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Allelopathic effects of *Eucalyptus* on native and introduced tree species

Chaojun Chu [a], P.E. Mortimer [b,c], Hecong Wang [a], Yongfan Wang [a], Xubing Liu [a], Shixiao Yu [a,*]

[a] Department of Ecology, School of Life Sciences/State Key Laboratory of Biocontrol/Key Laboratory of Biodiversity Dynamics and Conservation of Guangdong Higher Education Institutes, Sun Yat-sen University, Guangzhou 510275, China
[b] World Agroforestry Centre, East and Central Asia Office, Kunming, 650201 Yunnan, China
[c] Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming, 650201 Yunnan, China

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ABSTRACT

Allelopathy is widely considered to be one of the causes of biodiversity reduction in *Eucalyptus* plantations. However, most research conducted on the allelopathic effects of *Eucalyptus* is performed in the laboratory with weeds and crops as receptors, which fail to fully reflect natural ecosystems. In this study, we conducted two field trials and a greenhouse trial to assess the influence of soil allelopathy, allelochemical volatilization, and foliage litter decomposition on seed germination and seedling growth of three native (*Acmena acuminatissima*, *Cryptocarya concinna* and *Pterospermum lanceaefolium*) and one introduced (*Albizia lebbeck*) tree species in a *Eucalyptus urophylla* and *Pinus elliottii* plantation. In order to avoid confounding factors relating to management strategies and environmental influences, only one plantation of each species was used for experimentation. In the allelopathy experiment, the root length of the three native species was significantly inhibited in the *E. urophylla* plantation compared with that in the *P. elliottii* plantation. In the volatilization experiments, the seedling survival rate of the three native species was greater in the *E. urophylla* plantation, but significant differences were found for *A. acuminatissima* and *C. concinna*. Root length and dry weight of *P. lanceaefolium* increased significantly in the *E. urophylla* plantation, in the foliage litter decomposition experiments. There was no significant difference between the two plantations for *A. lebbeck*, except that the seedling survival rate was greater in the *E. urophylla* plantation in the foliage litter decomposition experiment. We concluded that allelopathy in the *E. urophylla* plantation was selective, which inhibited the growth of the native tree species but had no significant influence on the introduced *A. lebbeck* species. Allelopathy from volatilization and foliage litter decomposition contributed little to the inhibitory effects. We suggest that the introduced nitrogen-fixing species, *A. lebbeck* could be a potential choice for the establishment of mixed stands with *Eucalyptus*.

1. Introduction

The widespread establishment of *Eucalyptus* plantations for the commercial production of timber and fiber products has generated worldwide controversy (Tang et al., 2007; Zhao et al., 2007). *Eucalyptus* plantations are easily established and fast growing, and can be highly profitable, even in areas that are traditionally poor in timber production. However, there are also negative environmental impacts in planting *Eucalyptus*, such as loss of biodiversity in the understory and soil degradation (Molina et al., 1991; Michelsen et al., 1996; Bone et al., 1997; Forrester et al., 2006; Gareca et al., 2007; Wang et al., 2010).

Understory plants make a substantial contribution to the overall species diversity in plantations since many species are restricted to this layer and others must pass through it during their seedling stage (Ramovs and Roberts, 2003). Biodiversity reduction in fast-growing *Eucalyptus* plantations has been a crucial issue for the long-term sustainability of native ecosystems and allelopathy has been considered a factor for the loss of biodiversity in *Eucalyptus* plantations (Sasikumar et al., 2001; Ahmed et al., 2008; Zhang and Fu, 2009).

Eucalyptus, indigenous to Australia, was first introduced into China in the 1890s. It is now estimated that 300 varieties have been planted in south China, making China the second largest producer of *Eucalyptus* with an area of 3,680,000 ha (Chen et al., 2013). However, the proliferation of *Eucalyptus* plantations in south China has resulted in many problems for the local environment, the major one being the decrease in biodiversity, which many studies have attributed to the allelopathic effects of *Eucalyptus* (May and Ash, 1990; Lisanework and Michelsen, 1993; Fang et al., 2009; He et al., 2014).

* Corresponding author. Tel./fax: +86 20 39332980.
E-mail address: lssysx@mail.sysu.edu.cn (S. Yu).

