edwards, L.J.; Muller, K.E.; Sen, P.K.; Styner, M.A. A linear exponent AR(1) family of correlation structures. *Stat. Med.* **2010**, *29*, 1825–1838. [CrossRef] [PubMed]
81. Yang, L.; Qin, G.; Zhao, N.; Wang, C.; Song, G. Using a generalized additive model with autoregressive terms to study the effects of daily temperature on mortality. *BMC Med. Res. Methodol.* **2012**, *12*, 165. [CrossRef] [PubMed]
82. Weng, C.F. First Order Autoregressive Mixed Effects Zero Inflated Poisson Model for Longitudinal Data—A Bayesian Approach. Ph.D. Thesis, University of Maryland, College Park, MD, USA, 2014; p. 12.
83. Mellor, A.F.P.; Cey, E.E. Using generalized additive mixed models to assess spatial, temporal, and hydrologic controls on bacteria and nitrate in a vulnerable agricultural aquifer. *J. Contam. Hydrol.* **2015**, *182*, 104–116. [CrossRef] [PubMed]
84. Temesgen, H.; Gadow, K.V. Generalized height-diameter models—An application for major tree species in complex stands of interior British Columbia. *Eur. J. For. Res.* **2004**, *123*, 45–51. [CrossRef]
85. Pretzsch, H. Re-Evaluation of Allometry: State-of-the-Art and Perspective Regarding Individuals and Stands of Woody Plants. In *Progress in Botany 71*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 339–369.
86. Pretzsch, H.; Dieler, J. Evidence of variant intra-and interspecific scaling of tree crown structure and relevance for allometric theory. *Oecologia* **2012**, *169*, 637–649. [CrossRef]

87. Franceschini, T.; Schneider, R. Influence of shade tolerance and development stage on the allometry of ten temperate tree species. *Oecologia* **2014**, *176*, 739–749. [CrossRef]
88. Blanchard, E.; Birnbaum, P.; Ibanez, T.; Boutreux, T.; Antin, C.; Ploton, P.; Vincent, G.; Pouteau, R.; Vandrot, H.; Hequet, V.; et al. Contrasted allometries between stem diameter, crown area, and tree height in five tropical biogeographic areas. *Trees* **2016**, *30*, 1953–1968. [CrossRef]
89. Russo, S.E.; Wiser, S.K.; Coomes, D.A. Growth–size scaling relationships of woody plant species differ from predictions of the Metabolic Ecology Model. *Ecol. Lett.* **2007**, *10*, 889–901. [CrossRef]
90. Watt, M.S.; Kirschbaum, M.U. Moving beyond simple linear allometric relationships between tree height and diameter. *Ecol. Model.* **2011**, *222*, 3910–3916. [CrossRef]
91. Bragg, D.C. Potential relative increment (PRI): A new method to empirically derive optimal tree diameter growth. *Ecol. Model.* **2001**, *137*, 77–92. [CrossRef]
92. King, D.A. Linking tree form, allocation and growth with an allometrically explicit model. *Ecol. Model.* **2005**, *185*, 77–91. [CrossRef]
93. Pommerening, A.; Muszta, A. Methods of modelling relative growth rate. *For. Ecosyst.* **2015**, *2*, 5. [CrossRef]
94. Klingenberg, C.P. Multivariate Allometry. In *Advances in Morphometrics*; Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P., Slice, D.E., Eds.; Plenum: New York, NY, USA, 1996; pp. 23–49.
95. Vospernik, S.; Monserud, R.A.; Sterba, H. Do individual-tree growth models correctly represent height: diameter ratios of Norway spruce and Scots pine? *For. Ecol. Manag.* **2010**, *260*, 1735–1753. [CrossRef] [PubMed]
96. Pretzsch, H.; Dauber, E.; Biber, P. Species-specific and ontogeny-related stem allometry of European forest trees: evidence from extensive stem analyses. *For. Sci.* **2013**, *59*, 290–302. [CrossRef]
97. Ryan, M.G.; Yoder, B.J. Hydraulic limits to tree height and tree growth. *Bioscience* **1997**, *47*, 235–242. [CrossRef]
98. Weiner, J. Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology. *Evol. Syst.* **2004**, *6*, 207–215.
99. Bonser, S.P.; Aarssen, L.W. Plastic allometry in young sugar maple (*Acer saccharum*): adaptive responses to light availability. *Am. J. Bot.* **1994**, *81*, 400–406. [CrossRef]
100. Martínez-Vilalta, J.; Vanderklein, D.; Mencuccini, M. Tree height and age-related decline in growth in Scots pine (*Pinus sylvestris* L.). *Oecologia* **2007**, *150*, 529–544. [CrossRef]
101. Botero, C.A.; Weissing, F.J.; Wright, J.; Rubenstein, D.R. Evolutionary tipping points in the capacity to adapt to environmental change. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 184–189. [CrossRef]
102. Cañellas, I.; Río, M.; Roig, S.; Montero, G. Growth response to thinning in *Quercus pyrenaica* Willd. coppice stands in Spanish central mountain. *Ann. For. Sci.* **2004**, *61*, 243–250. [CrossRef]
103. Jiang, L.; Ye, M.; Zhu, S.; Zhai, Y.; Xu, M.; Huang, M.; Wu, R. Computational identification of genes modulating stem height-diameter allometry. *Plant Biotechnol. J.* **2016**, *14*, 2254–2264. [CrossRef] [PubMed]

3.5 Estimation of Biomass Dynamics and Allocation in Chinese Fir Trees Using Tree Ring Analysis in Hunan Province, China

sustainability

MDPI

Article

Estimation of Biomass Dynamics and Allocation in Chinese Fir Trees Using Tree Ring Analysis in Hunan Province, China

Xiaojun Xu [1,†], Fengfeng Ma [2,3,†], Kangying Lu [4], Baoqi Zhu [1], Shuaichen Li [1], Kangqi Liu [1], Qianmin Chen [1], Qingfen Li [1,*] and Cheng Deng [1,*]

[1] College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China
[2] Ministry of Education Key Laboratory of Silviculture and Conservation, Beijing Forestry University, Beijing 100083, China
[3] Hunan Academy of Forestry, Changsha 410018, China
[4] School of Ecological Engineering, Guangdong Eco-Engineering Polytechnic, Guangzhou 510520, China
* Correspondence: qfli@scau.edu.cn (Q.L.); cdeng@scau.edu.cn (C.D.)
† These authors contributed equally to this work.

Abstract: Studying tree biomass dynamics and allocation is crucial to understanding the forest carbon cycle and the adaptation of trees to the environment. However, traditional biomass surveys are time-consuming and labor-intensive, so few studies have specifically examined biomass formation in terms of the increase in individual tree biomass, and the role that tree age and site conditions play in this process, especially tree roots, is unclear. We studied the tree ring characteristics of 87 sample trees (8–40 years old) from 29 Chinese fir plantations with different site conditions and measured the biomass of their stems, crowns, and roots. The biomass increment at various age stages during tree growth was determined via using tree ring analysis, and a generalized additive mixed model (GAMM) was used to analyze biomass formation and allocation, as well as the specific impact of site conditions on them. The results showed that the biomass increment of Chinese fir trees first increased and then decreased with age, and improving site conditions delayed the carbon maturation of the trees. The proportion of stem biomass increased with age, while the proportion of crown biomass decreased and the proportion of root biomass increased and then decreased. The effect of the site conditions on the tree biomass allocation showed a nonlinear trend. Tree ring analysis provides a feasible and effective method for assessing tree growth and biomass dynamics. Forest managers can use the findings of this study to scientifically optimize the management of increasing forest carbon sequestration.

Keywords: tree ring analysis; biomass; allocation; tree age; site condition

Citation: Xu, X.; Ma, F.; Lu, K.; Zhu, B.; Li, S.; Liu, K.; Chen, Q.; Li, Q.; Deng, C. Estimation of Biomass Dynamics and Allocation in Chinese Fir Trees Using Tree Ring Analysis in Hunan Province, China. *Sustainability* **2023**, *15*, 3306. https://doi.org/10.3390/su15043306

Academic Editors: Zhongke Feng and Zixuan Qiu

Received: 28 December 2022
Revised: 5 February 2023
Accepted: 7 February 2023
Published: 10 February 2023

1. Introduction

Forests provide a variety of vital ecosystem services that contribute to maintaining the global climate system, mitigating global warming, and adjusting the global carbon balance [1–3]. One such service is carbon sequestration. Growing forests can capture atmospheric carbon dioxide through assimilation and release oxygen, and these processes can be utilized to mitigate climate change [4]. Forest carbon storage accounts for 82.5% of the total carbon storage of land vegetation, and this is an important indicator of a forest's carbon sequestration capacity [5]. However, global warming has caused an increase in the occurrence of natural disasters, redistribution of global precipitation, melting of glaciers and permafrost, sea level rise, and other hazards that pose a significant threat to human society [6]. To avoid extreme environmental harm, more than 70 countries have committed to reaching net-zero carbon emissions by 2050 or even earlier [7]. In terms of carbon sequestration, forests are pollution-free, low input, and sustainable. Hence, implementing

afforestation programs has become an important means for achieving ecological carbon sequestration enhancement, and the planting area of plantations is increasing globally [8,9].

Currently, a forest's carbon storage capacity is usually calculated by multiplying the forest's biomass by a carbon coefficient. Thus, it is critical to obtain an accurate understanding of a forest's biomass to determine its carbon sequestration capacity and evaluate the regional forest carbon balance [10,11]. The most common factors which affect forest biomass are stand age, community structure, growth environment, management measures, and natural disturbances [12–14]. Previous studies have shown that the factors that directly influence biomass in the short term are water, heat, and soil nutrient conditions, whereas in terms of long-term community structure succession, the determining factors are the biota, site conditions, and development time [15]. Due to different growth environments, trees adopt different survival strategies and different resource allocation and utilization patterns [16,17], which impact the biomass allocation of various organs. However, most current forest biomass research focuses on explaining the static research on the overall biomass of trees at a certain time node, while research on the specific formation process of biomass is rare. For example, in the relative growth relationship model, the relative relationship between biomass and external morphological indicators of trees is generally considered to be a constant, whereas in the stand volume model the ratio of biomass to volume is generally considered a constant [18]. Although destructive sampling can be used to accurately estimate biomass, it is difficult, expensive, and time-consuming, and it requires cutting down a large number of trees for detailed measurements. Predicting belowground biomass is also difficult [19]. Therefore, only a limited number of studies have focused on biomass allocation changes in different growth environments, especially in tree roots, and few studies have examined biomass allocation tradeoffs related to site conditions, particularly increases in the biomass of individual trees [20–26]. Without such knowledge, it is extremely challenging to accurately assess a forest's carbon storage capacity and to develop and implement management and regulation measures that will improve the carbon sequestration capacity of forests.

Tree ring analysis is a retrogressive growth measurement method, the great advantage of which is that a single field sampling program can produce annual growth data that can be traced back almost to the time the tree was established [27]. Tree rings can be considered long-term growth records, and are thus valuable tools for understanding past growth drivers and predicting future forest change [28]. Through the analysis of tree rings, tree growth tracks can be reconstructed, and information obtained about a tree's growth dynamics, such as the tree diameter at breast height and the tree height [29]. Tree ring analysis can be performed on trees growing in a wide range of conditions, including different stand or soil conditions, and over a substantial period of time. Hence, in combination with the use of the appropriate allometric equation, tree ring analysis has become an important tool for the rapid assessment of forest productivity at different stand ages and for understanding forest species growth, biomass allocation, and climate characteristics [30]. It has been widely applied to estimate the biomass accumulation and interannual increment of individual trees and their responses to climate factors [31–34].

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is the principal native tree species extensively planted for the economic purpose of timber production and for environmental purposes, such as carbon sequestration and soil erosion control, in subtropical areas of southern China [20]. According to the results of the Ninth National Forest Resources Continuous Inventory, Hunan Province is one of the main Chinese fir production areas, and its Chinese fir plantations account for 21.09% of the total area of Chinese fir plantations in China [35]. Therefore, clarifying the dynamics of the Chinese fir's biomass and its components will deepen our understanding of the carbon sequestration mechanism and potential of Chinese fir plantations, and provide empirical evidence and theoretical guidance for management measures to increase carbon storage. Here, a biomass equation has been constructed, and the dynamics of the Chinese fir biomass and its allocation have

been studied using tree ring analysis to provide a more feasible and effective method for researching Chinese fir biomass and carbon storage.

2. Materials and Methods

2.1. Study Area

The study was conducted in Chinese fir plantations in Hunan Province (24°38′ N–30°08′ N, 108°47′ E–114°15′ E), China. Hunan Province is a key forest region in southern China, with a forest coverage rate of 49.69%, ranking ninth in the country. The Chinese fir forest area accounts for 26.14% of the arbor forest area. The zonal soil is mainly red-yellow soil and yellow soil, with scattered red soil, purple soil, and yellow-brown soil. The soil texture is mainly loam and clay, and the parent materials are mainly slate and shale. The climate is a subtropical humid monsoon climate. The annual average temperature is 16.0–18.5 °C, the daylight hours are 1300–1800 h, the annual precipitation is 1200–1800 mm, and the altitude of most areas is 100–800 m. Jindong Forest Farm, Paiyashan Forest Farm, Huangfengqiao Forest Farm, Shichangxi Forest Farm, and Xishan Forest Farm were selected as the sampling areas in this study (Figure 1).

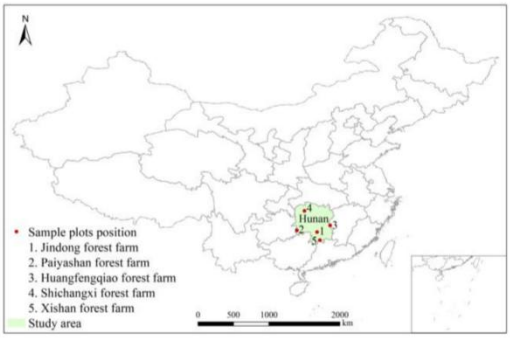


Figure 1. Distribution of the study area and the forest farms selected for sampling.

2.2. Forest Inventory and Sample Collection

The study was conducted in August 2021. We established 29 temporary sample plots (20 m × 20 m) in Chinese fir plantations. All trees in each sample plot with a diameter at breast height greater than 5 cm were numbered. The total height and diameter at breast height were measured, and the main stand characteristics of the sample plots were recorded (Table 1). The site index was represented by stand dominant height at a given base age [36]. In each sample plot, three average trees selected as sample trees according to the average diameter at breast height, average total tree height, and average stem form were felled from the ground level, and all the roots were dug out. Before felling the sample trees, the north direction was marked on the stem. Then, for trees with tree heights greater than 10 m, the discs were cut at 0, 1.3, 3.6, 5.6 m, and so on, until the treetop was reached. For trees with heights of less than 10 m, the discs were cut at 0, 0.5, 1.3, 1.5, 2.5 m, and so on, until the treetop was reached. The discs were approximately 5 cm thick, and the north direction was marked on each disc. The obtained discs were used for tree ring analysis. In addition, the stem was divided into upper, middle, and lower sections of equal length, and a disc was cut from the middle of each section. The stem (upper, middle, and lower sections), bark, leaves, branches, and roots of each sample tree were weighed, and samples were collected for biomass estimation. A total of 87 Chinese fir trees from 29 sample plots aged 8–40 years were included in this analysis, including 9 young-stage trees, 21 middle-stage trees, 21 near-mature trees, 24 mature trees, and 12 over-mature trees.

