

MIXED ALLELOPATHIC EFFECT OF *EUCALYPTUS* LEAF LITTER AND UNDERSTOREY FERN IN SOUTH CHINA

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YANG L, CHEN Y, HUANG Y, WANG J & WEN M. 2016. Mixed allelopathic effect of *Eucalyptus* leaf litter and understorey fern in South China. Exotic *Eucalyptus*, introduced to China, has been widely planted because of its fast growth and versatile uses. In *Eucalyptus* plantation ecosystems in South China, understorey vegetation is often composed of the dense heliophyte fern, *Dicranopteris dichotoma*. Both *Eucalyptus* and *D. dichotoma* have been reported to have strong allelopathic effects. However, traditionally it is believed that native tree seedlings grow poorly in *Eucalyptus* plantations with understorey fern *D. dichotoma* and cannot be used for rehabilitation. Thus, bioassay and greenhouse experiments were conducted to test the allelopathic effects of *Eucalyptus* and *D. dichotoma* and the growth performance of tree seedlings in South China. Both species, individually and in combination, had strong allelopathic potential. The allelopathic effects varied with concentration and donor species used. The results suggested that *D. dichotoma* had antagonistic effect at higher concentration, while *Eucalyptus* individually and two species together had synergistic effect at lower concentration. These results contradicted traditional views and suggested that the negative effects of *Eucalyptus* and *D. dichotoma* mainly came from their allelopathic properties. Thus, *Eucalyptus* plantations could be reconstructed by transplanting seedlings of native, evergreen, broad-leaved species, after clearing the understorey ferns, to avoid competition and to create multi-species mixed forests which could improve ecosystem services.

Keywords: Rehabilitation, growth performance, bioassay, greenhouse, plantation

INTRODUCTION

Allelopathy is defined as inhibitory and stimulatory effects among plants (Rice 1984). Species with allelopathic potential influence the seed germination and seedling growth of other plants through allelochemicals, which can act by foliage leaching, root washing, rhizosphere transformation or volatilization (Horsley 1993). Allelopathy may play an important role in plant–plant interactions. Many studies have considered the allelopathic effects of a single species in the laboratory or at field sites. However, multi-species allelopathy occurs ubiquitously and simultaneously in natural environments and few studies have investigated the mixed effect of two or more species with allelopathic potential on plant survival or growth.

Exotic *Eucalyptus* (Myrtaceae), native to Australia, was introduced to China and has been

widely planted because of its fast growth and versatile uses, such as extracting essential oil from the leaves and making paper and artificial boards from the lumber. *Eucalyptus* has become one of the most important economic tree species in China. *Eucalyptus* is planted in 16 provinces of China with a total area of 200,000 ha, second to Brazil, the largest planter of *Eucalyptus* (Zhao 2007). However, the allelopathic effects of *Eucalyptus* via various allelopathic chemicals have been widely reported (del Moral & Muller 1970, May & Ash 1990, Ahmed et al. 2005, Zhang & Fu 2009), resulting in simple structure and lower biodiversity (Ren et al. 2002). In *Eucalyptus* plantation ecosystems in South China, the understorey vegetation is often composed of dense *Dicranopteris dichotoma*, a heliophyte fern that is an indicator species of acidic soil in

subtropical humid climate conditions. It often forms single-species communities and appears on barren slopes after deforestation through clonal growth and a strong allelopathic effect. It has been reported that phenolic acid may be the most important allelopathic chemical (Ye & Hong 1987, Flora of China Editorial Committee 1999, Lin 2004, Yuan & Li 2007, Zhou 2007).

Both of these species, *Eucalyptus* and *D. dichotoma*, have strong allelopathic effects. However, the mixed allelopathic effect of the two species is unknown. Thus, a bioassay experiment was conducted to determine the allelopathic potential of the species individually and when combined. Then, potted allelopathic experiments in a greenhouse were carried out to investigate the mixed allelopathic effect of *Eucalyptus* and *D. dichotoma*, and to find out which species could adapt to the allelopathic environment for the purpose of creating a multi-species mixed forest to improve the ecosystem services of *Eucalyptus* plantations.