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In general, there are four ecological processes by which allelopathic chemicals are released into the environment, namely volatilization, leaching, foliage litter decomposition and root exudation (May and Ash, 1990; Fang et al., 2009; Zhang and Fu, 2009). A number of laboratory-based experiments have focused on the effects of leaf sap, volatile compounds, foliage decomposition and root exudation on seed germination and the early growth stages of various receptor species (Molina et al., 1991; Lisanework and Michelsen, 1993; Fang et al., 2009). However, the techniques employed in these experiments often do not resemble natural ecological processes (May and Ash, 1990). Coupled with this is the fact that field trials investigating allelopathic effects under natural conditions are rare (Jose et al., 2006), and studies focusing on the allelopathic effects of *Eucalyptus* on broad-leaved tree species even rarer (Fang et al., 2009). It has been reported that mixed plantations of *Eucalyptus* and other tree species enhance biodiversity and productivity (Forrester et al., 2006; Zhang and Fu, 2009), thus it is also important to screen for species with a high tolerance to the allelopathic effects of *Eucalyptus*. Furthermore, past research has focused on sensitive crops and weeds, which are not characteristic of the vegetation found within *Eucalyptus* plantations, thus emphasizing the need to study the effects of *Eucalyptus* allelopathy on relevant associated species, *in situ* (Sasikumar et al., 2001; Ahmed et al., 2008).

This paper studies the allelopathic effects of volatile compounds and foliage decomposition on seed germination and seedling growth of four tree species in *Eucalyptus* plantations in comparison to the same four species in a *Pinus elliottii* plantation. The aims are first, to identify the allelopathic effects of volatile compounds, foliage decomposition and root exudation in *Eucalyptus urophylla* plantations; secondly, to characterize the differences between different allelochemicals release processes in an *E. urophylla* plantation, and thirdly, to identify tree species that are suitable for the establishment of mixed stands with *E. urophylla*.

2. Materials and methods

2.1. Site description

The field trial was undertaken at Shuilian Mountain Forest Park, Dongguan city, Guangdong Province, China, situated at 22°58′N and 113°42′E, 50–378 m altitude. The climate of this region is subtropical marine monsoon with a mean annual precipitation of 1780 mm and a rainy season from April to September. The average annual temperature is 22.2 °C, with a maximum monthly mean temperature of 28.5 °C in July and a minimum of 14.1 °C in January. The soils are latosol developed on granite with a pH of 3.8. Shuilian Mountain Forest Park has extensive *E. urophylla* and *P. elliottii* plantations that are two of the most popular plantations in south China. The dominant native understorey species in the *E. urophylla* and *P. elliottii* plantations are *Callicarpa pedunculata* and *Psychotria rubra*, respectively. The two plantations are both over 15 years old with similar environmental characteristics, and have not received fertilizer application, canopy thinning or weed control. In order to control for these specific management and environmental factors, only one plantation of each species was used for experimentation. The total area of the plantations are about 50 ha and 15 ha respectively, and were of sufficient size to allow for spatially segregated plots.

2.2. Soil properties

In both *E. urophylla* and *P. elliottii* plantations, ten 20 m × 20 m sampling plots, spatially separated were established at five different elevation. Two plots were randomly selected at each elevation and the space between these two plots was over 100 m. Soil samples at two depths (0–20 cm and 20–40 cm) were randomly taken from five sampling points in each plot. All soil samples were thoroughly mixed, then was dried and ground to pass a 1 mm sieve following the removal of roots and debris. Soil pH was measured electrometrically with a glass electrode (soil: water = 1:2.5). The organic materials content (OM) (dichromate oxidation titration – heating), total nitrogen (TN) (diffusion method), hydrolysable nitrogen (HN) (alkali-hydrolyzed reduction diffusing method), total phosphorous (TP) (acid dissolved − Mo–Sb colorimetry), available phosphorous (AP) (ammonium fluoride-hydrochloric acid extraction), total potassium (TK) (flame photometry) and available potassium (AK) (1 mol/L ammonium acetate-flame photometry) in the soil were also analyzed (SPC, 2007).

