Plant Soil (2008) 305:145–156
DOI 10.1007/s11104-007-9533-9

REGULAR ARTICLE

Effect of soil biota on growth and allocation by *Eucalyptus microcarpa*

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Received: 10 October 2007 / Accepted: 19 December 2007 / Published online: 15 February 2008

Abstract We examined growth of *Eucalyptus microcarpa* seedlings in soil collected from four sites in southeastern Australia, in which retired pasture land has been revegetated with mixed plantings of *Eucalyptus* and *Acacia* species. Revegetation of farm land in southeastern Australia is an area of major investment. The focus of the study was to examine the influence of soil biota on seedling growth and its possible interaction with soil enrichment from a legume (*Acacia*) and decomposition rates. We used a soil freezing treatment (−80°C for 3 days) to retard the soil biota, with the expectation that invertebrates in particular would be killed. Soil freezing did not cause a nutrient pulse, but did reduce the level of ammonium in soil. Nitrate levels increased with time in pots, regardless of the soil treatment. Decomposition rates measured using cellulose substrate were significantly reduced by the freeze treatment, but only for approximately 90 days. *Eucalyptus microcarpa* seedlings grown in freeze-treated soil were approximately 40% smaller (total biomass), had marginally lower LAR (leaf area ratio), and significantly lower LMA (leaf mass per area). Low LMA indicates that leaves are either thinner in cross-section or less dense. We hypothesise that both the poor growth of seedlings and production of less robust leaves are consequences of reduced availability of soil nutrients due to the diminished soil biota after freeze treatment. Litter under *Acacia* was richer in nitrogen than litter under *Eucalyptus* but there was no difference in nitrogen content of soil, and consequently no soil source effects on plant growth or decomposition. We suggest that variation in the soil biota has the potential to greatly enhance or hinder the success of revegetation on retired agricultural land, but enrichment of soil by decomposition of nitrogen rich litter in these sites requires longer than the 8–15 years since they were revegetated.

Keywords Allocation · Growth · Leaf · Legume · Nitrogen · Revegetation

Responsible Editor: Barbara Wick.

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Introduction

In the last decade or so, it has become widely recognised that soil biota play an important role in plant growth (Bonkowski et al. 2000; Poveda et al. 2005) and community composition (Bever et al. 1997; Bradford et al. 2002; De Deyn et al. 2004). There is now a greater appreciation of the inter-dependence of

aboveground and belowground subsystems and the interactions between soil biota and plants (Bardgett 2004; Wardle et al. 2004). The primary mechanisms underpinning the effects of soil biota on plant growth are the mineralisation of plant residues by decomposer organisms and the release of nutrients into the soil in plant-available form, and the capacity of the soil biota to directly assist plants in nutrient uptake. These processes are influenced by soil microbial biomass and composition, which is, in turn, dependent on the amount and quality of inputs of plant litter (Wardle 2002; Rantalainen et al. 2004).

The invertebrate component of the decomposer biota affects plant growth and resource allocation via indirect processes including: (1) alteration of microbial abundance and community composition (Klironomos et al. 1992, but see McLean et al. 1996); (2) enhancement of nutrient mineralisation and transfer (Haimi et al. 1992; Schmidt and Curry 1999; Baker et al. 2003); (3) influencing competition between plants (Kreuzer et al. 2004; Endlweber and Scheu 2006); (4) redistribution of organic matter and nutrients (Cortez and Bouché 1998) and (5) enhancing leaf herbivory (Scheu et al. 1999; Poveda et al. 2005). Furthermore, root herbivore invertebrates can influence plant growth and resource allocation both directly (Wardle 2002) and via indirect mechanisms such as the release of organic compounds via plant wounds and faecal pellets that subsequently become available for plants (Yeates et al. 1999).

The majority of research on the effects of soil biota on plant growth and community structure has been undertaken in Europe and North America, but Australian environments are often quite different. Australian soils tend to be old, strongly leached and nutrient-poor (McKenzie et al. 2004). The Australian flora is notable for the abundance of sclerophyllous species, which produce leaf litter that is tough (Read et al. 2005), and rich in plant secondary metabolites (Cooper 2001). The decomposer biota may be highly seasonally variable in diversity and abundance, because they are limited by soil moisture (Spain and Hutson 1983). Decomposition in the drier areas tends to be sporadic, contingent upon rainfall events (Wood 1974; Hutson and Veitch 1985) and fire (Radho-Toly et al. 2001). There are, therefore, strong reasons to suspect that northern hemisphere studies provide a poor basis for understanding aboveground–belowground interactions in Australia.