Table 1. The main stand characteristics of the temporary Chinese fir plantation sample plots.

Sampling Forest Farm	Plot No.	Stand Age	Age Group	Site Index	Mean Diameter at Breast Height (cm)	Average Total Tree Height (m)	Stand Density (Trees ha^{-1})
Jindong	JD-1	17	II	14	12.1	10.8	2550
	JD-2	31	IV	12	14.9	12.2	1675
	JD-3	25	III	16	19	15	1200
	JD-4	40	V	14	19.9	16.5	950
	JD-5	27	IV	18	20.4	17.4	1175
	JD-6	8	I	14	8.3	6.4	3250
Paiyashan	PYS-1	18	II	20	18.4	17.2	1875
	PYS-2	39	V	20	25.2	23	975
	PYS-3	28	IV	14	15.8	12.9	1475
	PYS-4	21	III	12	11.9	9.9	2025
	PYS-5	36	V	16	24	19	1150
	PYS-6	29	IV	20	27.7	20.4	800
	PYS-7	24	III	12	17	11.8	1350
Huangfengqiao	HFQ-1	33	IV	16	22.3	18.7	900
	HFQ-2	24	III	18	22.5	17.9	1325
	HFQ-3	18	II	16	16.2	12.5	2125
	HFQ-4	14	II	14	11.4	9	2650
	HFQ-5	24	III	18	22.2	16	1200
Shichangxi	SCX-1	13	II	16	14.8	10.3	2700
	SCX-2	31	IV	18	27.2	17.9	1075
	SCX-3	27	IV	18	25	16.7	925
	SCX-4	8	I	12	7.9	6.1	3350
	SCX-5	17	II	16	17.2	11.8	2175
	SCX-6	23	III	16	19.8	13.3	1325
Xishan	XS-1	9	I	12	8.5	6.4	2850
	XS-2	39	V	14	22	14.7	825
	XS-3	13	II	12	11	8	2750
	XS-4	31	IV	14	21.8	14.6	950
	XS-5	21	III	18	22	14.7	1150

I represents young stage (≤10 years); II, middle stage (11–20 years); III, near mature (21–25 years); IV, mature (26–35 years); V, over mature (≥36 years).

2.3. Biomass Estimation and Tree Ring Analysis

The discs to be used in the tree ring analysis were polished, and two diameter lines were drawn on each through the pith: one from east to west and one from north to south (Figure 2). The rings on each disc were then counted, and according to an age gradation of every three or five years, the diameter of each age gradation on the east–west and north–south diameter lines of each disc was measured with a ruler, and the average diameter of the same age gradation in two directions was recorded as the diameter of the age gradation. The number of years required for a tree to grow to the height of the section was calculated as the difference between the age of the tree and the number of annual rings on each disc. The tree height at each age gradation was calculated via an interpolation method using the height of the section and the number of years required to grow to the height of the section [37]. The stem, bark, branch, leaf, and root samples collected in the field were transferred to the laboratory within three days of collection. After the deactivation of enzymes in an oven at 105 °C, the samples were dried to constant weight in an incubator at 85 °C, and the dry weight was measured. The following formula was used to calculate the biomass of each whole tree and each organ:

$$W_i = \left(M_{sf} - M_{sd}\right) / M_{sf} \quad (1)$$

$$B_i = M_{tf} \times (1 - W_i) \quad (2)$$

where W_i is the moisture content of each organ sample, M_{sf} is the fresh weight of each organ sample, M_{sd} is the dry weight of each organ sample, M_{tf} is the total fresh weight of each organ sample, and B_i is the biomass of each organ sample.

Figure 2. A representative polished disc showing the east–west and north–south diameter lines.

Stem biomass is equal to wood biomass plus bark biomass, crown biomass is equal to branch biomass plus leaf biomass, root biomass only includes the root biomass, and total biomass is equal to stem biomass plus crown biomass plus root biomass. The basic information of the sample trees is presented in Table 2.

Table 2. Basic information of the sample trees.

Age Group	Num.	Site Index Range	Mean Diameter at Breast Height	Mean Height	Total Biomass	Stem Biomass	Crown Biomass	Root Biomass
I	9	12–14	8.1	6.1	11.88	6.37	3.75	1.77
II	21	12–20	14.4	11.6	55.45	35.06	9.68	10.71
III	21	12–18	19.2	14.2	107.27	69.68	15.61	21.97
IV	24	12–20	21.9	16.8	180.9	120.99	23.04	36.87
V	12	14–20	22.7	17.6	171.29	120.48	16.35	34.46

I represents young stage (≤10 years); II, middle stage (11–20 years); III, near mature (21–25 years); IV, mature (26–35 years); V, over mature (≥36 years).

2.4. Correction of Diameter at Breast Height of Each Age Gradation

The diameter at breast height calculated using the cumulative tree ring data was not equal to that measured in the field using a diameter tape, and the diameter at breast height of other age gradations measured by tree ring analysis did not include bark thickness. Thus, Formula (3) from Zhang et al. [38] and Wang et al. [39] was applied to eliminate the error by calibrating tree ring width at different age gradations:

$$\theta_k = (D_{mk} - D_{tk})/N_k \quad (3)$$

where θ_k is the corrected value (cm) for each tree ring width of the kth tree, D_{mk} is the diameter of the kth tree measured at breast height using a diameter tape (cm), D_{tk} is the diameter at breast height (with bark thickness) calculated from the cumulative tree diameter (m) of the kth tree, and N_k is the tree age (year) of the kth tree.

Formula (4) from Tang et al. [40] was applied to estimate bark thickness at each age gradation:

$$BR_k = D_{bk}/D_{mk} \quad (4)$$

where BR_k is the bark ratio of the kth tree, and D_{bk} is the bark thickness of the kth tree.

Assuming that the θ_k and BR_k of each sample tree are constant, then the corrected diameter at breast height value can be obtained according to Formulas (3) and (4):

$$D_{cik} = (D_{nik} + N_{ik} \times \theta_k)/(1 - BR_k) \quad (5)$$

where D_{cik} is the corrected diameter at breast height value of the ith age gradation of the kth tree, and D_{nik} is the diameter at breast height (without bark thickness) calculated from the cumulative tree diameter (m) of the ith age gradation of the kth tree.

2.5. Construction of the Biomass Model

Using the methods described above, biomass data were obtained for each component of the sample trees, and the diameter at breast height and tree height at each age gradation were determined. Next, we needed a model to estimate the biomass of each component at each age gradation. For this, a biomass model of tree height (h) and diameter at breast height (dbh) was constructed based on the allometric growth equation. The basic model form is as follows:

$$B = a \times dbh^b \times h^c \quad (6)$$

where a, b, and c are the fitted coefficients.

Considering that the prediction of the model should be additive (compatible), that is, the sum of the biomass of the stem, crown, and root should equal the total biomass, an aggregation method was introduced to build an additive biomass model. The resultant model form is as follows:

$$B_s = a_1 \times dbh^{b_1} \times h^{c_1} \quad (7)$$

$$B_c = a_2 \times dbh^{b_2} \times h^{c_2} \quad (8)$$

$$B_r = a_3 \times dbh^{b_3} \times h^{c_3} \quad (9)$$

$$B_t = B_s + B_c + B_r \quad (10)$$

where B_s, B_c, B_r, and B_t is the biomass of the stem, crown, root, and total tree, respectively. The estimated values of each parameter are shown in Table 3.

Table 3. The estimated values of each parameter in the biomass model.

	a	***b***	***c***	$\mathbf{R^2}$
B_s	0.02730	2.21501	0.48488	0.9522
B_c	0.02181	2.54169	−0.38784	0.7601
B_r	0.00911	2.24540	0.41587	0.9545
B_t	-	-	-	0.9484

2.6. Statistical Analysis

Two-way analysis of variance (ANOVA) and Tukey's multiple comparisons were used to separate the significant differences ($p < 0.05$) among tree age and site index if the homogeneity of variance and normal distribution tests were passed. If not, the data were treated with inverse sine transformation, logarithmic transformation, etc.

The generalized additive mixed model (GAMM) in the "mgcv" package of the R 4.2.1 software was used to analyze the effects of age and site condition on the biomass of the individual trees and each biomass component, annual increment, and allocation [41]. GAMM is a semi-parametric model with linear predictors that involves the sum of smooth

functions of covariates, which allows flexible functional dependence of outcome variables on covariates through nonparametric regression, and uses random effects to explain the correlation between observations [42,43]. The model is expressed as follows:

$$E_{ijk} = K_0 + S_i\left(Age_{ijk}\right) + S_i\left(Site\ index_{ijk}\right) + R_{ijk} + \varepsilon_{ijk} \quad (11)$$

where E_{ijk} is the dependent variable (total and each component biomass, annual biomass increment, proportion of each component biomass), K_0 is the overall intercept, $S_i\left(Age_{ijk}\right)$ is a smooth function of tree age, $S_i\left(Site\ index_{ijk}\right)$ is a smooth function of site index, R_{ijk} is a random effect assumed to be distributed as $N\left(0, \sigma^2\right)$ with a variance component σ^2, and ε_{ijk} is an error vector.

3. Results

3.1. Growth and Change in Total Biomass

The results of the variance analysis showed that age, site index, and their interaction all had a significant effect on the total biomass and its annual increment of each individual tree ($p < 0.001$). The total biomass of the individual trees increased with age and site index, while the annual biomass increment first increased and then decreased with tree age. Improvement in the site conditions was found to significantly enhance biomass accumulation (Figure 3). Under different site conditions, differences were observed in both the total biomass and its annual increment. When the site conditions were poor, the biomass increased at a low rate after the age of 20 years. On the contrary, when the site conditions were good, the annual biomass increment rapidly declined after reaching the maximum. However, the annual increment was still positive at the age of 40 years, and higher than that recorded when the site conditions were poor.

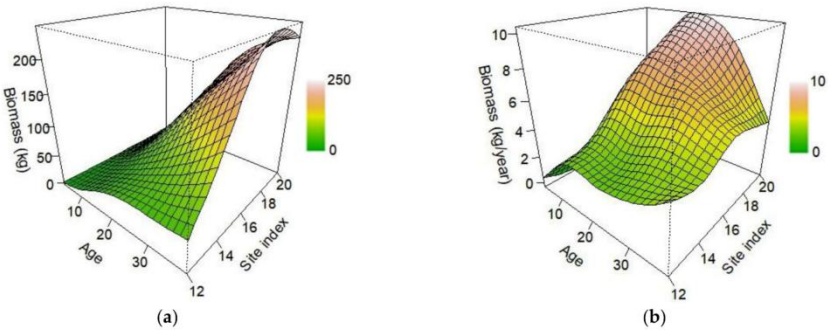


Figure 3. Tree-level biomass dynamics: (**a**) total biomass, and (**b**) annual biomass increment.

3.2. Growth and Change in the Biomass of the Tree Components

The results of the variance analysis showed that age, site index, and their interaction had a significant effect on the biomass of tree components as well as on the annual biomass increment of each component ($p < 0.001$). Significant and strong nonlinearity was observed between the biomass/annual biomass increment of each component and age/site index (Figure 4). The influence of age and site condition on the biomass and annual biomass increment of each component was found to follow the order of stem > root > branch. As age increased, the biomass of each component increased, while the annual biomass increment of each component first increased and then decreased. The annual biomass increments of the stem and root began to decline after 15 years, and that of the crown after 10 years (Figure 4a,c). The site index was found to have the same effect on the biomass and its

increment of each component (Figure 4b,d). When the site index rose from 14 to 20, an improvement in the site conditions was found to significantly enhance the biomass and its increment; however, when the site index increased from 12 to 14, the positive effect was not so obvious.

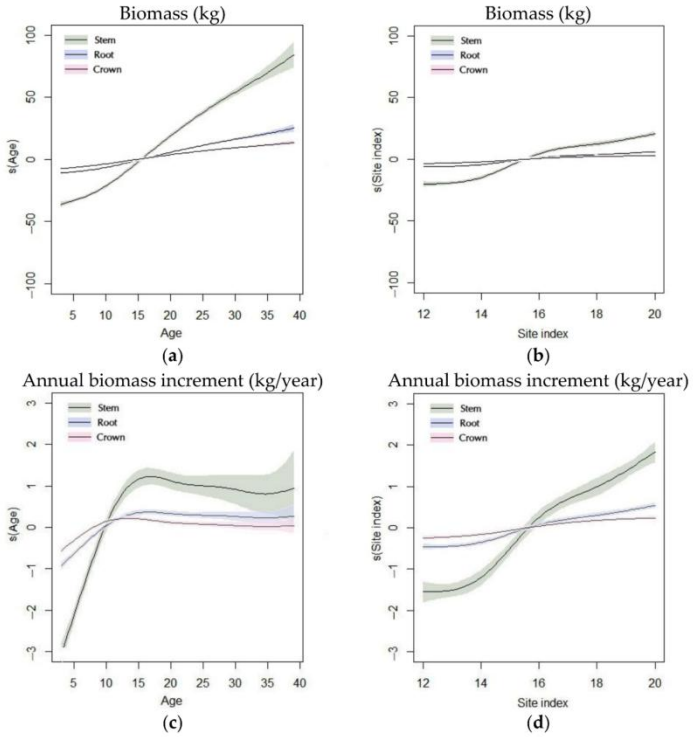


Figure 4. Estimated degrees of freedom with smooth spline functions on observed organ biomass ((**a**) Age and (**b**) Site index) and their annual increments ((**c**) Age and (**d**) Site index). The different color shades indicate the 95% confidence intervals.

3.3. Biomass Allocation within the Tree Components

As shown in Figure 5, the stem made up the largest proportion of the total biomass (49–70%), and the proportion increased with age. The root made up about 16–20% of the total biomass, and this proportion kept an obvious increasing trend only at a young age. The proportion of the crown decreased from 35% at age 8 years to 9% at age 40 years. After nine years, the root biomass made up a greater proportion of the total biomass than that of the crown, which was second only to that of the stem. The GAMM results generated by fitting the age, site condition, and biomass proportion data showed that the proportions of the stem, root, and crown biomasses increased or decreased relatively quickly before the tree reached 10 years of age (Figure 6). Tree age and site index positively contributed to the proportion of root biomass before the trees matured. After the trees matured, the proportion of root biomass decreased with age, and site index still positively contributed to it. The effect of site index on biomass allocation was nonlinear. When the site index

rose from 14 to 16 or from 18 to 20, it significantly impacted the increase or decrease in the proportion of the stem, root, and crown biomass.