2.3. Plant species

We tested the seed germination and seedling growth of four common broad-leaved tree species: *Acmena acuminatissima, Pterospermum lanceaefolium*, *Cryptocarya concinna* and *Albizia lebbeck*. The former three tree species are dominant or common tree species in lower subtropical evergreen broad-leaved forest while *A. lebbeck* plantation is one of popular plantation in south China. Seeds were obtained between late 2009 and early 2010 from Dinghushan National Nature Reserve, Guangdong (23°10′N and 112°34′E, 120–1008 m altitude). The first three tree species are indigenous evergreen species, whilst *A. lebbeck* is an introduced nitrogen (N)-fixing species known to improve soil fertility (Chen et al., 1999). The seeds of *P. lanceaefolium* and *A. lebbeck* are orthodox seeds and can be stored dry, whilst the seeds of *A. acuminatissima* and *C. concinna* are recalcitrant and must be kept in moist sand (Roberts, 1973).

2.4. Experimental design

The allelopathy and volatilization experiments were conducted in the field with shrubs and weeds removed prior to the start of the trial, whilst the foliage litter decomposition experiment was conducted in the greenhouse. The three experiments were designed as follows:

(1) Full allelopathy experiment: In order to receive all of the allelopathic chemicals, seeds were planted directly in the soil of both *E. urophylla* and *P. elliottii* plantations in natural conditions in 1 m × 1 m plots. For each plantation, we select a replicate site (4 m × 1 m) for each two 20 m × 20 m sampling plots. There were totally five replicate sites and four plots (1 m × 1 m) in each replicate site. The aforementioned four tree species were randomly assigned to a plot in each replicate site. One hundred seeds were sown uniformly in each plot.
(2) Volatilization experiment: Seeds were sown in pots (diameter: 15 cm; depth: 8 cm) under a transparent mat awning in both plantations in order to receive volatile compounds only. The soil in the pots was obtained from the margins of the plantations to avoid interference from root exudation. There were five replicates for each target species and 30 seeds of each species were sown uniformly in each pot.
(3) Decomposition experiment: Seeds were sown in soil that had been covered with fallen leaves and were placed in pots in the greenhouse thus receiving allelopathic chemicals derived from foliage litter decomposition only. Soil source, number of replicates and seeds were as for (2).

The experiments were conducted from April 2010 to August 2010. Each treatment was supplied with sufficient water. At the end of the fourth month, plants and roots were excavated and the survival rate, root length, shoot length, fresh weight and dry weight of the seedlings were measured.

2.5. Statistical analyses

All measured variables (mean ± SE) were subjected to an independent-samples *t*-test to assess differences in soil properties, seedling survival and seedling growth between *E. urophylla* and *P. elliottii* plantations. Homogeneity of variance was tested by the Levene's test. All analyses were performed using SPSS 16.0 software for Windows.

3. Results

3.1. Soil properties

For the topsoil (0–20 cm depth), the TN, HN, TK and AK concentrations were all significantly greater in the *E. urophylla* plantation than those in the *P. elliottii* plantation. However, the TP content was lower in comparison to *P. elliottii*. The OM, TN, HN, TK and AK sampled from the 20–40 cm depth were significantly greater in the *E. urophylla* plantation (Table 1).

3.2. Full allelopathy experiment

Seedling survival of three native and one introduced tree species under the released progress of full allelopathy was not significantly affected in the *E. urophylla* plantation when compared to that in the *P. elliottii* plantation (Fig. 1), while seedling growth of the three indigenous tree species decreased significantly in the *E. urophylla* plantation when compared to that in the *P. elliottii* plantation. As for *A. lebbeck*, there was no significant difference between the *E. urophylla* and *P. elliottii* plantations (Fig. 2). Root length of *A. acuminatissima*, *P. lanceaefolium* and *C. concinna* were significantly inhibited by 25.3%, 23.0% and 23.6%, respectively, in the *E. urophylla* plantation (Fig. 2). Shoot length of *A. acuminatissima* was significantly suppressed in the *E. urophylla* plantation compared with that observed in the *P. elliottii* plantation (Fig. 3). Fresh and dry weight of *C. concinna* was also significantly reduced under the allelopathy treatment. However the fresh weight of *P. lanceaefolium* was found to increase significantly (Figs. 4 and 5).