MATERIALS AND METHODS

Field site description

The study was conducted at the Heshan National Field Research Station of Forest Ecosystems (60.7 m asl, 112° 50' E, 22° 34' N) located in Heshan City, Guangdong, China. Growing in the red laterite soil, the climax plant community is a low subtropical monsoon evergreen, broad-leaved forest, including families *Lauraceae*, *Euphorbiaceae* and *Fagaceae*. As a result of serious and long-term human disturbance, the

soil has been severely eroded and the original vegetation almost disappeared. The station was established in 1983 and the post-fire degraded land was planted with many native and exotic species to test their restoration effects. It is one of the core stations of the Chinese Ecological Research Network (CERN) and occupies 40 ha, of which 20 ha are *Eucalyptus* plantations (5 years, 2.5 m space between plants and 10.11 ± 1.54 cm diameter at breast height (dbh) with dense *D. dichotoma* (Figure 1). The mean annual temperature is 22.6 °C and the mean annual precipitation is 1700 mm. The annual solar radiation is 4350.5 MJ m⁻². There are distinctive wet hot (April–September) and dry cold (October–March) seasons, and the wet season is the main period of plant growth. The soil organic carbon and soil total nitrogen were measured at 14.5 and 0.52 g kg⁻¹, respectively (Sun et al. 2009). The light penetration is $32.3 \pm 4.9\%$.

In vitro bioassay experiment

Fresh leaf litters of *Eucalyptus urophylla* were collected from litter traps at field sites, and leaf litters of *D. dichotoma* of lower height with adhesive property on branches, were collected by hand, in January 2011. All the leaf litters were rinsed with distilled water and taken to the laboratory where they were ground to a fine powder after air drying. The leaf powder, 0.2, 0.5 and 2 g of each species was soaked in 100 ml distilled water for 24 hour and filtrated. The concentrations were 0.002, 0.005 and 0.02 g dry weight (DW) mL⁻¹ (dry weight litter powder (g) in 1 ml distilled water).



Figure 1 *Eucalyptus urophylla* plantation with dense fern, *Dicranopteris dichotoma*, in South China

The concentration, 0.02 g DW mL⁻¹, simulated the natural situation based on calculations from annual leaf litter produced and soil water content in a 1 m² area (Zhang & Fu 2010). Also, the aqueous extracts of the two species were combined at volume ratio 1:1. The aqueous extracts of each species and the combined extracts were kept in a 4 °C refrigerator.

Seeds of *Lactuca sativa*, from Guangdong Academy of Agricultural Sciences, were used to test germination responses to the aqueous extracts of the two species (Zhou 2007). The seeds were sterilised with 0.5% HgCl₂ for 5 min and placed in Petri dishes (9 cm diameter) with two layers of filter paper (9 cm diameter). Thirty seeds were placed in each Petri dish with 5 ml aqueous extract. The Petri dishes (five replications for each extract) were incubated in climate chambers under general conditions (26 °C and 238 µmol m⁻² s⁻¹ light for 14 hour in the daytime, 24 °C with no light for 10 hour at night). The bioassay experiment was designed as three aqueous extract treatments (*D. dichotoma*, *E. urophylla* and *D. dichotoma* + *E. urophylla*) at three concentrations, 0.002, 0.005 and 0.02 g DW mL⁻¹ × 5 petri dish replicates × 30 *L. sativa* seeds. Distilled water treatment was used as control. The germination rate was measured every day for the first two weeks. The lengths of the hypocotyls and radicals were measured on the 14th day.