The genera *Acacia* and *Eucalyptus* dominate the Australian flora, often co-occuring. They each have mechanisms to cope with drought, fire and low levels of soil nutrients, especially nitrogen and phosphorus (Adams and Attiwill 1984; Gill 1994). The *Acacia* are legumes, often pioneer species, with symbiotic, rhizobial N-fixing capacity (Stock et al. 1995; Thrall et al. 2000), while *Eucalyptus* have mutualisms with mycorrhiza whereby they acquire N and P (Launonen et al. 2004). *Eucalyptus* are noted for their sclerophyllous foliage (another adaptation to nutrient poor soils) but many *Acacia* species produce less sclerophyllous leaves (or phyllodes) with higher nitrogen content. In this way the two genera usually differ in the quality of leaf litter they drop. The nitrogen economy (Wright et al. 2004) is also expected to greatly influence growth responses to environmental variation, potentially affecting both the rate of leaf production (with positive feedback into future growth) and the structure of the leaves produced.

Broad-scale revegetation, including natural regeneration, environmental plantings and agroforestry, is a major activity in the restoration and rehabilitation of degraded Australian landscapes (Dorrough and Moxham 2005; Vesk and Mac Nally 2006), involving revegetation targets of several millions of hectares nationwide, at a cost roughly in the order of Aust $2,000–6,000 dollars per hectare (McCarthy and Lindenmayer 2007). *Eucalyptus* spp. are commonly the dominant element of revegetated sites and *Acacia* spp. are commonly co-planted in order to provide a source of nitrogen (Thrall et al. 2005). Many studies of mixed *Eucalyptus* and *Acacia* plantations have demonstrated superior growth compared to monocultures, but the outcomes are context dependent (Forrester et al. 2006). The interactions between plants and soil biota in relation to growth and resource allocation are therefore of considerable environmental and economic significance in relation to ecological restoration in Australia.

One approach to assess the overall role of soil organisms as drivers of plant response is to examine the effect of soil sterilisation. Various methods have been used in order to retard populations of the soil biota. Selective chemical biocides for invertebrates, fungi and bacteria have been used to examine the effects of these taxa on decomposition and mineralisation in the field (Beare et al. 1992; Hu et al. 1995).

Others have used gamma-irradiation (Troelstra et al. 2001). For this experiment we chose to use soil freezing because it posed the least risk of introducing direct effects on soil nutrient status (such as associated with chemical intervention) and because it is expected to have a differential effect on invertebrates. Freezing at the conditions we used is likely to kill invertebrates outright (Bardgett and Chan 1999) while spores of fungi and bacteria are likely to remain viable (Vishnivetskaya et al. 2000; Walker et al. 2006).

We used pots containing soil and soil biota from revegetation sites established on retired pasture land in order to measure decomposition rates and the growth responses of Grey Box, *Eucalyptus microcarpa*, a common tree species in south-eastern Australia. We compared effects of a soil freezing treatment and a nitrogen supply treatment in the form of soil taken from under the legume, *Acacia pycnantha*.

The purpose of the study was to ask:

(1) Do soil biota affect growth of *E. microcarpa* seedlings, as well as the allocation of resources to different parts of the plant?
(2) Does soil sourced from under a legume (*Acacia pycnantha*) provide more nitrogen for growth of *E. microcarpa* seedlings, and is availability of this nitrogen influenced by the soil freezing treatment?
(3) Does the soil source or soil freezing treatment influence decomposition rates in soil?

Materials and methods

Sites, soil sampling and construction of pots

Soil and litter was collected from sites located in north-central Victoria, Australia; near Charlton (36° 25′S, 143°27′ E), Bendigo (36°54′S, 144°00′E), Heathcote (37°02′S, 144°43′E) and St Arnaud (36° 51′S, 143°04′E) between 12 April and 14 April 2005. These were revegetation sites on retired grazing land, planted with a mixture dominated by *Eucalyptus* and *Acacia* species. All sites had both grey box, *Eucalyptus microcarpa* and golden wattle, *Acacia pycnantha*. Site characteristics were (in the following order: Charlton, Bendigo, Heathcote, St Arnaud), year planted: 1997, 1994, 1996, 1990; range of diameter (cm at breast height) of *E. microcarpa* individuals from under which soil was sampled: 6–10, 12–17, 8–15, 4–17; mean distance (m) between canopies: 2, 0, 4, 1.

At each site, soil and litter were collected beneath four each of *A. pycnantha* and *E. microcarpa* trees. We selected an area of 0.25 m^2 that represented the most complete litter cover within 2 m of the base of each tree, and then collected separate fractions of leaf litter, shallow soil (0–5 cm) and deep soil (5–15 cm). Each fraction was sealed in a plastic bag and transported to the laboratory in insulated coolers.