3.3. Volatilization experiment

The seedling survival rate of the native tree species was greater in the *E. urophylla* plantation under the volatilization treatment compared to that grown in the *P. elliottii* plantation, with the seedling survival rate of *A. acuminatissima* and *C. concinna* increasing significantly (Fig. 1). Within the volatilization experiment, the seedling survival rate of the native tree species A. *acuminatissima* and C. *concinna* in the *E. urophylla* plantation were significantly greater than in the *P. elliottii* plantation (Fig. 1).

3.4. Decomposition experiment

Seedling survival rates of the three indigenous tree species did not show any significant difference between the *E. urophylla* and *P. elliottii* plantations, but the seedling survival rate of *A. lebbeck* was significantly greater in the *E. urophylla* plantation (Fig. 1). Seedling growth of the three native species was affected significantly in the foliage litter decomposition treatment (Fig. 2), whilst only root length and dry weight of *P. lanceaefolium* showed a significantly better performance in the *E. urophylla* plantation.

4. Discussion

The present study demonstrates that the inhibitory effects relating to allelopathy in the *E. urophylla* plantation vary according to the tree species present. The three indigenous tree species experienced diminished seedling survival rates and growth, whereas no significant changes were noted for the introduced species, *A. lebbeck*.

This decline in the survival rate and growth performance of native tree seedlings planted in the *E. urophylla* plantations, despite the improved soil nutrition within these sites, provides strong evidence for the allelopathic affects attributed to the *Eucalyptus* trees. This is in agreement with past studies which found that seedlings planted in *Eucalyptus* plantations were affected by allelopathic chemicals from volatilization, leaching, foliage litter decomposition and root exudation (May and Ash, 1990; Sasikumar et al., 2001). The inhibitory effects on native tree species observed in our study provides an explanation for the decrease in biodiversity of understory species within these plantations , as much of the understory is comprised of tree seedlings and saplings, which is consistent with the work of both Alexander (1989), Tang et al. (2007).

Contrary to our overall findings of a decline in seedling growth due to *Eucalyptus* induced allelopathy, the volatilization of allelochemicals from *Eucalyptus* stimulated seed germination in *A. acuminatissima* and *C. concinna*, and foliage litter decomposition of *Eucalyptus* stimulated root length and dry weight in *P. lanceaefolium*. This suggests that the inhibitory effects of allelopathy on native species was not induced by volatilization or foliage litter decomposition alone. This stimulative effect is in agreement with several studies that claim that lower allelochemical concentrations could stimulate the survival and growth of other plant species. This is further confirmed by the work of Xu and Zhang (2006), and He et al. (2014) who found that allelochemical concentrations were lower under natural conditions in comparison to those produced in laboratory experiments (Qiu et al., 2007; Reigosa et al., 2000). Additional evidence is provided by Chen (1999) who reported that certain allelochemicals can stimulate plant growth.

Given the stimulative effects of the volatile and litter-derived allelochemicals, the negative impact of allelopathy on the survival and growth of the indigenous tree seedlings tested seems likely to be the result of a cumulative effect of the various compounds involved. However, the specific mechanism by which these allelo-

Table 1
Soil chemical properties from two soil depths (0–20 and 20–40 cm) in *E. urophylla* and *P. elliottii* plantations. Abbreviations: OM-organic materials concentration; TN-total nitrogen; HN-hydrolysable nitrogen; TP-total phosphorous; AP-available phosphorous; TK-total potassium; AK-available potassium.

Soil sources		OM %	TN %	HN mg kg^{-1}	TP %	AP mg kg^{-1}	TK %	AK mg kg^{-1}
0–20 cm	*E. urophylla*	4.41 ± 0.09	0.15 ± 0.005*	160 ± 0*	0.027 ± 0.0004*	2.18 ± 0.19	1.69 ± 0.01*	93.8 ± 1.69*
	P. elliottii	4.68 ± 0.25	0.12 ± 0.002	120 ± 0	0.029 ± 0.0004	2.32 ± 0.20	1.30 ± 0.01	26.0 ± 1.92
20–40 cm	*E. urophylla*	2.97 ± 0.03*	0.10 ± 0.003*	120 ± 0*	0.025 ± 0.0007	0.64 ± 0.16	1.59 ± 0.07*	68.0 ± 0.71*
	P. elliottii	2.59 ± 0.05	0.08 ± 0.001	84.8 ± 1.1	0.025 ± 0.0007	1.09 ± 0.16	1.08 ± 0.03	20.8 ± 3.06

The values refer to means ± S.E.
* Shows the means of *E. urophylla* are significantly different with that of *P. elliotti* at $P < 0.05$ level by Independent-Samples *T* – Test.