Potted allelopathic experiment in the greenhouse

Schima superba (Theaceae), *Elaeocarpus apiculatus* (Elaeocarpaceae) and *Cinnamomum burmanni* (Lauraceae) were used for the potted experiment. These were climax evergreen, broad-leaved species that grew relatively fast and considered excellent for reforestation in South China. The species ranged widely in the subtropics of China and frequently planted in artificial forests for the rehabilitation of degraded land. For the experiment, 1-year-old seedlings of the three species were purchased from Heshan Institute of Forestry Science and transplanted into plastic pots (20 cm diameter and 15 cm height) containing local red soil from the field site, in April 2011. The soil was amended with organic fertiliser. After two weeks of steady survival and growth, the experiment was carried out, designed

as 3 extract treatments × 3 concentrations × 5 seedling replicates. The three treatments and three concentrations were the same as those in the bioassay experiment. Distilled water treatment was used as control. The seedlings were watered with 20 mL aqueous extracts every 2 days and no other water was added. Weeds of other species were removed from the pot. The survival and growth (basal diameter and shoot height) were measured at the beginning (April 2011) and end (September 2011) of the experiment, which lasted about six months. After harvesting all of the seedlings, every individual was cut into leaf, stem and root sections and dried for 96 h to a constant weight. The total biomass and organ biomass was measured using an electronic balance.

Data analysis

All results were presented as means ± standard error (SE). One-way ANOVA was used to compare the effects of the treatments on germination rate, shoot length and root length of *L. sativa* at the end of the *in vitro* bioassay experiment. Shoot height and basal diameter were also compared with one-way ANOVA at the end of the potted experiment. Least significant difference tests were used for *post hoc* multiple comparison when an effect was determined to be significant at $\alpha = 0.05$. Three-way ANOVA was used to compare the effects of species, treatment, concentration and their interactions on shoot height, basal diameter, total biomass, leaf biomass, shoot biomass and root biomass at the end of the potted experiment. SYSTAT 11.0 was used for statistical analysis.

RESULTS

In vitro bioassay experiment

The germination rate of *L. sativa* under the four treatments increased with time at concentrations 0.002 and 0.005 g DW mL⁻¹, but *L. sativa* did not germinate at a concentration of 0.02 g DW mL⁻¹ under the treatments D, E and D+E (Figure 2c). At the end of the bioassay experiment, the rankings of the germination rates among the four treatments were W > D > D+E ≥ E at 0.002 g DW mL⁻¹, and W > D ≥ D+E ≥ E at 0.005 g DW mL⁻¹.

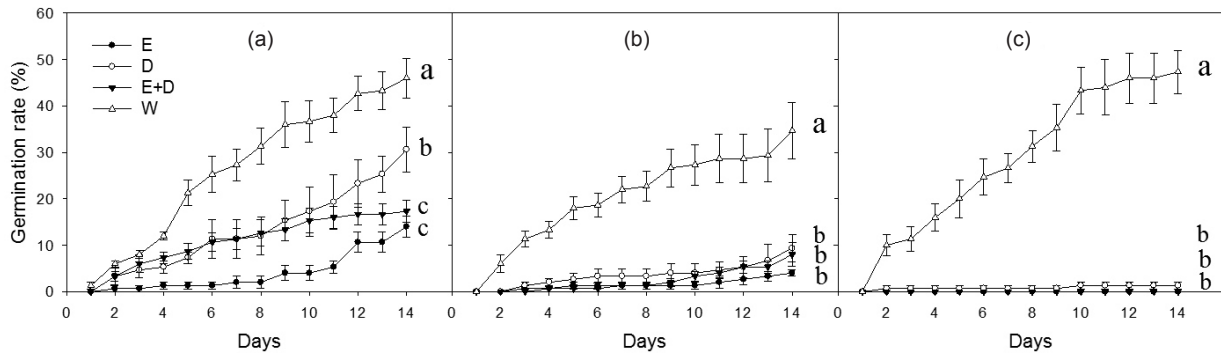


Figure 2 Germination rate of *L. sativa* at different concentrations, (a) 0.002 g DW mL⁻¹, (b) 0.005 g DW mL⁻¹, (c) 0.02 g DW mL⁻¹, of four aqueous extract treatments; D = *D. dichotoma*, E = *E. urophylla*, D+E = *D. dichotoma* + *E. urophylla*, W = distilled water as control, in the first two weeks during *in vitro* bioassay experiment, the same letter showed no significant difference at $p > 0.05$

Treatment with EU significantly reduced the shoot length, while treatment with EU and DD reduced the shoot length at concentration 0.005 g DW mL⁻¹ (Figure 3a). Treatment with EU+DD significantly increased root length at concentration 0.002 g DW mL⁻¹ and treatment with EU significantly decreased root length at concentration 0.005 g DW mL⁻¹ (Figure 3b).