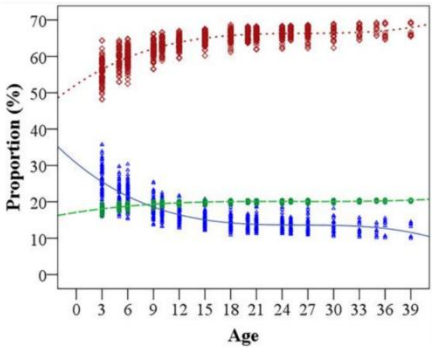


Figure 5. Tree-level dynamics of the biomass proportion of each component. The red squares and short dotted line represent the stem and its fitted line, the green triangles and long dotted line represent the root and its fitted line, and the blue circles and line represent the crown and its fitted line.

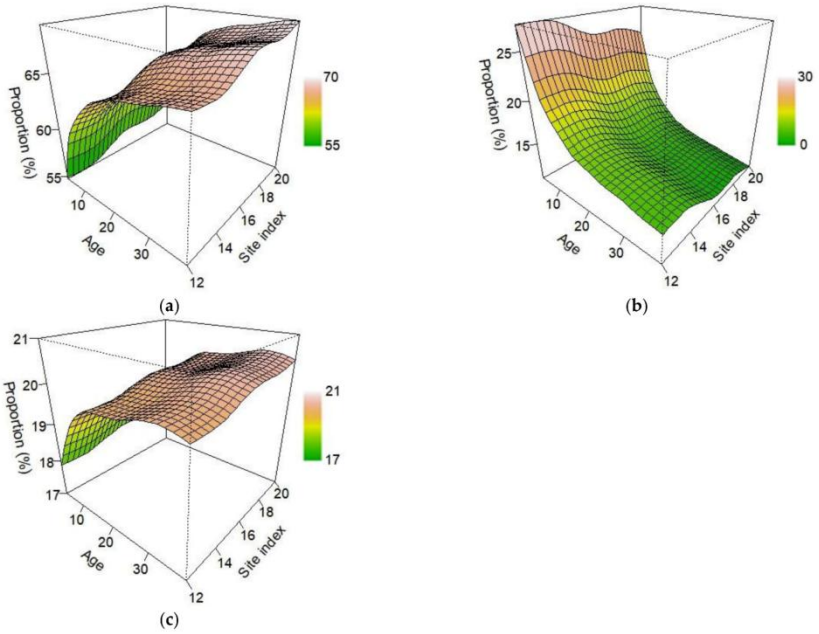


Figure 6. Observed biomass proportion of each component with age and site index interactions: (**a**) proportion of stem biomass, (**b**) proportion of crown biomass, and (**c**) proportion of root biomass.

4. Discussion

4.1. Dynamics of Total Biomass and Its Increment

It is widely recognized that the biomass of a single tree increases with tree age and good site conditions and that the tree biomass increment may reach its maximum at the early stage of succession due to tree recruitment and size increase. When a certain age is reached, biomass increases at a low rate for a period and then decreases. Similarly, the annual increment shows a trend of first increasing and then decreasing [40]. Others have shown that the increase in above-ground biomass of trees in plots with better site conditions is significantly higher than that of trees in plots with poorer site conditions [44,45]. This pattern was also observed in our study, in which the continuous age variable and site index variable were used. However, there were some differences due to the additive or interactive effects of age and the site conditions on tree growth and biomass accumulation. Such effects have been recognized by others, but have not been well explained [22]. If nutrient requirements are not met during rapid tree growth, tree biomass will be negatively affected [46]. Our findings show that the total biomass may stop increasing or increase only slightly after 20 years in plots with poor site conditions. In contrast, the total biomass still has growth potential after 40 years in plots with better site conditions. This indicates that site conditions affect the time at which maximum total biomass is reached; that is, improving site conditions delays the age at which the tree reaches carbon sequestration maturation. This is similar to the consensus that trees growing at sites with good site conditions have a longer natural maturation period than trees subjected to poor site conditions [47]. Nowadays, timber production is no longer the main aim of forest management. Instead, the focus is on modern forestry development with ecological considerations, such as increased carbon sequestration and the maintenance of biodiversity [48,49]. Our findings show that when considering only carbon sequestration gains, management measures should be taken to promote stand growth after 20 years in plots with poor site conditions. It is also clear from our findings that in plots with good site conditions where trees have a long natural maturity age, the carbon storage capacity of the whole ecosystem can be increased by implementing measures such as interplanting broadleaved species to improve carbon sequestration [50,51]. Unfortunately, the maximum age of the sample trees in our study was 40 years; thus, no conclusions can be drawn or recommendations made based on our results about the growth of trees after 40 years. Older sample trees should be included in future studies to improve our understanding of the full tree growth cycle.

4.2. Biomass Dynamics and Increment Tree Components

By dividing trees into stems, crowns, and roots, we analyzed the tree-level biomass dynamics of Chinese fir plantations, which contained trees of different ages and in different plots, thus providing a higher resolution of biomass dynamics [40]. Other studies usually consider only age variables and tend to ignore root biomass and the effect of site conditions on biomass dynamics [52,53]. A recent study conducted by Tang et al. in Chinese fir forests used a method similar to that used in this study [40]. However, that study focused on Chinese fir trees of the same age, growing at the same site condition, and with different dominant grades. In addition, root biomass was not taken into account, and the trees were not separated into components for analysis. In our study, the dynamic changes in the biomass and the increment of each component caused by age and site conditions were found to be very similar to those observed in the total biomass. On the whole, increases in site index positively contributed to increases in biomass; however, the relationship was not linear. In contrast to the site conditions, which were found to contribute to the continuous increase in total biomass, the effect of the site index rising from 12 to 14 on the biomass and its increment was not as obvious as when the site index rose from 14 to 20. These findings indicate that when the site index rose from 12 to 14, the site index may not have been the main contributing factor to biomass change and that biomass dynamics may be influenced by other factors.

4.3. Dynamic of Proportion of Tree Components Biomass

Since carbon allocation is an important factor in predicting forest growth, it is critical to study biomass allocation patterns [54]. Our findings showed that tree age and site condition significantly influenced the allocation of a tree's biomass. The aboveground allocation pattern observed in this study, in which the proportion of biomass found in the stem increased and that found in the crown decreased with tree age, is consistent with that reported in previous studies [20,55,56]. However, some investigators found that the proportion of root biomass decreases with stand age [38], and others have reported that the proportion of root biomass increased slightly but remained relatively stable after the tree matured [57,58]. However, our study found that the proportion of root biomass increased with age and then gradually decreased after the trees matured, which is consistent with the results of our other study [59]. The reason for the increase in the proportion of root biomass with age may be that the part below the ground from the cutting place was treated as roots in our study, which resulted in roots usually containing part of the stem. One of the most recognized concepts used to explain this allocation pattern is "optimal allocation theory", in which plants preferentially allocate biomass to the organ that can maximize the use of the most limited resource [60]. According to this theory, more organic matter will be allocated to organs with storage and reproductive functions than to organs with growth functions [61,62]. Our findings support this theory: we observed a rapid accumulation of root biomass before the age of 10 years, and the allocation of biomass changed from stem > crown > root to stem > root > crown. This was mainly due to the rapid expansion of leaves and branches in the early growth period of the trees, the transportation of large quantities of organic substances produced by photosynthesis to other organs for growth in the crown, and the rapid increase in diameter at breast height and the total height of the trees. The proportion of root biomass gradually increases, as part of a general strategy to help trees become fixed in the soil to resist lodging and improve the nutrient absorption capacity to support sustainable growth [23].

Site conditions comprise the terrain, landform, climate, soil, and other environmental factors at the tree location, and they are closely related to the growth and development of trees [63]. In our study, biomass accumulation varied with tree age and site quality. The stem, crown, and root components represented 49–70%, 9–35%, and 16–20% of the total biomass, respectively, depending on tree age and site condition. The stem was found to be the main contributor to the total biomass throughout the tree life cycle. The proportion of crown biomass was higher than that of the root before the age of 10 years, but thereafter the root biomass was the second largest contributor to the total biomass [64,65]. Most studies have shown that tree biomass allocation is significantly different among trees at high-quality sites and low-quality sites [66]. Our results showed that the effects of tree age and site conditions on tree biomass allocation were nonlinear. When the site index rose from 12 to 14 or from 16 to 18, the site conditions may not have been the main factor affecting biomass allocation, and other environmental or non-environmental factors should be considered when attempting to improve biomass allocation. Similar conclusions were obtained using measured biomass data from sample trees [59], implying that tree ring analysis can be used to simulate growth trends in tree biomass.

5. Conclusions

Improving the understanding of tree growth processes is important for developing a comprehensive knowledge of carbon storage formation processes. We retrospectively studied the total and incremental biomass dynamics and allocation of whole trees and their components (i.e., the stem, crown, and root) using tree ring analysis. It is effective and feasible to analyze the dynamics and allocation patterns of forest biomass using tree ring analysis. Compared to traditional destructive sampling that can only obtain biomass at a given age, tree ring analysis can obtain biomass growth and changes at various ages during tree growth, which is conducive to a better understanding of the formation and change processes of forest biomass and carbon stocks. The results showed that the annual

biomass increment of Chinese fir trees tended to first increase and then decrease with age and that the biomass of the tree and each component increased nonlinearly with age. However, improving the site conditions delayed the age at which the tree reached carbon sequestration maturation. The effect of the site conditions on the tree biomass allocation was also nonlinear. These findings can be used by forest managers to help them fully understand the specific processes of biomass formation and provide guidelines for optimizing management measures to increase forest carbon storage.

Author Contributions: Conceptualization, C.D. and X.X.; formal analysis, B.Z., Q.C., S.L. and K.L. (Kangqi Liu); investigation, C.D., X.X., F.M., B.Z. and S.L.; data curation, X.X. and Q.L.; resources, C.D., F.M. and K.L. (Kangying Lu); methodology, C.D. and Q.L.; supervision, F.M. and Q.L.; visualization, C.D. and X.X.; writing—original draft preparation, X.X.; writing—review and editing, C.D. and Q.L.; project administration, Q.L. and F.M.; funding acquisition, F.M. and K.L. (Kangying Lu). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Forestry Science and Technology Innovation Fund Project of Hunan Province (XLK202104-1), the European Investment Bank Loan for Hunan Forest Quality and Efficiency Improvement Demonstration Project (OT-S-KTA2), the Hunan Forestry Engineering Science and Technology Support Project (ly2021-02) and the Youth Innovative Talents Project of Guangdong Province (2019GKQNCX060).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

Acknowledgments: We appreciate the staff of five forest farm for the help during the field survey and the editors and anonymous reviewers for their constructive comments.

Conflicts of Interest: The authors declare no conflict of interest.

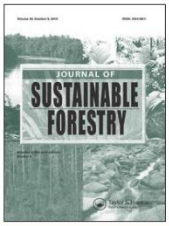
References

1. Bustamante, M.; Robledo-Abad, C.; Harper, R.; Mbow, C.; Ravindranat, N.H.; Sperling, F.; Haberl, H.; de Siqueira Pinto, A.; Smith, P. Co-benefits, trade-offs, barriers and policies for greenhouse gas mitigation in the agriculture, forestry and other land use (AFOLU) sector. *Glob. Chang. Biol.* **2014**, *20*, 3270–3290. [CrossRef] [PubMed]
2. Houghton, R.A. Aboveground forest biomass and the global carbon balance. *Glob. Chang. Biol.* **2005**, *11*, 945–958. [CrossRef]
3. Olson, J.S.; Watts, J.A.; Allison, L.J. *Carbon in Live Vegetation of Major World Ecosystems*; ORNL: Oak Ridge, TN, USA, 1983.
4. Foster, J.R.; Finley, A.O.; D'Amato, A.W.; Bradford, J.B.; Banerjee, S. Predicting tree biomass growth in the temperate–boreal ecotone: Is tree size, age, competition, or climate response most important? *Glob. Chang. Biol.* **2016**, *22*, 2138–2151. [CrossRef] [PubMed]
5. Chris, S.; Heimann, M.; Artaxo, P.; Bakker, D.; Chen, C.-T.A.; Field, C.; Gruber, N.; Le Quéré, C.; Prinn, R.; Richey, J.; et al. Current status and past trends of the global carbon cycle. In *The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World*; Island Press: Washington, DC, USA, 2004; Volume 62, pp. 17–44.
6. Houghton, J. Global warming. *Rep. Prog. Phys.* **2005**, *68*, 1343. [CrossRef]
7. Gore, A. Measure emissions to manage emissions. *Science* **2022**, *378*, 455. [CrossRef]
8. Wang, W.; Duan, Y.; Zhang, L.; Wang, B.; Li, X. Review on forest carbon sequestration counting methodology under global climate change. *J. Nanjing For. Univ.* **2016**, *40*, 170–176.
9. Wei, X.; Zhang, J.; Liu, G.; Liu, S.; Wang, W.; Liu, Y.; Blanco, A.J. The concept and application of carbon sequestration potentials in plantation forests. *Shengtai Xuebao* **2015**, *35*, 3881–3885.
10. Dixon, R.K.; Solomon, A.M.; Brown, S.; Houghton, R.A.; Trexier, M.C.; Wisniewski, J. Carbon pools and flux of global forest ecosystems. *Science* **1994**, *263*, 185–190. [CrossRef]
11. Vashum, K. Methods to estimate above-ground biomass and carbon stock in natural forests—A review. *J. Ecosyst. Ecography* **2012**, *2*, 1–7. [CrossRef]
12. Satō, T.; Madgwick, H.A.I. *Forest Biomass*; Martinus Nijhoff/Dr. W. Junk Publishers: The Hague, The Netherlands, 1982.
13. Top, N.; Mizoue, N.; Kai, S. Estimating forest biomass increment based on permanent sample plots in relation to woodfuel consumption: A case study in Kampong Thom Province, Cambodia. *J. For. Res.* **2004**, *9*, 117–123. [CrossRef]
14. Reinikainen, M.; D'Amato, A.W.; Bradford, J.B.; Fraver, S. Influence of stocking, site quality, stand age, low-severity canopy disturbance, and forest composition on sub-boreal aspen mixedwood carbon stocks. *Can. J. For. Res.* **2014**, *44*, 230–242. [CrossRef]
15. Luo, Y. *Biomass and Its Allocation of Forest Ecosystems in China*; China Forestry Publishing House: Beijing, China, 2013.