Soil and litter layers were reassembled in pots (14 cm diameter×15 cm deep; 2.2 l) within 5 days of collection. Deep soil was placed into pots to a depth of 10 cm, followed by 5 cm of shallow soil, followed by approximately 40 g of litter. The soil and litter sampled from under each tree was used to construct one pot for freeze-treatment, one control, and contributed half the material for the mixed pot (Fig. 1). For the mixed pots we combined soil, 1:1, from one *Eucalyptus* tree with soil from one *Acacia* tree. This latter treatment was included to help distinguish between two possible mechanisms associated with an *Acacia* soil benefit. If soil under *Acacia* has more plant-available nitrogen, one would predict that the plant response would be dosage dependent, with seedlings in mixed pots intermediate between those in the 'pure' soil treatments. Alternatively, *Acacia* soil might be beneficial because it is free of *Eucalyptus* specific organisms that harm growth (e.g. root feeding nematodes). In this case one would predict that the mixing of soils inoculates pots with harmful organisms, so that seedling performance in mixed pots would be more like that seen in pure *Eucalyptus* soil than in pure *Acacia* soil.

Experimental design

Soil treatment (freezing vs. control) and soil source (soil from under *E. microcarpa* or *A. pycnantha* or mixed) were applied orthogonally, creating 6 unique combinations. With four replicates of each, there were 24 pots in each block. Pots were arranged on benches in a greenhouse in a randomised block design (Fig. 1). Combining site with greenhouse block allowed for a strong test of the treatments of interest, but limited our capacity to distinguish between site effects (from the field) and block effects (from the greenhouse). Blocks were rotated clockwise within the greenhouse fort-

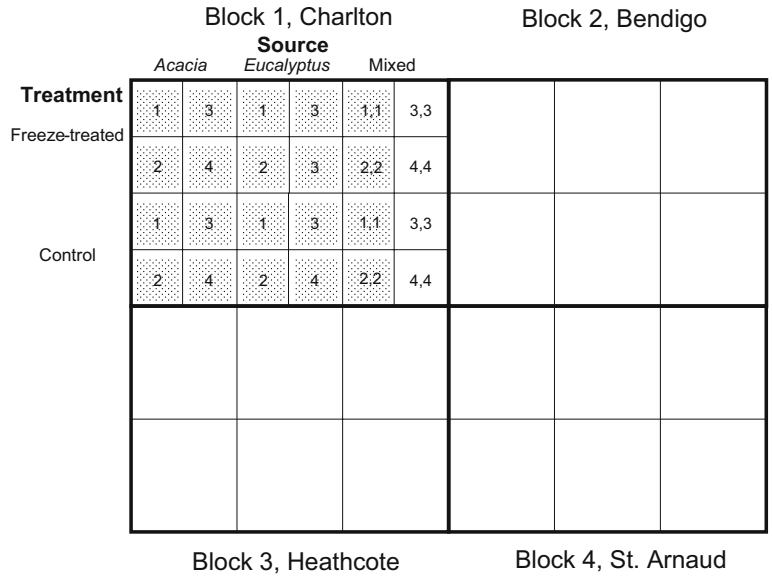

Fig. 1 Study design for greenhouse pot trial. Blocks refer to the collecting sites and also the layout of pots in the greenhouse. Source refers to the tree species from under which soil and litter were collected, and treatment refers to freezing versus controls. Numbers *1–4* refer to individual trees at each site, and indicate the distribution into pots of soil and litter that was sampled from under each tree, e.g. 1,1,=half the soil in the mixed pot came from *A. pycnantha* tree 1 and half from *E. microcarpa* tree 1. *Shaded cells*, soil in pots that were planted with *E. microcarpa* seedlings. *Unshaded cells*, pot not planted with seedling but was used for monitoring of soil nitrogen. Only the first block is illustrated in detail, as all other blocks follow the same model

nightly to minimise positional effects. Contamination of pots by crawling invertebrates was minimised by applying fluon to the rim of the pots.

Soil freezing treatment and planting

To retard biological activity in the soil, particularly of invertebrate fauna, half the pots were freeze-treated before planting by holding assembled pots of soil and litter at −80°C for 3 days. Pots were subsequently kept in a greenhouse where the temperature ranged from 5 to 33°C during the course of the experiment (April to September). Pots were kept well watered before seedlings were planted.

Eucalyptus microcarpa seeds were supplied by the National Tree Seed Centre (ENSIS, Canberra). To minimise genetic variation we used seed collected from the same mother tree on the same date. Seeds were germinated in 1:1 vermiculite/perlite and then provided with liquid fertiliser for about 2 weeks prior to planting. Seedlings were planted in pots when they reached a height of 5 mm. Germination and early growth took longer than anticipated, so pots were left in the greenhouse for some time after the soil freezing treatment and before seedlings were planted. After planting, pots were watered close to saturation twice weekly. We judged that pots were saturated by feeling the weight of pots, and observing drainage.