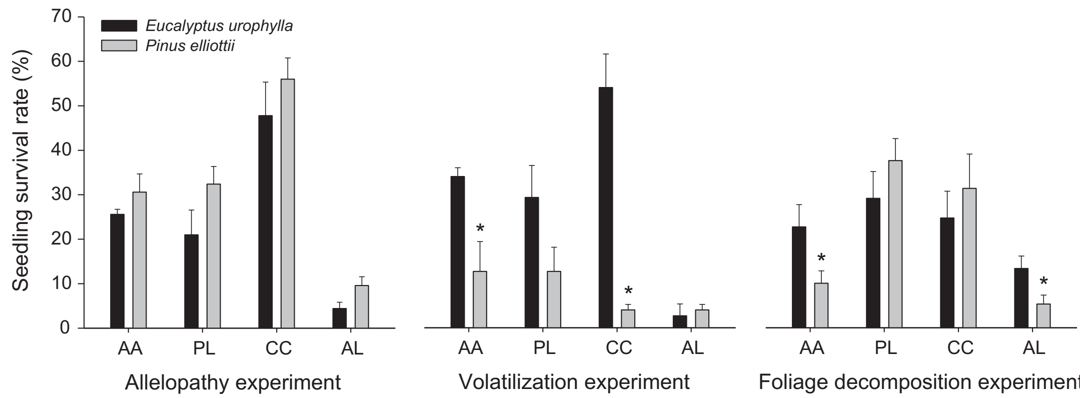

Fig. 1. Seedling survival of three native (AA: *Acmena acuminatissima*, PL: *Pterospermum lanceaefolium* and CC: *Cryptocarya concinna*) and one introduced (AL: *Albizia lebbeck*) tree species under different released progress of allelopathy (soil allelopathy, allelochemical volatilization, and foliage litter decomposition) after seed-sowing in *Eucalyptus urophylla* and *Pinus elliottii* plantations for four months. Error bars represent standard errors, and an "*" denotes significant differences between *E. urophylla* and *P. elliottii* plantations, based on independent-samples *t*-tests at the 0.05 level.

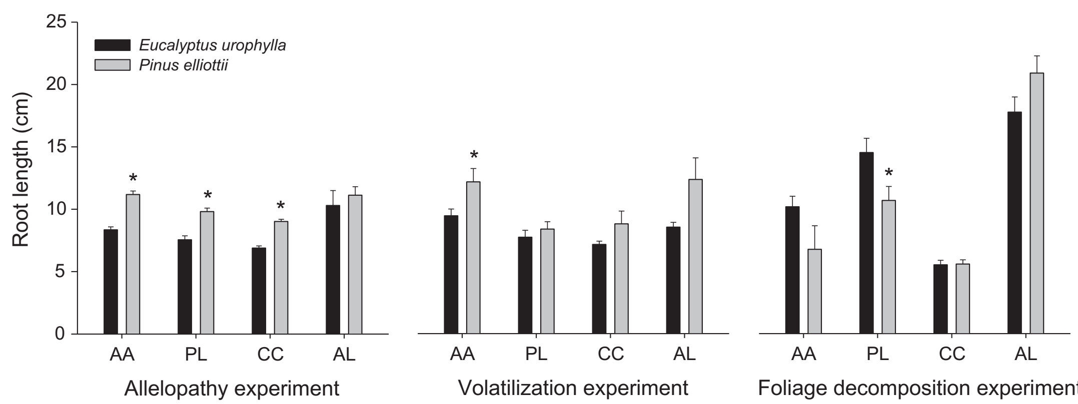

Fig. 2. Seedling root length of three native (AA: *Acmena acuminatissima*, PL: *Pterospermum lanceaefolium* and CC: *Cryptocarya concinna*) and one introduced (AL: *Albizia lebbeck*) tree species under different released progress of allelopathy (soil allelopathy, allelochemical volatilization, and foliage litter decomposition) after seed-sowing in *Eucalyptus urophylla* and *Pinus elliottii* plantations for four months. Error bars represent standard errors, and an "*" denotes significant differences between *E. urophylla* and *P. elliottii* plantations, based on independent-samples *t*-tests at the 0.05 level.