16. Henry, H.A.L.; Aarssen, L.W. The interpretation of stem diameter–height allometry in trees: Biomechanical constraints, neighbour effects, or biased regressions? *Ecol. Lett.* **1999**, *2*, 89–97. [CrossRef]
17. Chave, J.; Andalo, C.; Brown, S.; Cairns, M.A.; Chambers, J.Q.; Eamus, D.; Fölster, H.; Fromard, F.; Higuchi, N.; Kira, T.; et al. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. *Oecologia* **2005**, *145*, 87–99. [CrossRef] [PubMed]
18. Song, J. Research on Temporal and Spatial Changes of Forest Carbon Storage and Forest Landscape Pattern in Qilian Mountains. Ph.D. Thesis, Gansu Agricultural University, Lanzhou, China, 2021. [CrossRef]
19. Weiskittel, A.R.; MacFarlane, D.W.; Radtke, P.J.; Affleck, D.L.R.; Temesgen, H.; Woodall, C.W.; Westfall, J.A.; Coulston, J.W. A call to improve methods for estimating tree biomass for regional and national assessments. *J. For.* **2015**, *113*, 414–424. [CrossRef]
20. Xiang, W.; Li, L.; Ouyang, S.; Xiao, W.; Zeng, L.; Chen, L.; Lei, P.; Deng, X.; Zeng, Y.; Fang, J.; et al. Effects of stand age on tree biomass partitioning and allometric equations in Chinese fir (*Cunninghamia lanceolata*) plantations. *Eur. J. For. Res.* **2021**, *140*, 317–332. [CrossRef]
21. Merganič, J.; Pichler, V.; Gömöryová, E.; Fleischer, P.; Homolák, M.; Merganičová, K. Modelling impact of site and terrain morphological characteristics on biomass of tree species in Putorana region. *Plants* **2021**, *10*, 2722. [CrossRef]
22. Vanninen, P.; Ylitalo, H.; Sievänen, R.; Mäkelä, A. Effects of age and site quality on the distribution of biomass in Scots pine (*Pinus sylvestris* L.). *Trees* **1996**, *10*, 231–238. [CrossRef]
23. Veronica, G.; Luis, P.; Gerardo, R. Allometric relations for biomass partitioning of Nothofagus antarctica trees of different crown classes over a site quality gradient. *For. Ecol. Manag.* **2010**, *259*, 1118–1126. [CrossRef]
24. Tian, D.; Xiang, W.; Chen, X.; Yan, W.; Fang, X.; Kang, W.; Dan, X.; Peng, C.; Peng, Y. A long-term evaluation of biomass production in first and second rotations of Chinese fir plantations at the same site. *Forestry* **2011**, *84*, 411–418. [CrossRef]
25. Yu, Y.; Song, T.; Zeng, F.; Peng, W.; Wen, Y.; Huang, C.; Wu, Q.; Zeng, Z.; Yu, Y. Dynamic changes of biomass and its allocation in *Cunninghamia lanceolata* plantations of different stand ages. *Chin. J. Ecol.* **2013**, *32*, 1660–1666. [CrossRef]
26. Zhou, L.; Shalom, A.-D.D.; Wu, P.; He, Z.; Liu, C.; Ma, X. Biomass production, nutrient cycling and distribution in age-sequence Chinese fir (*Cunninghamia lanceolate*) plantations in subtropical China. *J. For. Res.* **2016**, *27*, 357–368. [CrossRef]
27. Bowman, D.M.J.S.; Brienen, R.J.W.; Gloor, E.; Phillips, O.L.; Prior, L.D. Detecting trends in tree growth: Not so simple. *Trends Plant Sci.* **2013**, *18*, 11–17. [CrossRef]
28. Anderson-Teixeira, K.J.; Herrmann, V.; Rollinson, C.R.; Gonzalez, B.; Gonzalez-Akre, E.B.; Pederson, N.; Alexander, M.R.; Allen, C.D.; Alfaro-Sánchez, R.; Awada, T.; et al. Joint effects of climate, tree size, and year on annual tree growth derived from tree-ring records of ten globally distributed forests. *Glob. Chang. Biol.* **2022**, *28*, 245–266. [CrossRef] [PubMed]
29. Toro-Herrera, M.A.; Pennacchi, J.P.; Vilas Boas, L.V.; Honda Filho, C.P.; Barbosa, A.C.M.C.; Barbosa, J.P.R.A.D. On the use of tree-ring area as a predictor of biomass accumulation and its climatic determinants of coffee tree growth. *Ann. Appl. Biol.* **2021**, *179*, 60–74. [CrossRef]
30. Bouriaud, O.; Bréda, N.; Dupouey, J.-L.; Granier, A. Is ring width a reliable proxy for stem-biomass increment? A case study in European beech. *Can. J. For. Res.* **2005**, *35*, 2920–2933. [CrossRef]
31. Genet, H.; Bréda, N.; Dufrêne, E. Age-related variation in carbon allocation at tree and stand scales in beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) using a chronosequence approach. *Tree Physiol.* **2010**, *30*, 177–192. [CrossRef] [PubMed]
32. Mbow, C.; Verstraete, M.M.; Sambou, B.; Diaw, A.T.; Neufeldt, H. Allometric models for aboveground biomass in dry savanna trees of the Sudan and Sudan-Guinean ecosystems of Southern Senegal. *J. For. Res.* **2014**, *19*, 340–347. [CrossRef]
33. Gonzalez-García, M.; Hevia, A.; Anta, M.; Almeida, A.; Sanchez-Salguero, R.; Majada, J. A Multi-Scale Assessment of Biomass Production in Eucalyptus Nitens Plantations: A Spatio-Temporal Modeling from Tree-Ring Data. In Proceedings of the TRACE—Tree-Rings in Archaeology, Climatology and Ecology 2015, Sevilla, Spain, 20–23 May 2015.
34. Hember, R.A.; Kurz, W.A.; Girardin, M.P. Tree ring reconstructions of stemwood biomass indicate increases in the growth rate of black spruce trees across boreal forests of Canada. *J. Geophys. Res. Biogeosci.* **2019**, *124*, 2460–2480. [CrossRef]
35. National Forestry and Grassland Administration. *China Forest Resources Report*; China Forestry Publishing House: Beijing, China, 2019.
36. Yang, Y.; Huang, S.; Vassov, R.; Pinno, B.; Chhin, S. Climate-sensitive height-age models for top height trees in natural and reclaimed oil sands stands in Alberta, Canada. *Can. J. For. Res.* **2020**, *50*, 297–307. [CrossRef]
37. Meng, X. *Forest Mensuration*; China Forestry Publishing House: Beijing, China, 2006.
38. Zhang, Y.; Liu, Y.; Liu, S.; Zhang, X. Dynamics of stand biomass and volume of the tree layer in forests with different restoration approaches based on tree-ring analysis. *Chin. J. Plant Ecol.* **2012**, *36*, 117–125. [CrossRef]
39. Wang, T.R.S. Dynamic of carbon storage of *Pinus armandii* forest at different diameter levels based on tree ring data in the Baotianman National Nature Reserve, central China. *Chin. Sci. Bull.* **2014**, *59*, 3499–3507.
40. Tang, X.; Lu, Y.; Fehrmann, L.; Forrester, D.I.; Guisasola-Rodríguez, R.; Pérez-Cruzado, C.; Kleinn, C. Estimation of stand-level aboveground biomass dynamics using tree ring analysis in a Chinese fir plantation in Shitai County, Anhui Province, China. *New For.* **2016**, *47*, 319–332. [CrossRef]
41. Hu, S.; Chen, P.; Lin, H.; Xie, C.; Chen, X. Promoting carsharing attractiveness and efficiency: An exploratory analysis. *Transp. Res. Part Transp. Environ.* **2018**, *65*, 229–243. [CrossRef]
42. Lin, X.; Zhang, D. Inference in generalized additive mixed models by using smoothing splines. *J. R. Stat. Soc. Ser. B* **1999**, *61*, 381–400. [CrossRef]

43. Wood, S.N. Thin plate regression splines. *J. R. Stat. Soc.* **2003**, *65*, 95–114. [CrossRef]
44. Vanninen, P. Development of the Production and Biomass Structure of Scots Pine: Effects of Competition, Tree Age and Site Fertility. Ph.D. Thesis, University of Helsinki, Helsinki, Finland, 2003.
45. Keeling, H.C.; Baker, T.R.; Martinez, R.V.; Monteagudo, A.; Phillips, O.L. Contrasting patterns of diameter and biomass increment across tree functional groups in Amazonian forests. *Oecologia* **2008**, *158*, 521–534. [CrossRef]
46. Beets, P.; Madgwick, H. Above-ground dry matter and nutrient content of Pinus radiata as affected by lupin, fertiliser, thinning, and stand age. *N. Z. J. For. Sci.* **1988**, *18*, 43–64.
47. Gan, X.H.; Tang, C.B.; Wen, Z.B.; Gao, Y.J. Effects of site conditions on the biomass of *Phyllostachys bambusoides* f. shouzhu Yi. *J. Northwest A F Univ.* **2010**, *38*, 140–146.
48. Cintas, O.; Berndes, G.; Hansson, J.; Poudel, B.C.; Bergh, J.; Börjesson, P.; Egnell, G.; Lundmark, T.; Nordin, A. The potential role of forest management in Swedish scenarios towards climate neutrality by mid century. *For. Ecol. Manag.* **2017**, *383*, 73–84. [CrossRef]
49. Triviño, M.; Pohjanmies, T.; Mazziotta, A.; Juutinen, A.; Podkopaev, D.; Le Tortorec, E.; Mönkkönen, M. Optimizing management to enhance multifunctionality in a boreal forest landscape. *J. Appl. Ecol.* **2017**, *54*, 61–70. [CrossRef]
50. Busing, R.T.; Garman, S.L. Promoting old-growth characteristics and long-term wood production in Douglas-fir forests. *For. Ecol. Manag.* **2002**, *160*, 161–175. [CrossRef]
51. Gustafson, E.J. Relative influence of the components of timber harvest strategies on landscape pattern. *For. Sci.* **2007**, *53*, 556–561. [CrossRef]
52. Wang, X.; Huang, X.; Wang, Y.; Yu, P.; Guo, J. Impacts of site conditions and stand structure on the biomass allocation of single trees in Larch plantations of Liupan Mountains of Northwest China. *Forests* **2022**, *13*, 177. [CrossRef]
53. Xu, Z.; Du, W.; Zhou, G.; Qin, L.; Meng, S.; Yu, J.; Sun, Z.; SiQing, B.; Liu, Q. Aboveground biomass allocation and additive allometric models of fifteen tree species in northeast China based on improved investigation methods. *For. Ecol. Manag.* **2022**, *505*, 119918. [CrossRef]
54. Franklin, O.; Johansson, J.; Dewar, R.C.; Dieckmann, U.; McMurtrie, R.E.; Brännström, Å.; Dybzinski, R. Modeling carbon allocation in trees: A search for principles. *Tree Physiol.* **2012**, *32*, 648–666. [CrossRef]
55. Peichl, M.; Arain, M.A. Allometry and partitioning of above-and belowground tree biomass in an age-sequence of white pine forests. *For. Ecol. Manag.* **2007**, *253*, 68–80. [CrossRef]
56. Jagodziński, A.M.; Dyderski, M.K.; Gęsikiewicz, K.; Horodecki, P. Tree-and stand-level biomass estimation in a *Larix decidua* Mill. chronosequence. *Forests* **2018**, *9*, 587. [CrossRef]
57. Grier, C.C.; Vogt, K.A.; Keyes, M.R.; Edmonds, R.L. Biomass distribution and above-and below-ground production in young and mature *Abiesamabilis* zone ecosystems of the Washington Cascades. *Can. J. For. Res.* **1981**, *11*, 155–167. [CrossRef]
58. Hu, M.; Lehtonen, A.; Minunno, F.; Makela, A. Age effect on tree structure and biomass allocation in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* [L.] Karst.). *Ann. For. Sci.* **2020**, *77*, 90. [CrossRef]
59. Deng, C.; Ma, F.; Xu, X.; Zhu, B.; Tao, J.; Li, Q. Allocation patterns and temporal dynamics of Chinese fir biomass in Hunan Province, China. *Forests* **2023**, *14*, 286. [CrossRef]
60. Bloom, A.J.; Chapin, F.S.; Mooney, H.A. Resource limitation in plants-An economic analogy. *Annu. Rev. Ecol. Syst.* **1985**, *16*, 363–392. [CrossRef]
61. Niklas, K.J.; Enquist, B.J. Canonical rules for plant organ biomass partitioning and annual allocation. *Am. J. Bot.* **2002**, *89*, 812–819. [CrossRef] [PubMed]
62. King, D.A. Allocation of above-ground growth is related to light in temperate deciduous saplings. *Funct. Ecol.* **2003**, *17*, 482–488. [CrossRef]
63. Lehtonen, A. Estimating foliage biomass in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) plots. *Tree Physiol.* **2005**, *25*, 803–811. [CrossRef]
64. Zhang, X.; Cao, Q.; Xiang, C.; Duan, A.; Zhang, J. Predicting total and component biomass of Chinese fir using a forecast combination method. *Iforest Biogeosci. For.* **2017**, *10*, 687–691. [CrossRef]
65. Garrett, L.; Smith, C.; Beets, P.; Kimberley, M. Early rotation biomass and nutrient accumulation of Pinus radiata forests after harvest residue management and fertiliser treatment on contrasting types of soil. *For. Ecol. Manag.* **2021**, *496*, 119426. [CrossRef]
66. Zhao, D.; Bullock, B.P.; Montes, C.R.; Wang, M.; Westfall, J.; Coulston, J.W. Long-term dynamics of loblolly pine crown structure and aboveground net primary production as affected by site quality, planting density and cultural intensity. *For. Ecol. Manag.* **2020**, *472*, 118259. [CrossRef]

3.6 Determining the Ecological Compensation Standard Based on Forest Multifunction Evaluation and Financial Net Present Value Analysis: A Case study in Southwestern Guangxi, China



Taylor & Francis
Taylor & Francis Group

Journal of Sustainable Forestry

ISSN: 1054-9811 (Print) 1540-756X (Online) Journal homepage: https://www.tandfonline.com/loi/wjsf20

Determining the ecological compensation standard based on forest multifunction evaluation and financial net present value analysis: a case study in southwestern guangxi, china

Cheng Deng, Shougong Zhang, Yuanchang Lu & Qingfen Li

To cite this article: Cheng Deng, Shougong Zhang, Yuanchang Lu & Qingfen Li (2020): Determining the ecological compensation standard based on forest multifunction evaluation and financial net present value analysis: a case study in southwestern guangxi, china, Journal of Sustainable Forestry, DOI: 10.1080/10549811.2020.1723644

To link to this article: https://doi.org/10.1080/10549811.2020.1723644

Published online: 05 Feb 2020.