Potted experiment in the greenhouse

The survival rate was 100% for all species in the greenhouse potted experiment. According to three-way ANOVA analysis, the effect of species on the growth indices, including shoot height

and basal diameter, was significant, and the effect of concentration on basal diameter was obvious (Table 1). For all three species, the treatment and concentration had no obvious effect on shoot height (Figures 4a–c). Also, the treatment and concentration had no obvious effect on basal diameter for *S. superba* and *C. burmannii* (Figures 4d and f). For *E. apiculatus*, the EU and DD treatments increased basal diameter at concentration 0.002 g DW mL⁻¹ (Figure 4e).

According to three-way ANOVA analysis, the effects of species and the interaction between treatment and concentration on four biomass indices, including total biomass, leaf biomass, shoot biomass and root biomass, were

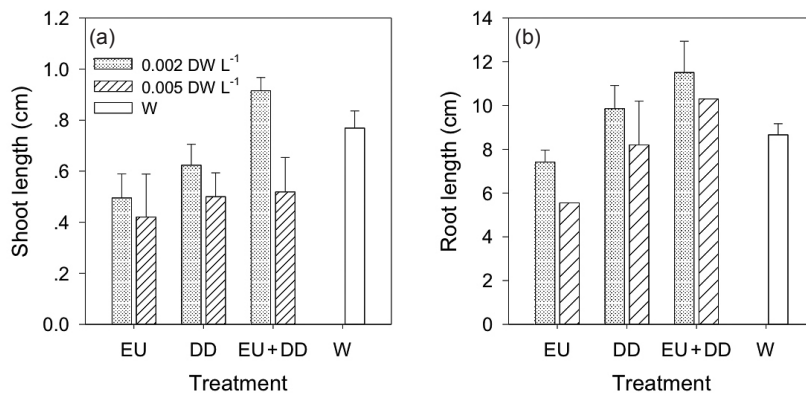


Figure 3 Shoot length and root length of *L. sativa* under different concentrations, 0.002, 0.005 and 0.02 g DW mL⁻¹, of four aqueous extract treatments; D = *D. dichotoma*, E = *E. urophylla*, D+E = *D. dichotoma* + *E. urophylla*, W = distilled water as control, at the 14th day in the *in vitro* bioassay experiment; no values were presented for the three aqueous extract treatments (D, E and D+E) at 0.02 g DW mL⁻¹ as no seedlings germinated; the same letter showed no significant difference at $p > 0.05$

Table 1 Summary of three-way ANOVA analysis of shoot height and basal diameter of the target species at the end of the potted experiment

Source	Shoot height		Basal diameter	
	F	p	F	p
SP	43.119	0.000	9.666	0.000
TR	0.122	0.885	1.356	0.261
CO	0.738	0.480	4.499	0.013
SP × TR	0.333	0.855	0.206	0.935
SP × CO	0.326	0.860	0.622	0.648
TR × CO	1.460	0.217	0.898	0.467
SP × TR × CO	0.819	0.587	0.509	0.848

SP = species, TR = treatment, CO = concentration, F = freedom, P = probability; the differences were not significant at $p > 0.05$

Table 2 Summary of three-way ANOVA analysis of total biomass, leaf biomass, shoot biomass and root biomass at the end of the potted experiment

Source	Total biomass		Leaf biomass		Shoot biomass		Root biomass	
	F	p	F	p	F	p	F	p
SP	90.849	0.000	111.756	0.000	73.894	0.000	32.054	0.000
TR	0.575	0.564	1.805	0.168	0.403	0.669	0.651	0.523
CO	4.864	0.009	4.990	0.008	2.337	0.100	4.308	0.015
SP × TR	1.045	0.386	2.988	0.021	0.054	0.995	1.217	0.306
SP × CO	2.459	0.048	2.429	0.050	1.254	0.291	2.365	0.055
TR × CO	5.506	0.000	2.985	0.021	3.875	0.005	6.129	0.000
SP × TR × CO	0.808	0.596	0.805	0.599	0.495	0.858	0.735	0.661