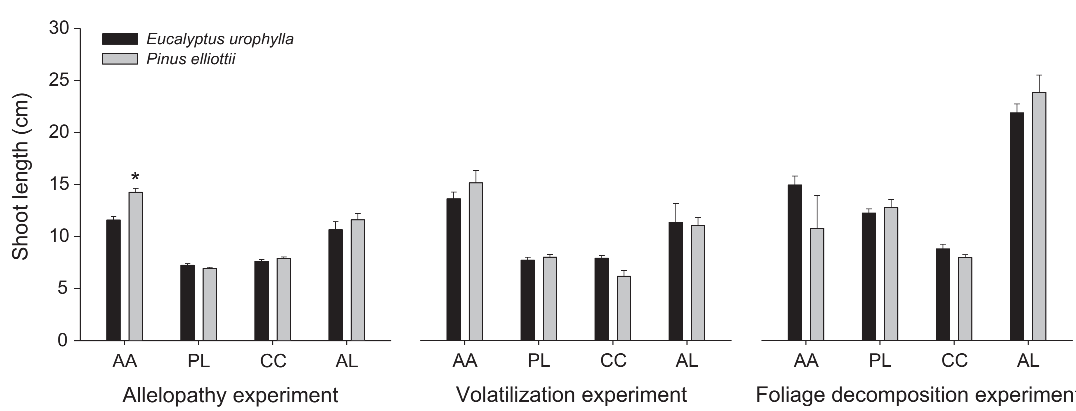

Fig. 3. Seedling shoot length of three native (AA: *Acmena acuminatissima*, PL: *Pterospermum lanceaefolium* and CC: *Cryptocarya concinna*) and one introduced (AL: *Albizia lebbeck*) tree species under different released progress of allelopathy (soil allelopathy, allelochemical volatilization, and foliage litter decomposition) after seed-sowing in *Eucalyptus urophylla* and *Pinus elliottii* plantations for four months. Error bars represent standard errors, and an "*" denotes significant differences between *E. urophylla* and *P. elliottii* plantations, based on independent-samples *t*-tests at the 0.05 level.

chemicals inhibited seedling growth remains unclear. Related studies on allelopathy have proposed various mechanisms for the allelopathic action, including the suppression of gibberellins and indole acetic acid synthesis (Zhang and Fu, 2009), interference with respiration by allelochemicals (Penuelas et al., 1996), poor photosynthesis through a reduction in chlorophyll content (Romagni et al., 2000), and inhibition of certain metabolic processes due to the production of phenolic acids (Moreland and Novitsky, 1987).

In the allelopathy experiment, seedling survival and growth of the three native species was suppressed to different extents under

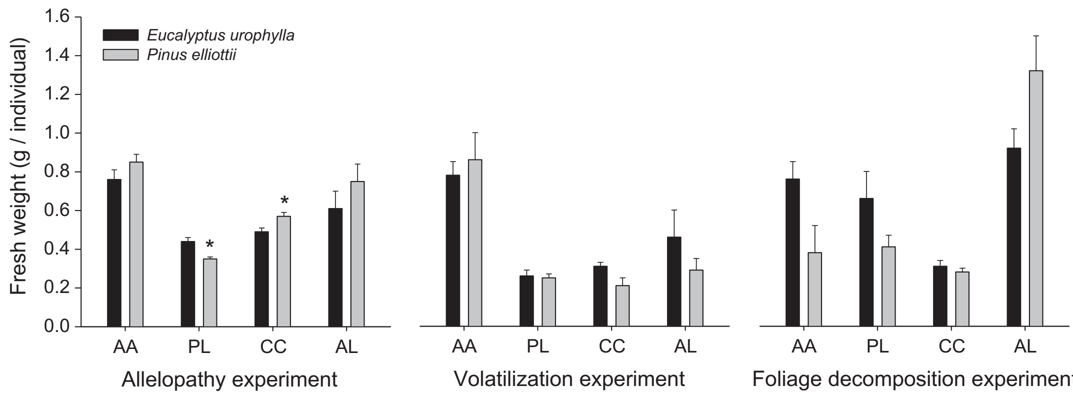

Fig. 4. Seedling fresh weight of three native (AA: *Acmena acuminatissima*, PL: *Pterospermum lanceaefolium* and CC: *Cryptocarya concinna*) and one introduced (AL: *Albizia lebbeck*) tree species under different released progress of allelopathy (soil allelopathy, allelochemical volatilization, and foliage litter decomposition) after seed-sowing in *Eucalyptus urophylla* and *Pinus elliottii* plantations for four months. Error bars represent standard errors, and an "*" denotes significant differences between *E. urophylla* and *P. elliottii* plantations, based on independent-samples *t*-tests at the 0.05 level.