Three blocks were planted 23 days after freezing treatment (17 May 2005) and the fourth block was planted 7 days later (24 May 2005). Sixteen pots with soil and litter from the mixed source were not planted with a seedling and were only used to detect any post-freezing nutrient pulse (discussed further below; Fig. 1).

Estimation of decomposition rates

We used filter paper discs (Whatman, 42.5 mm diameter) as a standard cellulose substrate to assess decomposition rates (Deacon 1985; Savoie and Goubière 1989; Carter et al. 1999). Discs were placed beneath the litter layer 33 days after freezing treatment, shortly after the seedlings were planted. All 80 pots containing seedlings were used. Discs were left in situ until those that decomposed most rapidly had almost disappeared (27 May–29 June). The remains were removed, dried (70°C for 24 h) and

weighed to determine mass loss. Observations were repeated, using fresh filter papers, between 29 June and 27 July (27 days) and 1–22 August (21 days).

Measurements of plant growth and biomass allocation

Plants were destructively sampled on 6th and 7th September, 106 days after planting. Plants in the fourth block grew for 1 week less (99 days) than plants in the other three blocks, which contributes another source of variation to the block effect. Plants were removed from pots and then separated into stems, leaves and roots. Soil was tipped out of the pots and root fragments retrieved for a standard 2 min search time per pot. Plant parts were dried at 60°C for 72 h before weighing. Leaf area was measured using a flatbed scanner prior to drying.

Over the course of the experiment four plants died from unknown causes (evenly among the treatments, with no more than one from any of the six categories), reducing the overall sample size to 76 plants available for harvest. Six plants (three each from control and freeze treated pots) did not produce enough leaf tissue to perform nitrogen analyses, therefore only 70 seedlings were measured for leaf nitrogen content.

Carbon and nitrogen analysis

To test if there was a soil nutrient pulse after freezing, a sample of approximately 10 g of shallow soil was taken during weeks 1, 2 and 4 following the treatment, from 16 pots containing mixed soil without any seedling planted in them. Mineral nitrogen from soil samples was extracted in 2 M KCl (Rayment and Higginson 1992) and analysed colorimetrically for nitrate and ammonium using an Alpkem continuous flow analyser.

Total carbon and total nitrogen content of shallow soil, leaf litter and leaves of *E. microcarpa* seedlings was analysed according to the method of Dumas (1981), using a mass spectrometer (Europa 20–20) with an automated nitrogen and carbon analysis preparation system. Repeated N analyses on 11 leaf samples and 24 litter and soil samples were used to assess accuracy. Regression of repetition 1 versus repetition 2 of these samples explained 99.3% for leaf N and 99.8% of variation for litter/soil N measurements respectively. Coefficient of variation for %N measurement of standards was 0.68% for leaves (n=42), 0.94% for litter (n=27) and 4.19% for soil (n=27). Together these assessments indicate a high level of accuracy in N analysis.

Data analysis

All data were analysed using GenStat (Lawes Agricultural Trust 2002). Data were log-transformed when necessary to meet the assumption of normal distribution of residuals. Where sample sizes were uneven (decomposition and plant allocation measurements) we used restricted estimates maximum likelihood (ReML) models with Wald significance tests (Payne 2005).

Freezing treatment and soil source were fixed factors and block (combining field site and greenhouse location) was a random factor for the analysis of plant growth, allocation, leaf traits and decomposition rates. Interaction terms were dropped if no significant interaction was found, but non-significant treatment effects were maintained. To analyse the effect of species (*E. microcarpa* vs *A. pycnantha*) on C and N content of leaves, litter and shallow soil material we used one-way analysis of variance (ANOVA) with species as a fixed factor and site as a random factor. To analyse the effect of freezing treatment on NO_3, NH_4 and total mineral nitrogen (NO_3 + NH_4) in soil, we used both one-way ANOVA and repeated measures ANOVA.

Results

Soil carbon and nitrogen and plant growth

Litter and soil used to construct pots varied in carbon and nitrogen content depending on its source. Litter beneath *A. pycnantha* was significantly higher in nitrogen ($F_{1,27}$=39.11, P<0.001; Fig. 2) and had a lower C/N ratio compared with litter beneath *E. microcarpa* ($F_{1,27}$=23.13, P<0.001; data not shown). Soil from under *A. pycnantha* had a significantly lower C/N ratio than soil from under *E. microcarpa* ($F_{1,27}$=12.04, P=0.002), but the difference was small, and the nitrogen content was not significantly different ($F_{1,27}$=1.61, P=0.22; Fig. 2). In spite of these differences in quality between soil and litter from under *A. pycnantha* and *E. microcarpa*, there were no significant effects on plant growth, plant