SP = species, TR = treatment, CO = concentration, F = freedom, P = probability; the differences were not significant at $p > 0.05$

significant. In addition, concentration had an obvious effect on total biomass, leaf biomass and root biomass. The interaction between species and treatment had an obvious effect on leaf biomass. The interaction between species and concentration had a clear effect on total biomass and leaf biomass. For *S. superba*, the EU + DD treatment increased total biomass and stem biomass at $0.005 \text{ g DW mL}^{-1}$ (Figures 5a and g), while the DD treatment decreased root biomass at $0.02 \text{ g DW mL}^{-1}$ (Figure 5j). For *E. apiculatus*, the DD treatment increased total biomass, leaf biomass and root biomass at $0.002 \text{ g DW mL}^{-1}$ (Figures 5b, e and k), and the EU treatment increased leaf biomass at $0.002 \text{ g DW mL}^{-1}$ (Figure 5e). For *C. burmanni*, the DD treatment decreased total biomass and root biomass at $0.02 \text{ g DW mL}^{-1}$ (Figure 5c and l), while the EU + DD treatment increased leaf biomass and stem biomass at $0.005 \text{ g DW mL}^{-1}$ (Figures 5f

and l), and the EU treatment increased stem biomass at $0.002 \text{ g DW mL}^{-1}$ (Figure 5i).

DISCUSSION

The allelopathic effect of *Eucalyptus*

The dispute about whether *Eucalyptus* plantations improved or destroyed the ecological environment has aroused great interest among ecologists in recent years (Peng et al. 2003, Zhang & Fu 2010, Zhang et al. 2010). Many studies have reported that *Eucalyptus* reduced the biodiversity of understory vegetation and restricted the seedling growth of other tree species by depleting the soil water, absorbing soil nutrients and releasing allelochemicals (Zeng & Li 1997, Fang et al. 2009, Zhang & Fu 2009, 2010, Zhang et al. 2010). Compared with distilled water treatment, *Eucalyptus* treatments reduced

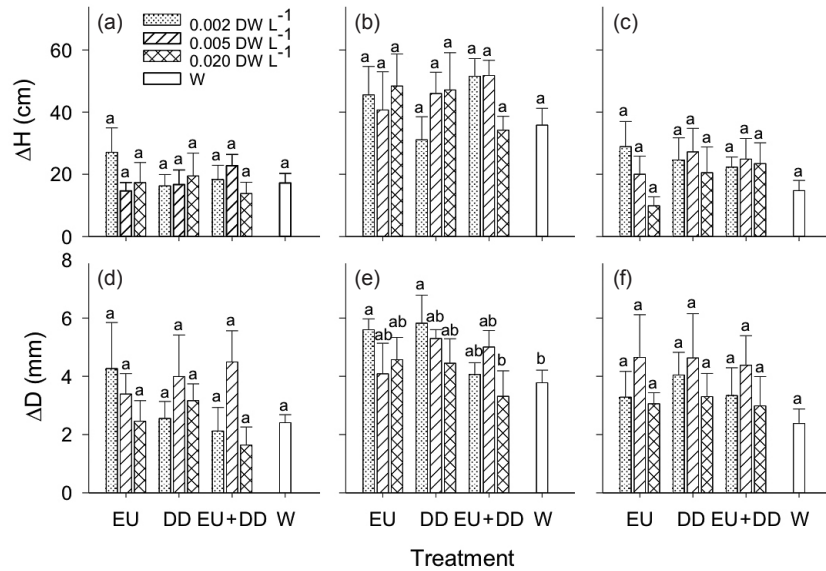


Figure 4 Increase of shoot height (a–c) and basal diameter (d–f) during potted experiment on three species, *S. superba* (a and d), *E. apiculatus* (b and e) and *C. burmanni* (c and f), at different concentrations, 0.002, 0.005 and 0.02 g DW L⁻¹ of four aqueous extract treatments; D = *D. dichotoma*, E = *E. urophylla*, D+E = *D. dichotoma* + *E. urophylla*, W = distilled water as control; the same letter showed no significant difference at $p > 0.05$