JOURNAL OF SUSTAINABLE FORESTRY
https://doi.org/10.1080/10549811.2020.1723644

Taylor & Francis
Taylor & Francis Group

Determining the ecological compensation standard based on forest multifunction evaluation and financial net present value analysis: a case study in southwestern guangxi, china

Cheng Deng [a], Shougong Zhang [b], Yuanchang Lu [c], and Qingfen Li [a]

[a]College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China; [b]Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China; [c]Research Institute of Resource Information, Chinese Academy of Forestry, Beijing, China

ABSTRACT

There is growing concern that public benefits from forests are underprovided in current forest management regimes and need to be increased through policy and economic measures that promote conservation. Ecological compensation is a type of institutional arrangement for the sustainable use of ecosystem services achieved by adjusting the distribution of costs and benefits among different stakeholders using economic measures. However, how to accurately and reasonably determine the compensation standard for ecological services has not been guided by scientific methods and theories. This study provides an estimation of the compensation standard for forest ecological services based on the forest multifunction evaluation and financial net present value analysis, and a case study was performed in Southwest China. The results showed that most forest types brought some economic loss to the managers but contributed great ecological benefits to the public when they were managed as ecological forests. It is crucial to incentivize forest managers to participate in voluntary conservation programs through ecological compensation. The results of this analysis can potentially guide sustainable forest management by both accurate quantification of the value of forest ecosystem services and an improved understanding of the costs of voluntary forest conservation schemes currently in use in many countries.

KEYWORDS

Ecological forest; conservation programs; ecological benefit; ecological compensation; forest multifunction evaluation; financial net present value analysis

Introduction

The forest ecosystem provides many kinds of ecological services, such as air environment improvement, carbon fixation, oxygen release, soil and water conservation, biodiversity protection, and others (Costanza et al., 1997; Pukkala, 2016). However, while the prices for "traditional" forest goods, such as timber and fuel-wood, have been decided for centuries by the market, ecological goods and services are not traded in markets and their real value is mostly unknown (Lazdinis, Mavsar, Weiss, & Lazdinis, 2009). The majority of these non-market goods and services are, from the social point of view, undervalued in economic terms as there is typically an expectation that these services should be offered for free and made available to various degrees to all as public goods (Merlo & Briales, 2000). An additional complication is that the absence of monetary values placed on forest

CONTACT Qingfen Li li63757416@163.com College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China

goods and services and their free availability may cause users to underestimate the value of these services and costs of their provision (Lazdinis et al., 2009). Destruction of the forest resources, ecosystem imbalance, and environmental deterioration can result when the self-regulating limit of the ecological environment is exceeded. If everybody is reluctant to pay for the improvement of the ecological environment, it is fatal for the sustainable development of human society (Yang, Zhang, Chang, Cheng, & Cao, 2018).

With the development of the social economy and increased public awareness of environment protection, many countries have established forest conservation programs. Some governments have developed strategies to motivate and steer the forest managers to provide ecological services through various forms of payment for ecosystem services (PES) schemes (Canova et al., 2019; Lindhjem & Mitani, 2012; Mayrand & Paquin, 2004). In the last decade, PES has emerged as a dominant economic instrument to adjust market failures and conserve forest ecosystem services (Kamaljit, Gladwin, & Siddappa, 2007; Wunder, 2005). Many countries have established PES programs in which governments pay rural communities and farmers in exchange for ecological services (Carlo, 2013; Kalacska, Sanchez-Azofeifa, Rivard, Calvo-Alvarado, & Quesada, 2008; Muñoz-Piña, Guevara, Torres, & Braña, 2008; Pagiola, 2008; Ruggiero, Metzger, Tambosi, & Nichols, 2019). In addition to these government initiatives, self-sustaining PES initiatives involving private companies and NGOs as buyers and sellers of ecosystem services have also been enacted (Landell-Mills & Porras, 2002; Yang et al., 2018).

Eco-compensation is mainly a public mechanism to promote environmental protection and restoration. Eco-compensation can include different mechanisms that employ monetary subsidies as an integral part of project support and are regarded as equivalent to payment for ecological services (Li, Xu, Wang, Ding, & Song, 2018). Eco-compensation has been hailed as one of the most important support measures to slow degradation and loss of soils, grasslands, and forests in many countries (Asian Development Bank, 2016; Feng, Wu, Liang, Li, & Zhao, 2018; Lazdinis et al., 2009). Eco-compensation approaches have become a core part of strategies for sustainable ecological environment protection (Arriagada, Villaseñor, Rubiano, Cotacachi, & Morrison, 2018; Lima, Krueger, & García-Marquez, 2017), and are credited with improving the environment of animal habitats and the conservation and restoration of biodiversity (Grima, Singh, Smetschka, & Ringhofer, 2016; Herzog et al., 2005; Huang, Shao, Liu, & Lu, 2018; Torres & Skutsch, 2012).

However, how to accurately and reasonably determine the compensation standard for forest ecological services has not been guided by scientific methods and theories (Sheng, Zhen, Xie, & Xiao, 2017). There are ongoing studies on the theories and methodologies of value assessment of forest ecosystem services, but approved and well-established accounting methods have not yet been established (Liu, Yang, & Min, 2018; Zheng et al., 2013). Thus, existing forest eco-compensation strategies have not been based on a scientific evaluation of forest ecosystem services (Fen et al., 2011). In practice, the main methods for determining the compensation standard for forest ecological services have been based on the forest area or management cost (Brown, Clarkson, Barton, & Joshi, 2014; Zhou et al., 2007). Most methods do not consider aspects of both input and output (cost and benefit) simultaneously. In addition, studies have focused more on the implementation of ecological compensation projects, which mainly focus on the subject and object of compensation, but few studies addressed the characteristics and effects of forest management from the standpoint of forest managers, and most ignored the underlying ecological process (Chen, Ni, & Zhang, 2018;

Clot, Grolleau, & Méral, 2017; Meineri, Deville, Grémillet, Lelu, & Séguin, 2015). This lack of study has made further implementation of current schemes problematic, with no uniform assessment indices or methods, no long-term eco-compensation strategy analysis, and inaccurate eco-compensation standards that do not meet the needs of forest owners, restricting improvement of ecological conservation programs.

This study provided an estimation of reasonable eco-compensation standards for forest ecosystem services based on forest multi-function evaluation and financial net present value analysis. We expect these findings to provide important insights into the effectiveness of eco-compensation systems and present useful implications for the design of ecological conservation policies for more sustainable forestry development and enhanced ecosystem services.

Materials and methods

Study area

The study area was the Experimental Center of Tropical Forestry, located in the southwest of Pingxiang City, in the Guangxi Zhuang Autonomous Region of China (21°57'~22°19' N, 106° 39'~106°59' E) (Figure 1). The Experimental Center of Tropical Forestry occupies about 22,922.02 ha, and the forest area covered a surface area of 18,430.47 ha, comprised 80.41% of the territory. Among the forest areas, there are 16,527.33 ha of arboreal forest area, 89.67% of

Figure 1. The location of the study area.

the total, and 1903.15 ha of shrub forest and other forests. The main coniferous tree species in this area are masson pine (*Pinus massoniana*) and Chinese fir (*Cunninghamia lanceolata*), and the main broad-leaved tree species are eucalyptus (*Eucalyptus*), Mytilaria laosensis (*Mytilaria laosensis* Hamamelidaceae), and Illicium (*Illicium verum* Hook.f.). These tree species comprised more than 70% of the species in the whole forest area.

Since the beginning of the twenty-first century, the forest management target of the Experimental Center of Tropical Forestry transformed from the sustainable utilization of wood to multifunctional management. At the same time, the Experimental Center of Tropical Forestry actively participated in various conservation programs of the country. Currently, there are 5,122.5 ha of forests that are managed as ecological forest of conservation programs, which accounts for 27.79% of the whole forest area (Figure 2). Commercial business activities are prohibited in the ecological forest. Moreover, the logging of ecological forest of conservation programs is strictly restricted, only can be carried out when: (1) the forest suffered a natural disaster and need to be cleaned up, (2) the forest quality need to be improved through thinning, (3) the forest over-ripening and need to be updated. Moreover, only the selective cutting method could be used, the volume of each cutting should not be more than 25% of the stock before cutting, and the canopy density should not be less than 0.5 after felling.

Forest management monitoring

A monitoring system for a multifunctional forest management plan at the forest management unit level was conducted in the Experimental Center of Tropical Forestry territory (Deng,

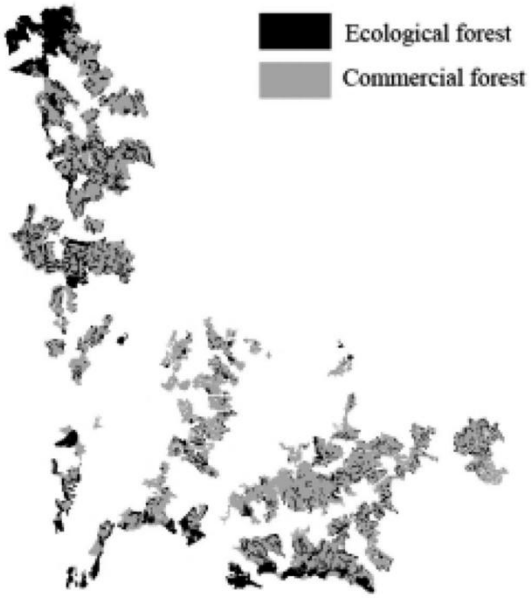


Figure 2. The distributions of ecological forests.

Zhang, Lu, & Tang, 2016). The monitoring system was based on inventory sampling of a 1-km grid, with a total of 236 permanent sample plots, including 61 assigned to ecological forest formations, 124 to commercial forest formations, and 51 to non-forest land. The information retrieved from the forest inventory sampling was then entered in a Geographical Information System (GIS) constructed using the forest map (Figure 3). Star cluster round sample plots were chosen for forests and shrublands. Each sample plot consisted of three circular sub-sample plots and the radius of each sub-sample plot was 6.51 m, with a total area of three sub-sample plots of 400 m^2. In each sub-sample plot, a 4 m × 4 m square plot was set for monitoring of bushes and saplings, and a 1 m × 1 m square plot was used to monitor herbs (Figure 4).

The most widespread forest types in the Experimental Center of Tropical Forestry territory were considered for the ecological services analysis. Eleven forest type groups were used based on the dominant tree species and silvicultural management of the sample plots: Massoniana pine forest (I), China fir forest (II), eucalyptus forest (III), Mytilaria laosensis forest (IV), slash pine forest (V), coniferous mixed forest (VI), Castanopsis hystrix forest (VII), broad-leaved mixed forest (VIII), conifer-broad-leaved mixed forest (IX), Illicium forest (X), and shrub forest (XI). The sample plots in non-forest land (XII) were used as comparison plots for part monitoring indexes.

Two inventories were made in 2012 and 2015 using the monitoring system of multi-functional forest described above. The monitoring data were collected through field plot

Figure 3. Layout of forest inventory sampling plots.

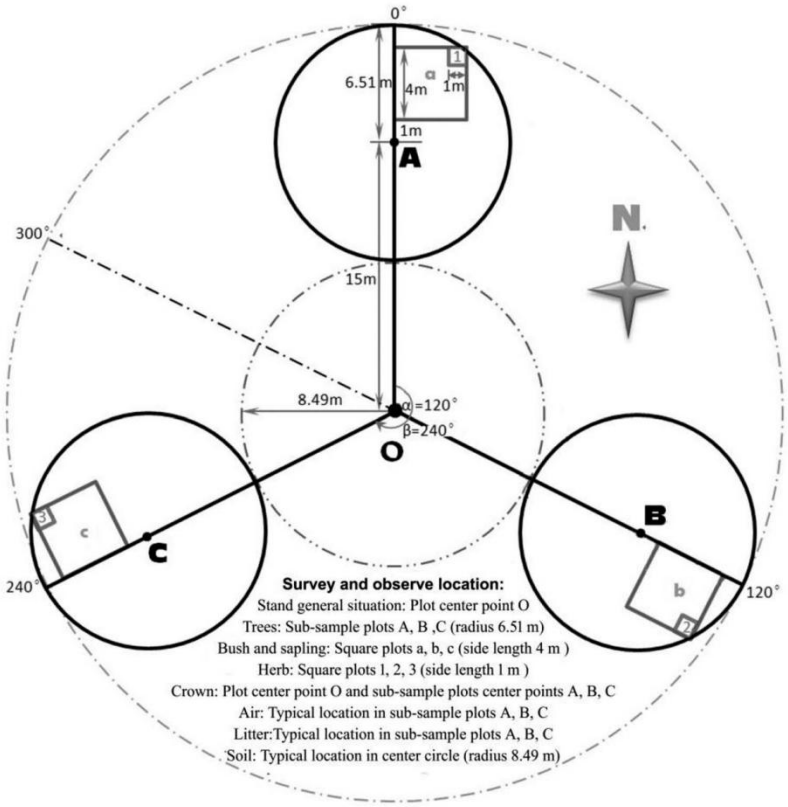


Figure 4. Diagram of circular shape plot clusters.

measurements and previous forest resource documents and management files. The main monitoring information of the ecological forest of conservation program as inventoried in 2015 is presented in Tables 1 and 2.

Forest multifunction evaluation

The ecological functions of each forest type were evaluated. Functions previously described in the literature were adapted to the Experimental Center of Tropical Forestry context to address the characteristics specific to that territory. These functions were defined and codified before the survey phase based on the result of the synthesis of data previously gathered and existing information including previous management plans and maps. Seven ecological functions were eventually considered: air quality improvement, carbon sequestration, oxygen release, water conservation, soil reinforcement, fertility maintenance, biodiversity conservation (Ritter & Dauksta, 2006).

Table 1. Main forest growth information of the ecological forest in 2015.