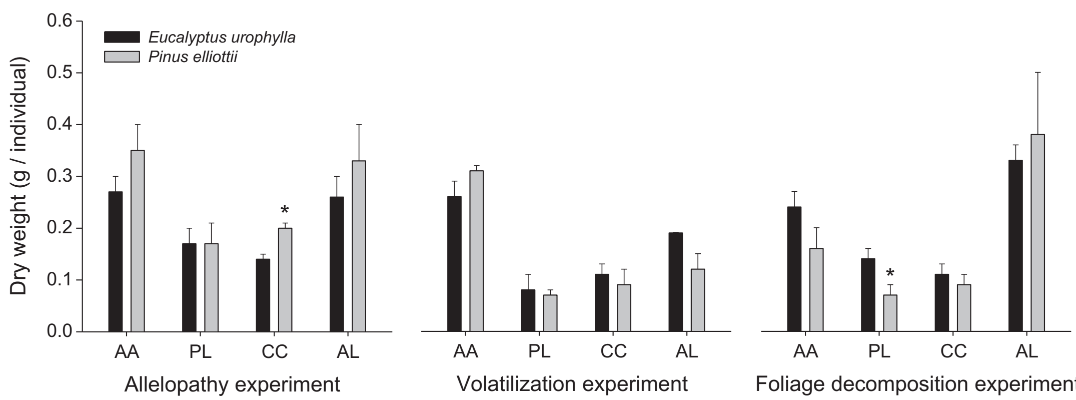

Fig. 5. Seedling dry weight of three native (AA: *Acmena acuminatissima*, PL: *Pterospermum lanceaefolium* and CC: *Cryptocarya concinna*) and one introduced (AL: *Albizia lebbeck*) tree species under different released progress of allelopathy (soil allelopathy, allelochemical volatilization, and foliage litter decomposition) after seed-sowing in *Eucalyptus urophylla* and *Pinus elliottii* plantations for four months. Error bars represent standard errors, and an "*" denotes significant differences between *E. urophylla* and *P. elliottii* plantations, based on independent-samples *t*-tests at the 0.05 level.

natural field conditions. However, seedling survival and growth of the introduced N-fixing species, *A. lebbeck,* was not inhibited in any of the treatments. Compared with *Eucalyptus* monocultures, mixed-species plantations of *Eucalyptus* with an N-fixing species have been reported to result in increased productivity, whilst maintaining soil fertility (Forrester et al., 2006). Therefore, as has been shown for other N-fixing tree species, *A. lebbeck* is a good choice for the establishment of mixed plantations with *Eucalyptus* in south China. This is in agreement with the findings of Forrester et al. (2006) who, in his review of the literature on N-fixing trees mixed with *Eucalyptus*, found that, in around half of the cases, the growth of *Eucalyptus* was better when it was combined with an N-fixing species, and in no case was it worse. However, further studies exploring the use of other, indigenous N-fixing tree species will be of value, as only we cannot conclude whether the results shown here are as a result of *A. lebbeck* being an N-fixing species or an introduced species.

It appears that the allelopathic affects of *Eucalyptus* influence the surrounding understory vegetation in various ways. Certain allelochemicals, such as those found in litter decomposition and volatilization experiments, stimulated seedling growth, whereas the total affects of allelopathy resulted in diminished seedling survival and growth. This is in contrast to the exotic, N-fixing tree which went unhindered by the allelopathic action of *Eucalyptus*. This response is not necessarily simply a reflection of indigenous versus introduced species, but is more likely the result of N-fixing and non N-fixing trees responding to the allelopathy differently. Thus the planting of N-fixing trees, such as *A. lebbeck* can be recommended for improving understory diversity and soil fertility in *Eucalyptus* plantations.

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