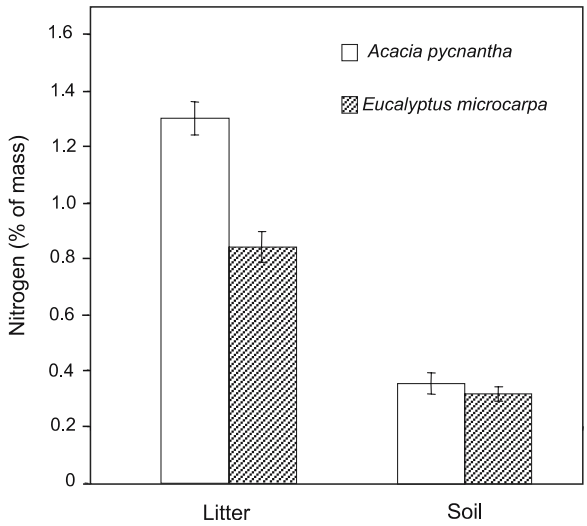

Fig. 2 Mean total nitrogen content of litter and soil (±SE) comparing material collected beneath *A. pycnantha* and *E. microcarpa*

allocation or decomposition associated with the soil source, including the mixed treatment.

There was no evidence of a soil nutrient pulse following the freezing treatment (Fig. 3). There was a marginally significant decrease in ammonium following the freeze-treatment ($F_{1,11}$=4.5, P=0.057) and no significant effect of time ($F_{2,28}$=1.2, P=0.314) or interaction with time. By way of contrast, nitrate was not significantly affected by freezing, but increased significantly over the 4 week period (Fig. 3, $F_{2,\ 28}$= 23.16, P<0.001) with no time-by-treatment interaction. The increase in nitrate content, combined with reduction in ammonium, suggests that nitrogen cycling activity recommenced in the soils relatively soon after freezing treatment.

Decomposition rates

Mass loss of filter paper loss ranged from 0 to 100% over all three observation periods. The pattern of loss varied from holes in otherwise complete paper discs to fragmentation of the paper. Increased fungal and bacterial activity was observed around areas of filter paper that had been decomposed. The fungal colonies present were consistent in appearance with *Rhizopus*, *Mucor*, *Fusarium* and *Stachybotrys* spp. (Celeste Linde, Australian National University, personal observations). Estimated mean daily loss of filter paper was greater in the control pots during the first 33 days

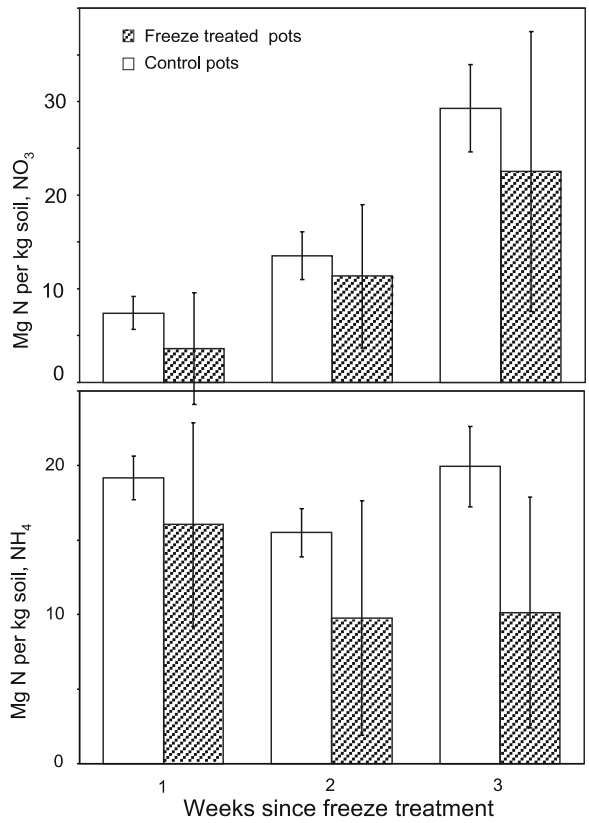

Fig. 3 Mean nitrate (NO_3) and ammonium (NH_4) content of soil in pots, 1, 2 and 3 weeks after freeze treatment. Values are means of $\log_e$-transformed data, back transformed for the figure. The standard errors were scaled to maintain the relationship to the mean after back transformation. Hatched columns show values for freeze-treated pots, open columns show values for control pots

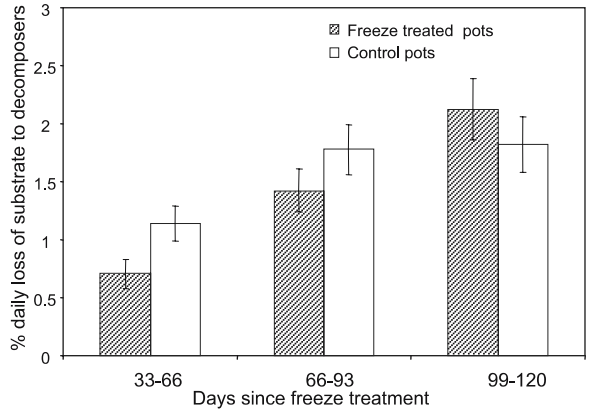

Fig. 4 Daily decomposition rate, estimated as percentage loss of filter paper (mean±SE) following freezing treatment of soils, as measured over three separate observation events, using fresh filter paper on each occasion