Forest type	Plots number	Stand age (year)	Stand density (Treesha^{-1})	Average DBH (cm)	Average height (m)	Volume (m^{3}ha^{-1})	Biomass of tree (Mg ha^{-1})	Biomass of bush (Mg ha^{-1})	Biomass of litter (Mg ha^{-1})
I	3	11	1,545	12.9	8.9	97.48	59.46	3.79	22.51
II	4	13	1,804	11.0	10.6	108.95	63.19	3.83	19.63
III	3	3	1,770	8.1	8.6	46.50	40.08	1.52	8.82
IV	5	10	1,487	14.1	12.2	131.80	210.88	2.17	10.29
V	4	15	1,816	11.6	8.5	92.31	55.02	3.93	18.82
VI	4	15	1,440	13.3	9.6	102.70	61.10	3.13	21.66
VII	5	16	1,269	11.7	7.6	70.53	112.85	3.22	11.78
VIII	7	17	1,085	13.0	9.8	58.64	93.83	4.11	11.25
IX	6	19	1,530	12.4	9.2	71.66	103.90	2.92	16.12
X	7	14	863	13.8	8.0	23.57	36.54	2.30	9.88
XI	13	-	-	-	-	14.88	18.60	8.79	13.92
Totality	61	13	1,332	12.4	9.1	75.23	67.16	3.83	17.24

The air quality improvement function can be evaluated from adjustment of air temperature and humidity, producing negative air ions. According to the electrical energy required by air conditioning to adjust to the same temperature as the forest can adjust the air temperature, the value of adjusting the air temperature function of the forest was calculated as follows (Peng, Wang, & Chen, 2005)

$$Q = C \cdot M_{\mathrm{a}} \cdot \Delta t \tag{1}$$

$$V_T = Q \times 0.0002778 \times 0.333 \cdot C_{\mathrm{E}} \cdot t \tag{2}$$

where Q represents the energy (kJ) required for air temperature change in unit time, C is the air specific heat capacity (typically, 1.01 kJ kg^{-1}), M_a is the total quality of the forest air (kg), and can be calculated according to the volume of forest air (forest area multiplied by stand height) and air density (1.2 kg m^{-3}), and Δ_t represents the adjusted air temperature by the forest (°C, this was equal to the difference between the forest air temperature and the average temperature of non-stocked land). V_T is the value of adjusting the air temperature of the forest, where 0.0002778 is the conversion coefficient of kilojoules to kilowatt-hours, 0.333 is the average electricity consumption (in kilowatt-hours) for the air conditioner to provide one kilowatt-hour of heat or cold energy, C_E is the cost of one kilowatt-hour (in this study it was 0.6 Yuan), t is the time (in hours). The volume of forest air was $10000AH$ m^3, where A is the forest area (ha), and H is the stand height (m), so formula (5) was then simplified to

$$V_T = 1.121A \cdot H \cdot C_{\mathrm{E}} \cdot \Delta t \cdot t \tag{3}$$

As a similar principle, the value of adjusting the air humidity function of forest could be calculated as follows:

$$V_H = 69.45A \cdot H \cdot C_E \cdot \Delta f \cdot t \tag{4}$$

where V_H represents the value of adjusting the air humidity and Δ_f was the adjusted air humidity by forest (%), which was equal to the difference between the forest air humidity and the average humidity of non-stocked land. In forests, the lifetime of negative oxygen ions is generally around 20 min, and the production cost of each negative oxygen ion is

Table 2. Main ecological environment information of the ecological forest in 2015.

Forest type	Average temperature (°C)	Average humidity (%)	Negative air ions (number)	Water holding rate of litter(%)		Leaf area index	Soil nutrient (g kg^{-1})				Soil bulk density (gcm^{-3})	Soil noncapillary porosity(%)	Biodiversity index		
				Max	Natural		N	P	K	OM			Trees	Shrubs	Herbs
I	30.3	77.1	1,522	139.62	31.96	2.87	1.07	0.26	8.07	12.61	1.29	10.43	0.36	1.85	1.08
II	30.1	78.9	2,226	162.34	36.24	3.87	1.30	0.49	1.58	11.11	1.23	8.19	0.54	1.73	1.39
III	30.9	79.5	1,506	196.75	24.96	2.05	0.96	0.31	11.75	8.10	1.37	7.22	0.00	1.05	0.75
IV	29.6	79.7	2,186	206.18	26.55	4.16	1.05	0.33	5.35	11.14	1.24	7.44	0.61	1.54	1.16
V	30.7	77.9	1,645	138.36	31.62	3.11	0.75	0.22	14.19	7.01	1.47	7.82	0.88	1.97	1.16
VI	27.9	81.6	2,106	147.51	33.28	3.22	1.03	0.23	13.99	16.81	1.34	12.05	1.24	1.67	1.17
VII	30.7	80.1	1,510	215.37	29.13	4.97	1.10	0.26	4.66	17.29	1.22	5.49	1.00	1.41	1.25
VIII	28.8	85.1	2,205	221.94	32.76	5.32	1.25	0.31	4.67	12.15	1.2	6.91	1.47	1.63	1.18
IX	28.6	85.2	2,871	153.33	34.07	4.25	0.86	0.22	16.07	8.19	1.43	10.68	1.30	2.13	1.06
X	28.3	83.1	2,541	201.01	35.45	3.17	1.35	0.37	3.34	17.01	1.09	7.29	1.13	1.76	1.62
XI	32.1	76.8	1,499	188.91	37.39	1.76	1.20	0.39	10.47	9.79	1.24	6.67	0.00	2.24	0.79
XII	32.6	76.6	816	-	-	-	-	-	-	-	-	-	-	-	-
Totality	30.1	79.6	1,811	150.37	30.74	2.86	1.07	0.29	9.09	11.75	1.30	9.13	2.88	4.46	2.53

approximately 8.73×10^{-18} Yuan (Shao & He, 2000). The annual average number and value of negative oxygen ions provided by a forest in 1 year can be determined as follows:

$$G_I = 2.628 \times 10^{14} \cdot n^- \cdot A \cdot H \tag{5}$$

$$V_1 = 0.002294A \cdot H \cdot (n^- - n_0{}^-) \tag{6}$$

where G_I represents the number of negative oxygen ions per year, n^- is the content of negative oxygen ions (number cm^{-3}) in forests, V_I is the value of negative oxygen ions, H is the stand height, and $n_0{}^-$ is the content of negative oxygen ions in non-stocked land.

Most of the fixed carbon in forests is found in trees and shrubs, some is in litter, and some is transferred to the soil with the decomposition of litter (Mcgarvey, Thompson, Epstein, & Shugart, 2016). The annual average amount and value of carbon sequestration of forests can be calculated as follows:

$$CS_\Delta = (CS_i - CS_{i-n})/n \tag{7}$$

$$CS_i = 0.5(B_{Ti} + B_{Bi} + B_{Li}) + SOCD_i \cdot A \tag{8}$$

$$SOCD_i = 1/1.724 \cdot RC_i \times h_i \times \rho_i/10 \tag{9}$$

$$V_C = CS_\Delta \times p_C \tag{10}$$

where CS_Δ is the annual average amount of carbon sequestration (Mg $year^{-1}$) of forests, Vc is the annual average value of carbon sequestration of forest, CS_i and $CS_{i\text{-}n}$ are the annual average amount of carbon sequestration at i years and i-n years, and 0.5 is the conversion coefficient of biomass to carbon. B_{Ti}, B_{Bi}, B_{Li} are the biomass (Mg) of trees, shrubs, and litter, respectively, at i years, and these values were estimated by existing biomass models (Yang & Guan, 2007; Zheng, Cai, Ming, Yu, & Li, 2014). n was the interval of inventory (year), $SOCD_i$ was the soil carbon density (Mg ha^{-1}) at i years, 1.724 was the Van Bemmelen factor (Chang & Jones, 2013), R_{Ci} is the soil organic matter content (g kg^{-1}) at i years, h_i is the soil thickness (m) at i years, ρ_i is the soil bulk density (g cm^{-3}) at i years, and P_C is the price of carbon calculated according to carbon taxation (Duff & Hsu, 2010), this study it was 921.42 Yuan Mg^{-1}.

According to the principle of plant photosynthesis, plants can emit 1.19 tonnes of oxygen for 1 tonne biomass, so the amount of oxygen released by the forest and the value of that oxygen can be calculated as shown below. Since the amount of oxygen released by the herbs was almost equal to the amount of oxygen consumed by litter decomposition, the calculation did not consider it.

$$OR_\Delta = 1.19(B_{Ti} + B_{Bi} - B_{T_{i-n}} - B_{B_{i-n}})/n \tag{11}$$

$$V_{O_2} = G_{O_2} \times p_{O_2} \tag{12}$$

where OR_{Δ} is the annual average amount of oxygen (Mg year^{-1}) released by forests, B_{Ti}, B_{Bi} are the biomass of trees and shrubs, respectively, at i years, n is the interval of the inventory, Vo_2 is the annual average value of oxygen released by forests, Po_2 is the average price of oxygen production (according to the market survey, it was 1000 Yuan Mg^{-1}).

The comprehensive water storage capacity method was used to calculate the water conservation capacity, which was considered the interception of the canopy layer, the water-holding capacity of the litter, and the water storage of soil. In theory, the forest litter begins to absorb rainwater only when the rainfall exceeds the interception capacity of the forest canopy. Similarly, the soil is considered to absorb rainwater only when the rainfall exceeds the interception capacity of the forest canopy and the litter absorb ability. The canopy interception was calculated using the rainfall interception model (Aston, 1979; Gómez, Giráldez, & Fereres, 2001)

$$W_I = \sum_{i=1}^{m} I_i \tag{13}$$

$$I_i = C_P S_{\max}(1 - e^{-kR_i/S_{\max}}) \times 10 \tag{14}$$

$$S_{\max} = 1.184 + 0.490LAI \tag{15}$$

$$k = 0.065LAI \tag{16}$$

where W_I represents the annual total rainfall interception (Mg ha^{-1} year^{-1}) per hectare forest, m is the number of rains in a year, I_i is the rainfall interception (Mg ha^{-1} year^{-1}) of per hectare forest of the i rain event (according to the statistical methods used by the meteorological department, precipitation within 1 year was divided into multiple single rain events on a daily basis, which starts at 8:00 on the first morning and ends at 8:00 on the second day), C_p is the crown cover (%), S_{max} is the maximum canopy storage capacity (mm), and k is a parameter that is closely related to leaf area. In theory, k was close to 1 when the canopy was very dense (completely covered), and all rainfall was intercepted by the canopy, R_i represents the gross precipitation (mm) of the i rain event (this was obtained from the local meteorological department), and LAI was the leaf area index.

The water-holding capacity of the forest litter can be calculated based on litter storage and its effective water-holding capacity as follows:

$$W_{Lmax} = (0.85R_{\max} - R_0)M_L \tag{17}$$

$$W_L = \begin{cases} W_{L_{max}} & R_i \times 10 - I_i \geq W_{L\max} \\ \sum_{i=1}^{m}(R_i - I_i) & R_i \times 10 - I_i < W_{L\max} \end{cases} \tag{18}$$

where W_{Lmax} is the maximum water holding capacity of the litter (Mg ha^{-1}), 0.85 represents the effective water holding coefficient, R_{max} and R_0 are, respectively, the maximum water-holding rate and average natural water-holding rate of litter (%), M_L is the stock of litter (Mg ha^{-1}), W_L is the annual total water-holding amount (Mg ha^{-1}

year^{-1}) of forest litter of per hectare forest, and 10 is the weight conversion coefficient of rainfall. Soil water storage can be calculated according to soil non-capillary porosity and soil thickness

$$W_{Smax} = 10000 P_N \cdot h \tag{19}$$

$$W_S = \begin{cases} W_{S\max} & R_i \times 10 - I_i - W_{L\max} \geq W_{S\max} \\ \sum_{i=1}^{m} (R_i - I_i - W_{L\max}) & R_i \times 10 - I_i - W_{L\max} < W_{S\max} \end{cases} \tag{20}$$

where W_{Smax} represents the maximum water storage capacity of soil (Mg ha^{-1}), P_N is the soil non-capillary porosity (%), h is the soil thickness (m), and W_S is the annual total water storage (Mg ha^{-1}) of per hectare soil. The value of water conservation can be calculated as shown below:

$$V_W = (W_I + W_L + W_S) \cdot A \cdot (C_R + C_P) \tag{21}$$

where V_W is the annual total value of water conservation of forests, C_R is the cost (Yuan) of 1 cubic meters of reservoir capacity construction, and C_P is the cost of purifying 1 cubic meters of water.

The capacity of preserving soil and limiting erosion can be calculated by the following equations.

$$S_R = (X_2 - X_1) \cdot A \tag{22}$$

$$V_S = (S_R/\rho) \cdot C_S \tag{23}$$

where S_R represents the annual capacity of preserving soil from erosion (Mg ha^{-1} year^{-1}), X_2 and X_1 are the soil erosion modulus (Mg ha^{-1} year^{-1}) of non-forest land and forest land, respectively, in the study area (Wang et al., 2013), V_S is the annual value of preserving soil from erosion of per hectare forest, ρ is the soil bulk density, and C_S is the cost of digging and transporting a tonne of soil.

The nitrogen (N), phosphorus (P) and potassium (K) in the soil preserved by the forest are converted into urea, superphosphate, and potassium chloride at corresponding rates. The amount of fertilizer maintained by the forest and its value can be calculated as below.

$$F_M = S_R(N/R_1 + P/R_2 + K/R_3 + R_C) \tag{24}$$

$$V_F = S_R(C_1 N/R_1 + C_2 P/R_2 + C_3 K/R_3 + C_4 R_C) \tag{25}$$

where F_M represents the annual amount of fertilizer (tonnes) maintained by the forest, V_F is the annual value of this fertilizer maintained by the forest, N, P, K, R_C represent the content of nitrogen, phosphorus, potassium, and organic matter in the soil, respectively, R_1 is the content of nitrogen in urea (46.67%), R_2 is the content of phosphorus in superphosphate (12.00%), R_3 is the content of potassium in potassium chloride (52.00%), C_1, C_2, C_3, and C_4 represent the price of urea, superphosphate, potassium chloride, and organic matter, respectively, and were derived from the local market.

The value of species conservation of forests was assessed according to the Shannon–Wiener index (H') and the corresponding cost standard

$$H' = -\sum_{i=1}^{s} p_i \ln p_i \tag{26}$$

$$V_B = C_B \cdot A \tag{27}$$

where S represents the species number, P_i is the important value proportion of species i, C_B is the value of the species conservation of a hectare forest as based on the Shannon–Wiener index. When $H'<1$, C_B was 2,000 Yuan ha^{-1} year^{-1}, $1\leq H'<2$, C_B was 5,000 Yuan ha^{-1} year^{-1}, $2\leq H'<3$, C_B was 10,000 Yuan ha^{-1} year^{-1}, $3\leq H'<4$, C_B was 20,000 Yuan ha^{-1} year^{-1}, $4\leq H'<5$, C_B was 30,000 Yuan ha^{-1} year^{-1}, $6\leq H'<6$, C_B was 40,000 Yuan ha^{-1} year^{-1}, $H' >6$, C_B was 50,000 Yuan ha^{-1} year^{-1} (Wang et al., 2008).