seed germination and seedling growth of lettuce, as the concentration increased, showing strong allelopathic potential in the *in vitro* bioassay experiment. The negative effects of *Eucalyptus* were always associated with its allelopathic effects, which have been broadly reported for various allelopathic chemicals that obviously impact biodiversity and productivity (del Moral & Muller 1970, May & Ash 1990, Ahmed et al. 2005, Zhang & Fu 2009). However, the species impacted by *Eucalyptus* were crops or herbaceous plant, and less of tree species. Aqueous extracts from *Eucalyptus* obviously restricted the growth of some crops and tree species (Zeng & Li 1997, Fang et al. 2009, Zhang & Fu 2009, Zhang & Fu 2010, Zhang et al. 2010). Many chemical components, including alkanes, alcohols, aldehydes, ketones, phenols, long-chain fatty acids and aromatic esters were found in the soil of *Eucalyptus* plantations in southwest China (Zhang et al. 2010). However, our potted experiment suggested that different *Eucalyptus* treatments had no obvious effects on survival and growth, regardless of species. The *Eucalyptus* treatment even increased the leaf biomass of *E. apiculatus* and stem biomass of *C. burmanni* at 0.002 g DW mL⁻¹ in the potted experiment. Fang et al. (2010) also found that *E. urophylla* had no obvious effect on the seed

germination and seedling growth of *S. superba*. The allelochemical effects of the donor varied with the dose applied, and species responded differently to the allelochemicals released by *Eucalyptus* (Fang et al. 2009, 2010).

The negative effects of *Eucalyptus* may not be solely from its allelopathic effect, but also from forest management (de Faria et al. 2006). *Eucalyptus* grows fast in the first three or four years and then more slowly. Thus, more water and nutrients are needed to support the greater biomass accumulation in earlier stages. However, *Eucalyptus* trees are always harvested at the same stage to increase profit. Besides the shorter harvest time, other negative effects of *Eucalyptus* come from large-scale cultivation, low fertilisation, monoculture and intensive planting. Every 1 kg of *Eucalyptus* biomass required 210 l water in South China, which is less than pines (1000 L) or Acacia trees (800 L), indicating water use efficiency of *Eucalyptus*. In some studies, the understory vegetation has been shown to grow vigorously in *Eucalyptus* plantations, and *Eucalyptus* can even be used as a nurse plant to improve seedling growth and plant biodiversity by buffering extreme temperatures and improving water use efficiency (Feyera et al. 2002, Lüttge et al. 2003). Other researchers have