(Wald=6.16, P=0.013; Fig. 4). During the second observation period (66–93 days post-freezing treatment) this difference was still marginally significant (Wald=2.74, P=0.098) but in the third period (99–120 days) there was no significant difference (Wald=0.75, P=0.387). Repeated measures ANOVA supported this analysis, showing a time-by-treatment interaction ($F_{2,177}$=3.53, P=0.033), and that daily loss of filter paper increased over the three observation periods ($F_{2,177}$=30.89, P<0.001). There was no effect of soil source on decomposition in any observation period.

Measurements of plant growth and allocation

Leaf, root and total biomass of *Eucalyptus microcarpa* seedlings grown in freeze-treated soil were each almost 40% lower than those grown in untreated soil (Table 1, Fig. 5a), although only leaf and total biomass reached statistical significance. There was no difference in stem mass between the treatments.

Leaf area and leaf area ratio (LAR; the ratio of leaf area to total plant weight) were lower in plants from freeze-treated soil compared with controls, but significance of this effect was marginal (Table 1). Leaf mass per leaf area (LMA, g/cm^2; Fig. 5b) was significantly lower in plants from freeze-treated soil (Table 1). In other words, *E. microcarpa* seedlings grown in freeze-treated soil produced smaller, flimsier leaves and less leaf tissue relative to other plant tissues. Other allocation variables were not significantly different between treatments (Table 1).

Nitrogen content per unit leaf mass showed a significant inverse correlation with LMA (R^2=0.26, P<0.001, n=70; Fig. 6). If N content per unit leaf area was completely independent of LMA then the relationship between LMA and N would have had a slope of −1, i.e. a doubling of LMA leads to a halving of N per mass. However, the slope of LMA versus N per mass was in fact less than −1, (Fig. 6) because of a countervailing positive correlation between LMA and N per unit leaf area (R^2=0.21, P<0.001, n=70; data not shown). Consequently, there was no significant effect of soil treatment on nitrogen content in leaves, such as one would have expected if LMA was the sole driver of nitrogen content. In other words, thinner (low LMA) leaves generally contained higher concentrations of nitrogen, but the slope and fit of this relationship was not great enough to lead to a significant effect of soil treatment on N per mass in leaves.

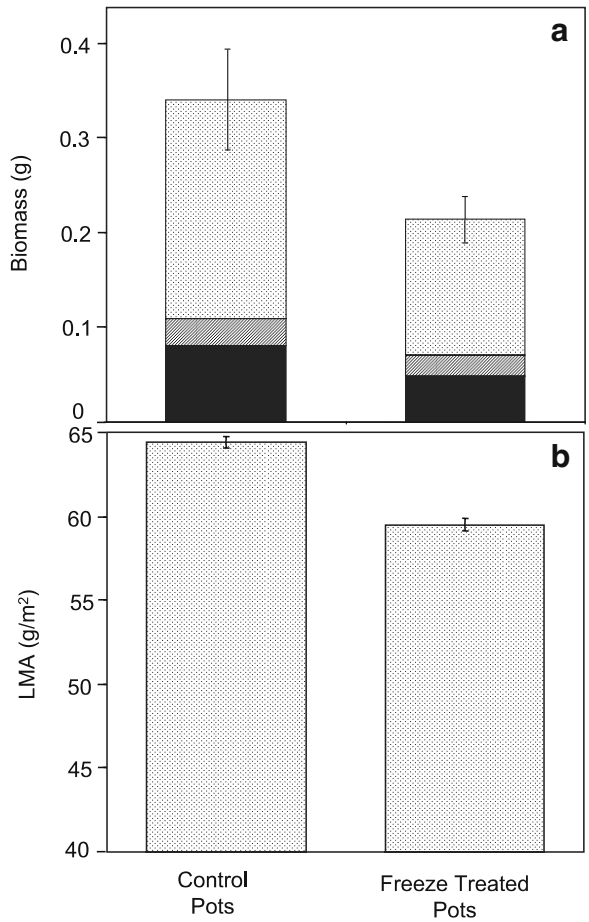

Fig. 5 Mean biomass and allocation to plant parts of *E. microcarpa* seedlings grown in freeze-treated and control pots. Values are means of $\log_e$-transformed data, back transformed for the figure. The standard errors were scaled to maintain the relationship to the mean after back transformation. **a** The total biomass of seedlings, and its allocation into leaves (*dots*), shoots (*hatched*) and roots (*solid*). The standard error applies to the total biomass. **b** Leaf mass area (LMA, g/m^2) of leaves from seedlings