Forest management benefit analysis

There are different investment indexes of various forest types. A forest of higher overall ecological services value does not represent a high investment benefit. Investment efficiency can differ for the same forest type with different management direction (as a commercial forest or as an ecological forest). To accurately understand the contribution of different management directions of various forest types to the managers, it is necessary to analyze the input and output of forest management. In addition, to allow a comparison of the investment benefit of all forest types, the financial net annual value ($FNAV$) was used to evaluate the financial benefit for each forest type. The rotation was used as the given period, and then the net cash flow of forest management within the given period was converted to the net annual value. A higher net annual value indicated a better investment benefit (Silva & Fontes, 2005). The net annual value was calculated as follows:

$$FNAV_j = [\sum_{t=0}^{n_j} (CI_j - CO_j)_t (P/F, i_c, t)](A/P, i_c, t) \tag{28}$$

where $FNAV_j$ represents the financial net annual value of forest type j; $(CI_j - CO_j)_t$ is the net cash flow of forest type j in t year, in which CI_j is the inward cash-flow of forest type j, which mainly includes thinning income, sales income of timber, and other forest products, and is mainly related to forest growth and forest yield, the price of timber, and other forest products, and CO_j is the cash outflow of forest type j, which mainly includes the cost of afforestation, forest management and protection, harvesting and transportation of timber and other forest products, and related taxes and fees. With different geographical locations, distance and traffic conditions will affect the input and output of forest management. To fully reflect the influence of these factors on forest management efficiency, the average cost of each forest type was calculated based on all sample plots of this forest type; $(P/F, i_c, t)$ represents the equivalent transformation coefficient between the final value F and the present value P; $(A/P, i_c, t)$ represents the equivalent transformation coefficient between present value P and annuity A; n_j is the rotation of forest type j, that was the time required for regeneration to harvest; i_c is the investment benchmark yield, here 8%.

Compensation standard for forest ecological services calculation

The compensation standards of an ecological forest were determined according to the financial net annual value of forest management and the annual average values of ecological services functions of each forest type. If the financial net annual value when the forest was managed as ecological forest of conservation programs was lower than the value when the forest was managed as commercial forest, the minimal compensation should fill this gap; otherwise, there would be little motivation for forest managers to manage a forest as an ecological forest in a conservation program. At the same time, the compensation should not exceed the total value of the ecological services provided by forest. In contrast, if the financial net annual value when the forest was managed as an ecological forest of conservation programs was higher than that when the forest was managed as a commercial forest, this would indicate a better economic benefit when the forest was managed as an ecological forest. In this case, managers would be motivated to manage the forest as an ecological forest even without compensation. If additional compensation were to be provided, the amount could be calculated from the value of the provided forest ecological services after deduction of the economic income of forest management. Therefore, the compensation standard of forest ecological benefits can be determined according to the following formulas.

$$CSVj = \begin{cases} -\Delta FV_j \sim EVA_j & \Delta FV_j < 0 \\ 0 \sim (EVA_j - \Delta FV_j) & \Delta FV_j \geq 0 \end{cases} \tag{29}$$

$$\Delta FV_j = FNAV_{ncj} - FNAV_{cj} \tag{30}$$

where CSV_j is the annual compensation standard value of forest type j, ΔFV_j is the difference between the financial net annual value of forest type j when it was managed as a commercial forest and the value when it was managed as an ecological forest, $FNAV_{cj}$ is the financial net annual value of forest type j when managed as a commercial forest, $FNAV_{ncj}$ is the financial net annual value of forest type j when managed as an ecological forest, and EVA_j is the annual average values of ecological services functions of forest type j when managed as an ecological forest.

Results

The value of forest ecological services

Conifer-broad-leaved mixed forest had the highest annual average value of ecological services functions in all forest types, followed by broad-leaved mixed forest, Mytilaria laosensis forest, coniferous mixed forest, Chinese fir forest, Eucalyptus forest, Castanopsis hystrix forest, Illicium forest, Massoniana pine forest, slash pine forest, and shrub forest. The values of each forest functions are shown in Table 3.

The financial net annual value of forest management

When managed as a commercial forest, the Illicium forest has the highest financial net annual value in all forest types, followed by Eucalyptus forest, Massoniana pine forest,

Table 3. Annual average values of ecological services functions per unit area.

Forest type	Ecological services functions value of per unit area (Yuan·ha^{-1}·a^{-1})							
	Total	Improve air quality	Fix carbon	Release oxygen	Conserve water	Reinforce Soil	Maintain fertility	Species conservation
I	48,574.25	3,529.63	1,500.69	4,391.93	23,470.95	952.05	1,729.00	13,000.00
II	59,392.84	5,597.12	3,280.92	9,601.96	26,190.23	1,019.61	703.00	13,000.00
III	56,846.62	4,216.08	5,767.76	16,879.92	15,611.82	954.04	2,417.00	11,000.00
IV	64,111.36	6,859.59	3,746.40	10,964.21	26,707.16	1,239.00	1,595.00	13,000.00
V	47,588.58	2,482.64	1,708.45	4,999.96	21,910.96	836.57	2,650.00	13,000.00
VI	62,946.97	10,002.88	1,774.15	5,192.24	27,206.88	926.82	2,844.00	15,000.00
VII	53,623.31	5,580.87	2,469.87	7,228.34	20,527.23	1,260.00	1,557.00	15,000.00
VIII	66,928.45	9,961.22	2,920.81	8,548.05	27,726.02	1,281.35	1,491.00	15,000.00
IX	71,445.66	10,245.14	2,036.84	5,961.03	28,861.78	971.87	3,369.00	20,000.00
X	51,837.63	10,001.32	1,782.51	5,216.70	18,310.12	787.98	739.00	15,000.00
XI	33,874.01	487.05	507.50	1,485.24	14,938.49	1,095.73	2,360.00	13,000.00
Average	53,602.14	5,290.15	1,998.25	5,848.07	23,118.73	1,009.25	2,022.47	14,315.22

Mytilaria laosensis forest, Castanopsis hystrix forest, slash pine forest, Chinese fir forest, coniferous mixed forest, conifer-broad-leaved mixed forest, broad-leaved mixed forest, and shrub forest. When managed as an ecological forest in conservation programs, the Illicium forest has the highest financial net annual value of all forest types, followed by eucalyptus forest, Shrub forest, Mytilaria laosensis forest, slash pine forest, broad-leaved mixed forest, Chinese fir forest, Massoniana pine forest, Coniferous mixed forest, Castanopsis hystrix forest, and conifer-broad-leaved mixed forest.

In all forest types, the Massoniana pine forest had the greatest difference in financial net annual value between management as a commercial forest or as an ecological forest. This was followed by Castanopsis hystrix forest, Mytilaria laosensis forest, Chinese fir forest, coniferous mixed forest, conifer-broad-leaved mixed forest, slash pine forest, broad-leaved mixed forest, Eucalyptus forest, Illicium forest, and shrub forest.

The financial net annual value and the annual average value of ecological services of different forest types are shown in Table 4.

The compensation standard of ecological services

According to the calculation, the lower limit of the compensation standard of different forest types should be between 0 and 3310.44 Yuan per year per hectare, with an average value of 993.99 Yuan per year per hectare. The upper limit of the compensation standard of different forest types should between 33711.48 Yuan and 71445.66 Yuan per year per hectare, with an average value of 56078.23 Yuan per year per hectare. The compensation standards of various forest types are shown in Table 4.

Discussion

The accurate and reasonable determination of forest ecological compensation standards is very important for the sustainable development of forestry and the benefits of economic society. Using both forest multifunction evaluation and financial net present value analysis, this study proposes a methodological system to determine forest ecological compensation standards. This method can calculate the value of forest ecological services and analyzes the contributions and losses of forest managers from the perspective of inputs

Table 4. The financial net annual value and annual average values of ecological services and compensation standard of ecological services for various forest types.

	Financial net annual value (Yuan·ha^{-1}·a^{-1})				The compensation standard (*CSV*) (Yuan·ha^{-1}·a^{-1})	
Forest type	Commercial forest ($FNAV_c$)	Ecological forest ($FNAV_{nc}$)	Difference (ΔFV)	Annual average values of ecological services function (*EVA*) (Yuan·ha^{-1}·a^{-1})	Lower limit	Upper limit
I	2,802.86	−507.58	−3310.44	48,574.25	3,310.44	48,574.25
II	799.20	−436.92	−1,236.12	59,392.84	1,236.12	59,392.84
III	3,928.51	3,843.69	−84.82	56,846.62	84.82	56,846.62
IV	1,397.20	107.33	−1,289.87	64111.36	1,289.87	64,111.36
V	807.76	103.97	−703.79	47,588.58	703.79	47,588.58
VI	651.74	−524.59	−1,176.33	62,946.97	1,176.33	62,946.97
VII	847.64	−558.97	−1,406.61	53,623.31	1,406.61	53,623.31
VIII	281.01	−415.76	−696.77	66,928.45	696.77	66,928.45
IX	347.74	−681.41	−1,029.15	71,445.66	1,029.15	71,445.66
X	1,4064.65	1,4211.30	146.65	51,837.63	0.00	51,690.98
XI	52.47	215.00	162.53	33,874.01	0.00	33,711.48
Average	2,361.89	1,396.01	−965.88	53,602.14	993.99	56,078.23

and outputs (cost and benefit). In different countries and regions, the cost of forest management and the value of forest ecological services function may be different, but the application principle of this method is the same, allowing its wide applicability.

The results revealed some contradictions between forest managers and government in the selection of forest types and the direction of forest management. For example, forest managers preferred to manage Illicium forests and Shrub forests as ecological forests in conservation programs due to the better economic benefits. The government prefers for forest managers to plant more Conifer-broad leaved mixed forests and broad-leaved mixed forests as ecological forests because they can provide more ecological benefits for society and the public. Forest managers did not want to manage other forest types as ecological forests in conservation programs. For the Illicium forest, the economic income of managers was obtained mainly through fruit picking rather than through timber harvesting, which was not restricted by the conservation activity. The shrub forest provides hardly any income to managers if they do not participate in conservation programs. Managers often lost income for other forest types when managed as ecological forests in conservation programs, due to the restriction of conservation activities. Thus, forest managers would prefer to manage most forests as commercial forests, and the governments prefer more ecological forests.

In order to reconcile these opposing viewpoints, implementation of an ecological compensation policy was necessary (Cuperus, Bakermans, Haes, & Canters, 2001; Fan, Chen, & Wang, 2019; Grillos, 2017). Government policies can be used to motivate and incentivize forest managers to participate in voluntary conservation programs and provide compensation to forest managers of ecological forests (Carlo, 2013; Kalacska et al., 2008; Muñoz-Piña et al., 2008; Pagiola, 2008; Ruggiero et al., 2019). Many studies indicated that the current forest ecological compensation standards are relatively low (Lei, Yang, & Liu, 2017; Li, Zhang, & Zhang, 2018; Sheng et al., 2017; Zhou, Zhou, Liu, & Xia, 2019), and our result appears to be consistent with these results. Recently, the Chinese government provided an average compensation of about 225 to 600 Yuan per year per hectare for ecological forests in conservation

programs (Deng, Zheng, Liu, & Liu, 2011). However, according to our results, this compensation standard is insufficient to compensate for the economic loss gap of forest managers.

In this study, the compensation standard for forest ecological services was determined based on the management characteristics of different forest types, which thus addresses the willingness and perception of the managers to accept the strategy (Canova et al., 2019; Lindhjem & Mitani, 2012). A forest can only be used as one of the ecological forests and commercial forests, so the lower limit of the compensation standard was based on the principle that a manager would not earn less when they participate in voluntary conservation programs. This also conforms to the principle that the opportunity cost should be considered for valuation in PES schemes (Canova et al., 2019; Zhou et al., 2019). The upper limit of the compensation standard was based on the ecological benefits of different forest types, which was closely related to the effectiveness of forest management. The financial net annual value was used to quantify and compare the forest management effect, so the compensation standard is determined with consideration of the input-output of forest management as well as the time value of money, which is reasonable and practical (Vodouhe et al., 2016).

The compensation standard established in this study provides a range rather than a single number. With consideration of the price factor, this provides an important reference for the staged and dynamic compensation of forest ecological services. Because of government fiscal restrictions and limited awareness of ecology, it is impractical and unrealistic to compensate all ecosystem services at a single time, as that would require a huge compensation fund beyond that affordable by the government. Because prices and inputs and outputs can change, the use of a staged ecological compensation scheme and dynamic compensation method is recommended and should be tailored to the local economic level and characteristics of each stage of economic development (Li, Xu, Wang, Ding, & Song, 2018; Li et al., 2018). At an early stage of economic development, the compensation payment is likely to focus only on making up for the loss of forest managers, and will not necessarily be high. With economic development, the payment can give more consideration to the contribution of managers to ecosystem services.

In addition, the approach of the forest multifunction evaluation and financial net present value analysis can provide effective means and tools for forest managers to accurately quantify and compare the management benefit of different forest types and then supply the data support and theoretical guidance for managers to choose the most appropriate forest management strategy. The results of this analysis strategy can serve as the basis for government to formulate other compensation standards and policies for improved forest ecological benefit.

The functions of forest ecological services are diverse, and their accurate valuation remains a challenging task, with particular difficulties in setting the upper limit (Li et al., 2018). As a case study, our study only quantified and evaluated the most common forest ecological service functions based on the characteristics of the study area. For areas with additional functions, more specific methods may be needed to quantify physical quantities and values. In addition, the implementation of a forest multi-functional monitoring system is generally complicated, and the accuracy of monitoring data will affect the overall accuracy of forest function evaluation results. Therefore, more accurate, fast, and efficient access to the forest ecological service data remains an important goal.

Conclusions

This study provided a scientific approach to the determination of the compensation standard of forest ecological services for conservation programs, and a case study was performed in Southwest China. This method was based on the forest multifunction evaluation and financial net present value analysis and considered not only the difference of management measures between different forest types, but also the effect of management. This approach enables the government to determine the differences in the ecological services provided by various forest types and the contribution of forest managers to the ecological environment, and then to formulate a more scientific compensation standard for conservation programs. The results of this study can provide important insights into the effectiveness of eco-compensation systems, and present useful implications for design of ecological conservation policies for more sustainable forestry development.

Acknowledgments

This work was funded by the Central Public-Interest Scientific Institution Basal Research Fund (No. IFRIT201501). We also appreciate the editors and anonymous reviewers for their constructive comments.

Competing interest

The authors have declared no conflict of interest.

ORCID

Cheng Deng http://orcid.org/0000-0002-6702-7772
Shougong Zhang http://orcid.org/0000-0001-9832-2558
Yuanchang Lu http://orcid.org/0000-0001-8972-2031
Qingfen Li http://orcid.org/0000-0001-6598-4598

References

ADB. (2016). Toward a national eco-compensation regulation in the People's Republic of China. Asian Development Bank. Mandaluyong City, Philippines.