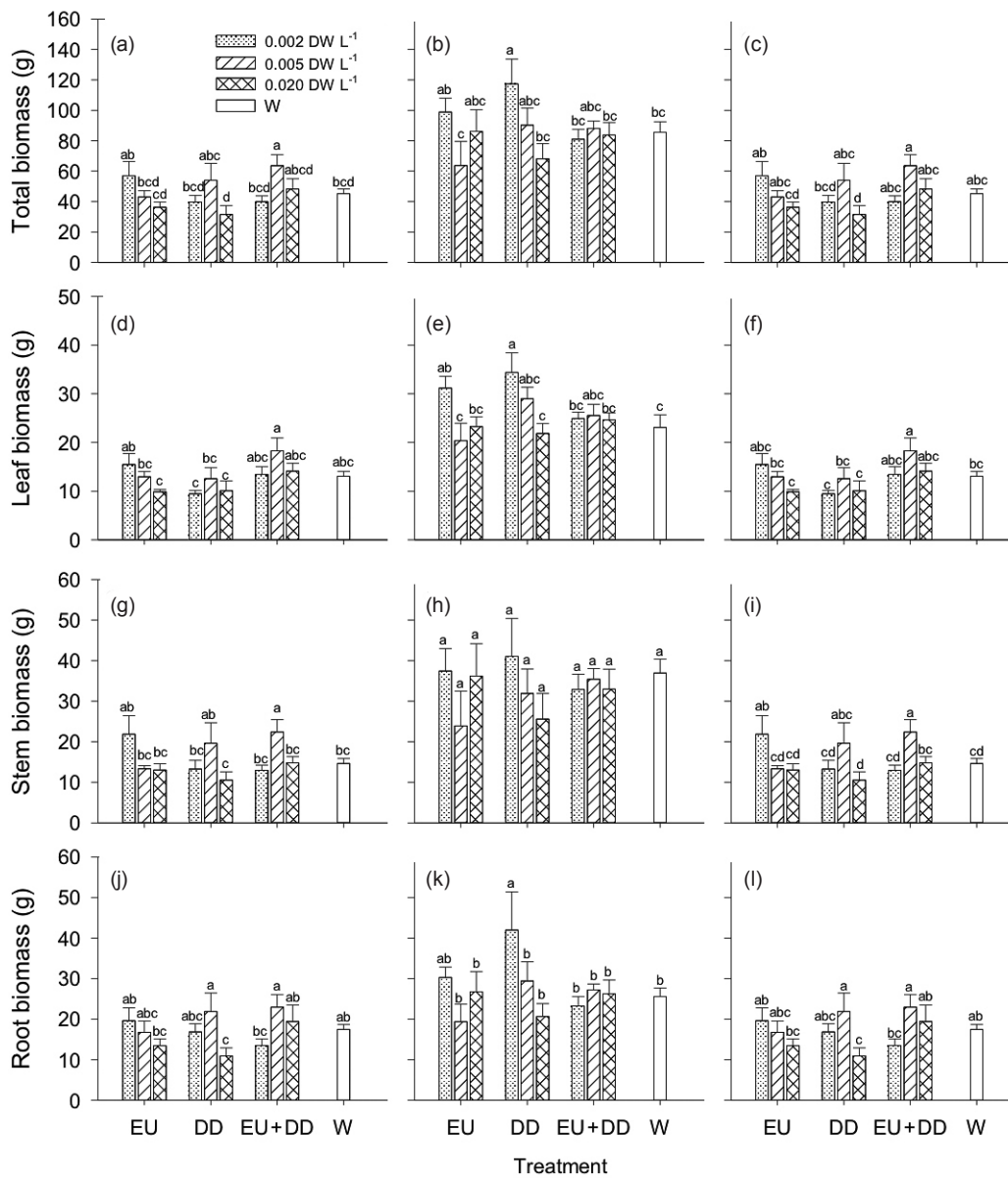


Figure 5 Total biomass (a–c), leaf biomass (d–f), stem biomass (g–i) and root biomass (j–l) during the potted experiment on three species, *S. superba* (a, d, g, and j), *E. apiculatus* (b, e, h, and k) and *C. burmanni* (c, f, i, and l), at different concentrations 0.002, 0.005 and 0.02 g DW mL⁻¹ of four aqueous extract treatments; D = *D. dichotoma*, E = *E. wrophylla*, D+E = *D. dichotoma* + *E. wrophylla*, W = distilled water as control; the same letter showed no significant difference at $p > 0.05$

found that *Eucalyptus* ecosystems in South China have lower soil erosion and higher understory vegetation biodiversity in the long term without artificial disturbance (Yu & Peng 1996). Native tree seedlings could grow with *Eucalyptus* in South China (Ren and Peng 2001) and *Eucalyptus* has been proven to nurse seedlings of native species *in situ*.

The allelopathic effect of *D. dichotoma*

Many ferns are considered to be allelopathic (Zhang et al. 2004). For example, the fern *Osmunda claytoniana*, growing on mesic sites of northern and eastern river valley slopes of the upper Mississippi River valley, significantly reduced seedling survival in red oak, *Quercus*