Discussion

The main effect of the soil freezing treatment on performance of *Eucalyptus microcarpa* seedlings was to significantly retard growth. This was evidenced by lower total and leaf biomass compared with controls, and the production of less robust leaves, as indicated by lower leaf mass per area (LMA). Together these effects suggest that the soil biota in untreated soils

Table 1 Effect of soil and litter treatment on growth and allocation of *E. microcarpa*

Variable	Wald statistic	*P* value
Leaf mass per area	6.41	0.011
Leaf mass	4.56	0.033
Total biomass	4.37	0.037
Leaf area	3.68	0.055
Root mass	3.43	0.064
Leaf area ratio	3.39	0.066
Stem mass	1.46	0.226
Root shoot ratio	0.06	0.800
Root mass ratio	0.06	0.809
Leaf mass ratio	0	0.965

Data were analysed using a ReML model, with n=76, 1 *df*, with soil treatment as a fixed effect, and block as a random effect. All data were $\log_e$-transformed before analysis.

was providing a net benefit to *E. microcarpa* seedlings, facilitating faster growth and development of more robust leaves.

Two lines of evidence support the hypothesis that the beneficial effect of the soil biotic community on plant growth is due to decomposition and nutrient cycling. The freezing treatment reduced the decomposing capacity of the soil, based on the filter paper bioassays, for at least 90 days, and reduced soil ammonium concentrations for at least 30 days. Higher rates of decomposition and nitrogen mineralisation in untreated soil may have been responsible for releasing more nitrogen in plant-available form. For *Eucalyptus*, ammonium availability appears to be particularly important in this regard. Several *Eucalyptus* spp. have been found to take up ammonium and the amino acid glycine considerably more rapidly and in greater amounts than nitrate (Warren 2006).

Plants have several mechanisms for avoiding rate-limited processes in soil nitrogen mineralisation. Where rates of nitrification are low, plants may take up ammonium in preference to nitrate. Where ammonification rates are low, plants may take up amino acids. Early successional species appear to have a higher capacity for nitrate uptake than species of later succession, which show preferential uptake of ammonium and amino acids (Chapin et al. 1993; Persson and Näsholm 2001). Ammonium and amino acids may be the dominant forms of nitrogen taken up by *Eucalyptus* spp. in Australian soils which can have quite high levels of ammonium and soluble organic nitrogen relative to nitrate (Turnbull et al. 1996; Garnett and Smethurst 1999).

The freezing treatment could also have harmed the mycorrhizal associates of *E. microcarpa*. Mycorrhiza of *Eucalyptus* spp. are common and widespread in Australia, are best known in relation to phosphorus nutrition of their host plants (Burgess et al. 1993) and can markedly increase growth of *Eucalyptus* seedlings (Chen et al. 2006). Mycorrhiza can also mobilise sources of nitrogen otherwise unavailable to plants (Read and Perez-Moreno 2003). Turnbull et al. (1995) found that mycorrhizal seedlings of *Eucalyptus* spp. grew well on a range of organic sources of nitrogen, whereas non-mycorrhizal seedlings could only grow on mineral sources of nitrogen or glutamine, indicating that mycorrhiza confer on *Eucalyptus* spp. the capacity to use nitrogen from a broad range of sources.

The capacity of *Eucalyptus* to exploit a wide range of nitrogen sources is likely to provide flexibility in exploiting a range of low nutrient environments. However, it may also mean they are ecologically linked to a wider range of soil organisms than solely the bacterial populations involved in nitrite oxidation. For example, they may be more affected by heterotrophic invertebrate populations and the fungi involved in decomposition of peptides and their

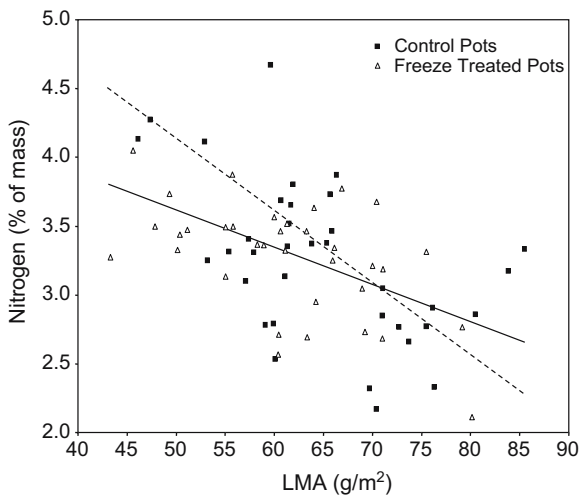

Fig. 6 The relationship between leaf mass per area (*LMA*) and nitrogen content on a mass basis. The dashed line shows the relationship that would hold if N per area was held constant. The solid line shows the relationship (from linear regression) in the data collected, pooling treated and untreated pots because no significant difference in slope was attributable to the treatment effect

ammonification, as well as bacteria involved in the various steps in the nitrification pathway.