Arriagada, R., Villaseñor, A., Rubiano, E., Cotacachi, D., & Morrison, J. (2018). Analysing the impacts of PES programmes beyond economic rationale: Perceptions of ecosystem services provision associated to the Mexican case. *Ecosystem Services*, *29*, 116–127. doi:10.1016/j.ecoser.2017.12.007

Aston, A. R. (1979). Rainfall interception by eight small trees. *Journal of Hydrology*, *42*(3), 383–396. doi:10.1016/0022-1694(79)90057-X

Brown, M. A., Clarkson, B. D., Barton, B. J., & Joshi, C. (2014). Implementing ecological compensation in New Zealand: Stakeholder perspectives and a way forward. *Journal of the Royal Society of New Zealand*, *44*(1), 34–47. doi:10.1080/03036758.2013.860377

Canova, M. A., Lapola, D. M., Pinho, P., Dick, J., Patricio, G. B., & Priess, J. A. (2019). Different ecosystem services, same (dis) satisfaction with compensation: A critical comparison between farmers' perception in Scotland and Brazil. *Ecosystem Services*, *35*, 164–172. doi:10.1016/j.ecoser.2018.10.005

Carlo, R. (2013). Ecological compensation in spatial planning in Italy. *Impact Assessment & Project Appraisal*, *31*(1), 45–51. doi:10.1080/14615517.2012.760228

Chang, W. A., & Jones, S. (2013). Assessing organic matter and organic carbon contents in soils of created mitigation Wetlands in Virginia. *Environmental Engineering Research*, *18*(18), 151–156. doi:10.4491/eer.2013.18.3.151

Chen, G., Ni, H., & Zhang, Q. (2018). Eco-*compensation practice in China's river basins*. 2017 3rd International Forum on Energy, Environment Science and Materials (IFEESM 2017). Atlantis Press.

Clot, S., Grolleau, G., & Méral, P. (2017). Payment vs. compensation for ecosystem services: Do words have a voice in the design of environmental conservation programs? *Ecological Economics*, *135*, 299–303. doi:10.1016/j.ecolecon.2016.12.028

Costanza, R., d'Arge, R., Groot, R. D., Farber, S., Grasso, M., Hannon, B., … Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, *387*(1), 253–260. doi:10.1038/387253a0

Cuperus, R., Bakermans, M. M. G. J., Haes, H. A. U. D., & Canters, K. J. (2001). Ecological compensation in dutch highway planning. *Environmental Management*, *27*(1), 75–89. doi:10.1007/s002670010135

Deng, C., Zhang, S. G., Lu, Y. C., & Tang, X. J. (2016). Research on the monitoring system of forest multifunctional at forest management unit level. *Journal of Northwest A&F University: Natural Science Edition*, *44*(7), 131–137.

Deng, H., Zheng, P., Liu, T., & Liu, X. (2011). Forest ecosystem services and eco-compensation mechanisms in China. *Environmental Management*, *48*(6), 1079–1085. doi:10.1007/s00267-011-9742-0

Duff, D., & Hsu, S. L. (2010). Carbon taxation in theory and in practice. *British Journal of Clinical Pharmacology*, *12*(4), 583–585.

Fan, M., Chen, L., & Wang, Q. (2019). Assessing the high impacts of land use change: Spatial characteristics of land uses and ecological compensation based on payment for ecosystem services model in a mountainous area, China. Mitigation and Adaptation Strategies for Global Change, 1–30.

Fen, L., Wenhua, L., Lin, Z., Heqing, H., Yunjie, W., & Naomi, I. (2011). Estimating eco-compensation requirements for forest ecosystem conservation: A case study in Hainan province, southern China. *Outlook on Agriculture*, *40*(1), 51–57. doi:10.5367/oa.2011.0028

Feng, D., Wu, W., Liang, L., Li, L., & Zhao, G. (2018). Payments for watershed ecosystem services: Mechanism, progress and challenges. *Ecosystem Health and Sustainability*, *4*(1), 13–28. doi:10.1080/20964129.2018.1434318

Gómez, J. A., Giráldez, J. V., & Fereres, E. (2001). Rainfall interception by olive trees in relation to leaf area. *Agricultural Water Management*, *49*(1), 65–76. doi:10.1016/S0378-3774(00)00116-5

Grillos, T. (2017). Economic vs non-material incentives for participation in an in-kind payments for ecosystem services program in Bolivia. *Ecological Economics*, *131*, 178–190. doi:10.1016/j.ecolecon.2016.08.010

Grima, N., Singh, S. J., Smetschka, B., & Ringhofer, L. (2016). Payment for ecosystem services (PES) in Latin America: Analysing the performance of 40 case studies. *Ecosystem Services*, *17*, 24–32. doi:10.1016/j.ecoser.2015.11.010

Herzog, F., Dreier, S., Hofer, G., Marfurt, C., Schupbach, B., Spiess, M., & Walter, T. (2005). Effect of ecological compensation areas on floristic and breeding bird diversity in Swiss agricultural landscapes. *Agriculture Ecosystems & Environment*, *108*(3), 189–204. doi:10.1016/j.agee.2005.02.003

Huang, L., Shao, Q., Liu, J., & Lu, Q. (2018). Improving ecological conservation and restoration through payment for ecosystem services in Northeastern Tibetan Plateau, China. *Ecosystem Services*, *31*, 181–193. doi:10.1016/j.ecoser.2018.04.005

Kalacska, M., Sanchez-Azofeifa, G. A., Rivard, B., Calvo-Alvarado, B., & Quesada, M. (2008). Baseline assessment for environmental services payments from satellite imagery: A case study from Costa Rica and Mexico. *Journal of Environmental Management*, *88*(2), 348–359. doi:10.1016/j.jenvman.2007.03.015

Kamaljit, S. B., Gladwin, J., & Siddappa, S. (2007). Poverty, biodiversity and institutions in forest-agriculture ecotones in the Western Ghats and Eastern Himalaya ranges of India. *Agriculture, Ecosystems and Environment*, *121*(3), 287–295. doi:10.1016/j.agee.2006.12.023

Landell-Mills, N, & Porras, I. T. (2002). *Silver bullet or fools' gold?: A global review of markets for forest environmental services and their impact on the poor*. London: IIED.

Lazdinis, I., Mavsar, R., Weiss, G., & Lazdinis, M. (2009). Trends in marketing of non-market forest goods and services in the Baltic States. *Baltic Forestry*, *15*(1), 65.

Lei, X. K., Yang, D. M., & Liu, B. (2017). The influencing factors of forestry farmers' satisfaction with the compensation standard of ecological public welfare forests-Taking Jiangxi Province as an example. *Xinjiang Land Reclamation Economy*, *08*, 62–68.

Li, D., Xu, D., Wang, Z., Ding, X., & Song, A. (2018). Ecological compensation for desertification control: A review. *Journal of Geographical Sciences*, *28*(3), 367–384. doi:10.1007/s11442-018-1478-9

Li, T., Zhang, Q., & Zhang, Y. (2018). Modelling a compensation standard for a regional forest ecosystem: A case study in Yanqing District, Beijing, China. *International Journal of Environmental Research and Public Health*, *15*(4), 565. doi:10.3390/ijerph15040565

Lima, L. S. D., Krueger, T., & García-Marquez, J. (2017). Uncertainties in demonstrating environmental benefits of payments for ecosystem services. *Ecosystem Services*, *27*, 139–149. doi:10.1016/j.ecoser.2017.09.005

Lindhjem, H., & Mitani, Y. (2012). Forest owners' willingness to accept compensation for voluntary conservation: A contingent valuation approach. *Journal of Forest Economics*, *18*(4), 290–302. doi:10.1016/j.jfe.2012.06.004

Liu, M., Yang, L., & Min, Q. (2018). Establishment of an eco-compensation fund based on eco-services consumption. *Journal of Environmental Management*, *211*, 306–312. doi:10.1016/j.jenvman.2018.01.037

Mayrand, K., & Paquin, M. (2004). Payment for environmental services: A survey and assessment of current schemes. *Journal of Helminthology*, *1*(2), 77–80.

Mcgarvey, J. C., Thompson, J. R., Epstein, H. E., & Shugart, H. H. (2016). Carbon storage in old-growth forests of the Mid-Atlantic: Toward better understanding the eastern forest carbon sink. *Ecology*, *96*(2), 311–317. doi:10.1890/14-1154.1

Meineri, E., Deville, A., Grémillet, D., Lelu, M. A., & Séguin, A. (2015). Combining correlative and mechanistic habitat suitability models to improve ecological compensation. *Biological Reviews of the Cambridge Philosophical Society*, *90*(1), 314. doi:10.1111/brv.12111

Merlo, M., & Briales, E. R. (2000). Public goods and externalities linked to mediterranean forests: Economic nature and policy. *Land Use Policy*, *17*(3), 197–208. doi:10.1016/S0264-8377(00)00017-X

Muñoz-Piña, C., Guevara, A., Torres, J. M., & Braña, J. (2008). Paying for the hydrological services of Mexico's Forests. *Ecological Economics*, *65*(4), 725–736. doi:10.1016/j.ecolecon.2007.07.031

Pagiola, S. (2008). Payments for environmental services in Costa Rica. *Ecological Economics*, *65*(4), 712–724. doi:10.1016/j.ecolecon.2007.07.033

Peng, J., Wang, Y., & Chen, Y. (2005). Economic value of urban ecosystem services: A case study in Shenzhen. *Acta Scicentiarum Naturalum Universitis Pekinesis*, *41*(4), 594.

Pukkala, T. (2016). Which type of forest management provides most ecosystem services? *Forest Ecosystems*, *3*(1), 1–16. doi:10.1186/s40663-016-0068-5

Ritter, E., & Dauksta, D. (2006 June, 18–23). *Ancient values and contemporary interpretations of European Forest Culture-reconsidering our understanding of sustainability in forestry*. "Small-scale forestry and rural development: the intersection of ecosystems, economics and society", Proceedings of IUFRO 3.08 Conference, hosted by Galway-Mayo Institute of Technology, Galway, Ireland.

Ruggiero, P. G., Metzger, J. P., Tambosi, L. R., & Nichols, E. (2019). Payment for ecosystem services programs in the Brazilian Atlantic Forest: Effective but not enough. *Land Use Policy*, *82*, 283–291. doi:10.1016/j.landusepol.2018.11.054

Shao, H. R., & He, Q. T. (2000). Forest and air anion. *World Forestry Research*, *13*(5), 19–23.

Sheng, W., Zhen, L., Xie, G., & Xiao, Y. (2017). Determining eco-compensation standards based on the ecosystem services value of the mountain ecological forests in Beijing, China. *Ecosystem Services*, *26*, 422–430. doi:10.1016/j.ecoser.2017.04.016

Silva, M. L. D., & Fontes, A. A. (2005). Discussion on the criteria of economical evaluation: Net present value (VPL), equivalent annual value (VAE) and soil expected value (VET). *Revista Árvore*, *29*(6), 931–936. doi:10.1590/S0100-67622005000600012

Torres, A. B., & Skutsch, M. (2012). Splitting the difference: A proposal for benefit sharing in reduced emissions from deforestation and forest degradation (REDD*). *Forests*, *3*(1), 137–154. doi:10.3390/f3010137

Vodouhe, F. G., Dossou-Yovo, H. O., Chadaré, F. J., Gélinas, N., Assogbadjo, A. E., & Coulibaly, O. (2016). Valuing the potential of non-timber forest products in financial valuation of savannah formation in Sudanian Region. *Universal Journal of Agricultural Research*, *4*(5), 183–197. doi:10.13189/ujar.2016.040504

Wang, B., Wei, J. S., Yu, S. B., Liang, J. P., Cai, H. D., & Nong, S. Q. (2013). Research on forest ecosystem services in Guangxi Zhuang Autonomous region. *Guihaia*, *33*(1), 46–51.

Wang, B., Yang, F. W., Guo, H., Li, S. N., Wang, Y., Ma, X. Q., … Wei, W. J. (2008). *Forest ecosystem service function evaluation criterion* (LY/T1721-2008). Beijing, China: China Standard Press.

Wunder, S. (2005). *Payments for environmental services: Some nuts and bolts*, Occasional Paper No 42. Bogor, Indonesia: Center for International Forestry Research (CIFOR).

Yang, K., & Guan, D. S. (2007). Selection of gaining quadrat for harvesting the undergrowth vegetation and its biomass estimation modeling in forest. *Acta Ecological Sinica*, *02*, 705–714.

Yang, Y., Zhang, X., Chang, L., Cheng, Y., & Cao, S. (2018). A method of evaluating ecological compensation under different property rights and stages: A case study of the Xiaoqing River Basin, China. *Sustainability*, *10*(3), 615. doi:10.3390/su10030615

Zheng, H., Robinson, B. E., Liang, Y. C., Polasky, S., Ma, D. C., Wang, F. C., … Daily, G. C. (2013). Benefits, costs, and livelihood implications of a regional payment for ecosystem service program. *Proceedings of the National Academy of Sciences*, *110*(41), 16681–16686. doi:10.1073/pnas.1312324110

Zheng, L., Cai, D. X., Ming, A. G., Yu, H. L., & Li, Z. G. (2014). Biomass Allocation of different species plantations in subtropical area of China. *Forest Research*, *04*, 454–458.

Zhou, J. X., Pen, Z. H., Fei, S. M., Li, D. X., Sun, Q. X., & Qi, L. H. (2007). A discussion on compensation of forest ecological engineering benefit. *Journal of Forestry Research*, *18*(2), 157–164. doi:10.1007/s11676-007-0033-9

Zhou, Y., Zhou, J., Liu, H., & Xia, M. (2019). Study on eco-compensation standard for adjacent administrative districts based on the maximum entropy production. *Journal of Cleaner Production*, *221*, 644–655. doi:10.1016/j.jclepro.2019.02.239

四、其他业绩

1. 个人荣誉

2024 年荣获林学与风景园林学院 2023-2024 学年“优秀班主任”

林学与风景园林学院 2023-2024 学年优秀班主任情况说明

根据《林学与风景园林学院关于开展 2023-2024 学年班主任考核及评优工作的通知》要求，经个人自评、学生评价及学院考核，以下 17 位老师被评为林学与风景园林学院 2023-2024 学年“优秀班主任”，特此说明。

序号	年级	班主任	班级	考核结果
1	2020 级	刘小蓓	20 园林 1 班	优秀
2	2020 级	汤辉	20 风景园林 1 班	优秀
3	2020 级	李青粉	20 林学 2 班	优秀
4	2020 级	张俊杰	20 中药资源与开发 1 班	优秀
5	2020 级	陈崇贤	20 风景园林 2 班	优秀
6	2021 级	王凌	21 城乡规划 1 班	优秀
7	2021 级	邓成	21 园林 3 班	优秀
8	2021 级	吴蔼民	21 林学丁颖班	优秀
9	2021 级	林毅颖	21 风景园林 1 班	优秀
10	2022 级	杨文越	22 城乡规划 2 班	优秀
11	2022 级	何茜	22 林学丁颖班	优秀
12	2022 级	林敏慧	22 旅游管理 2 班	优秀
13	2022 级	夏宇	22 风景园林 2 班	优秀
14	2023 级	叶昌东	23 城乡规划 2 班	优秀
15	2023 级	吴道铭	23 林学丁颖班	优秀
16	2023 级	徐锐	23 园林 4 班	优秀
17	2023 级	解加米	23 城规振兴班	优秀

林学与风景园林学院

2025 年 7 月 11 日

2. 社会服务

2021-2024 年参与社会服务 120 个工作量。