rubra (Hanson & Dixon 1987). It was also reported that the fern *Blechnum orientale* has a strong allelopathic effect which can be applied to control weeds in crop fields (Hong et al. 2003). *D. dichotoma* is the most common fern in South China, propagated by spores or clones and always formed a dense layer (Lin 2004). The average height of the fern is 45–90 cm. It has clone rhizome (2 mm thick) and chartaceous leaves with dark rusty hairs. The above ground biomass of *D. dichotoma* ranged from 1360 to 3411 kg ha⁻¹ and could reach 100% coverage in the understory (Liu et al. 2008). *D. dichotoma* has strong allelopathic effect. Ye et al. (1987) found that *D. dichotoma* had a strong allelopathic effect on most grazing plants and weeds. Also, Zhou (2007) found that the seed germination and seedling growth of *Ipomoea aquatica*, *Raphanus sativus*, *Cucumis sativus* and *Brassica pekinensis* showed a specific response to allelochemicals of *D. dichotoma*. The bioassay experiment also showed strong allelopathic potential of *D. dichotoma*. *D. dichotoma* reduced the root biomass of *S. superba* and the total biomass of *C. burmanni*, but increased the basal diameter and biomass of *E. apiculatus* in the potted experiment. The results showed that *D. dichotoma* had different allelopathic effects on different species, much like the species-specific allelopathic effects of *Eucalyptus*. Obviously *D. dichotoma* inhibited most plants except for *Pinus massoniana*, which was the dominant tree species in an earlier successional stage in South China (Ye et al. 1987).

The mixed allelopathic effect of *Eucalyptus* and *D. dichotoma*

Understory vegetation is often composed of the dense fern *D. dichotoma* in *Eucalyptus* plantation ecosystems in South China. The understory vegetation acted as a transitional layer that linked aboveground and belowground processes and restricted growth of tree seedlings as an ecological filter (George & Bazzaz 1999). For example, understory vegetation dominated by black crowberry reduced the microbial activity, litter decay rate and soil nitrogen content with strong allelopathic effects (Nilsson et al. 2000, Nilsson & Wardle 2005). Wang et al. (2009) found that understory vegetation reduced seedling survival by competing for soil water content and nutrients in a seeding and seedling

transplantation experiment in South China.

Previous study showed that the fronds of *D. dichotoma* differ from the frond-funnels of other ferns, and it intercepts litter-fall from tree species through interlaced fronds and stalks (Yang et al. 2014). Litter from the two species, *E. urophylla* and *D. dichotoma*, is often mixed together as both species have allelopathic effects. The results suggested that the mixed effect reduced seed germination in lettuce, but increased shoot length and root length in the bioassay experiment. The combined extracts increased biomass at 0.005 g DW mL⁻¹ in the potted experiment for *S. superba* and *C. burmanni*, indicating an antagonistic mixed action for growth characteristics. The results indicated that the competitive effect on seedling growth may not be from allelopathic effects in *Eucalyptus* plantations with the understory fern *D. dichotoma*. The mixed allelopathic effect of *E. urophylla* and *D. dichotoma* promoted the growth of tree seedlings by offsetting negative allelochemicals.

Implications for restoration

The current research proved that *Eucalyptus* and understory fern *D. dichotoma* had stronger allelopathic potential in bioassay experiment. However, the negative effect of allelopathy of *Eucalyptus* and *D. dichotoma* were not obvious in the potted experiment, even positive effects appeared to *E. apiculatus* at lower concentration. This contradicted the traditional view that native tree seedlings grow poorly in *Eucalyptus* plantations with understory fern *D. dichotoma* and cannot be used in their rehabilitation. Some researchers have suggested that the negative effects of *Eucalyptus* mainly come from problems with forest management, such as interspecific competition, over shading and unsuitable experiment design, and not just from its allelopathic effects (Xu & Zhang 2006, Ren et al. 2007). The allelopathic effects of *Eucalyptus* or *D. dichotoma* may not be the most important limiting factor. The focus on *Eucalyptus* problems should be how to manage *Eucalyptus* plantations to integrate economic, ecological and societal factors. Some traditional methods, including thinning to avoid intensive planting and applying fertiliser to prevent soil depletion, should be carried out. Additionally, monoculture has

always been used in *Eucalyptus* plantations. Adult *Eucalyptus* plantations should be reconstructed by clearing the understory vegetation to avoid competition and transplanting seedlings of native evergreen, broad-leaved species to create a multi-species mixed forest to improve ecosystem services.

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