Bardgett et al. (1998) completely eliminated nematodes, microarthropods and larger fauna from soils by freezing soil samples at −80°C and air drying. Only spore-forming microorganisms are likely to withstand these conditions (Hughes and Nobbs 2004). Antarctic soil metazoa can survive −80°C for years (Newsham et al. 2006), but lethal temperatures of −5°C to −30°C are the norm for invertebrates from temperature latitudes (Brown and Gaugler 1996; Addo-Bediako et al. 2000). Our study showed that decomposition rate and nitrate concentration in pots was lowest immediately after the freezing treatment, but then increased with time. Both patterns suggest that microflora recolonised pots via airborne spores or increased from populations that remained in a resting stage after freezing. It is much less likely that any invertebrates (or their eggs and cysts) could have survived the prolonged extreme low temperatures, but they could reestablish by airborne dispersal into the greenhouse, or by crawling across the fluon barrier. Dipteran larvae were observed in freeze-treated pots towards the end of the experiment.

Litter under *A. pycnantha* was approximately 50% richer in nitrogen than litter under *E. microcarpa*. Nevertheless, there was no evidence of soil or litter source effects in this study on plant growth or decomposition rate. This lack of a nitrogen enrichment effect might in part be explained by the fact that the difference in soil nitrogen concentration was so small as to be non-significant. It might be that decomposition rates in the field are slow, so that most of the nitrogen remains relatively unavailable in the litter, with no significant enrichment of the soil over the short time that these sites have been under revegetation. Alternatively, available nitrogen may have been assimilated into the soil biota, but not subsequently released in a plant available form. Decomposition rates in the natural environment are likely to be extremely temporally variable, because of the great variability in rainfall, but we probably increased decomposition rates in pots, relative to the field, by frequent watering. The small soil mass in pots (relative to the field) could have lead to higher temperatures, also increasing decomposition.

Absence of a soil and litter source effect also indicates that there were not significant numbers of *E. microcarpa* specialist root feeders to create a 'home site disadvantage'. Although root feeding has been important in some studies (De Deyn et al. 2004), we found that the abundance of plant feeding nematodes in our soil was relatively low (unpublished observations). Because there was no evidence of a home site disadvantage associated with soil from under *Eucalyptus*, or nitrogen enrichment of soil from under *Acacia*, one would expect that the mixed soil treatment would not be different to the pure soil treatments, and this was indeed the case.

Inoculation experiments, in which soil organisms are introduced into pots, have detected positive effects on plant growth associated with soil organisms (Alphei et al. 1996; Laakso and Setälä 1999; Liiri et al. 2002). Our study differs from these studies in focusing on a tree species rather than grasses or herbs, and in detecting an effect on leaf structure (i.e. LMA) as well as growth rates. Evolution of high LMA can be an adaptation to low nutrient and low rainfall environments, where selection favours robust long-lived leaves (Cunningham et al. 1999). However, when considering within-population variation in LMA, as in the present study, variation is more likely to reflect a developmental response. We suggest that low LMA in the plants grown in freeze-treated soils is a stress response to low nutrient availability. Low LMA leaves are less robust, and therefore less likely to remain functional for as long as high LMA leaves, so this response is unlikely to be adaptive. Leaf nitrogen analyses indicated that there was no difference in nitrogen concentration between treatments, so the LMA response does not appear to be primarily attributable to a nitrogen allocation strategy. There was, however, a correlation between LMA and N_{mass} and N_{area}, which was similar regardless of treatment, suggesting close coupling in these traits that may represent a constraint to variation in leaf structure.

Conclusions

This experiment demonstrates that the soil biota confers large benefits to the tree *Eucalyptus microcarpa*. Tree seedlings grown in freeze-treated soil not only suffered in terms of biomass, but also had less robust leaves. This effect of soil biota on leaf development underlines the potential for complex consequences of above-ground below-ground ecological linkages. Our data on the nitrogen dynamics and

decomposition rates support the hypothesis that the soil biota provided benefits through the release of nitrogen from complex compounds into plant-available forms. In this study we detected no legume enrichment of the soil, probably because insufficient time had passed since planting to allow decomposition of the nitrogen rich legume litter. We suggest that variation in the soil biotic communities has the potential to greatly enhance or hinder the success of revegetation on retired agricultural land. Benefits to the soil and soil fauna from legume enrichment are likely to occur, but may take more than a decade to be realised.

Acknowledgments We thank Alison Jeavons, Lindsay Ezard and Geoff Winsell (Department of Primary Industry, Victoria) for help with site selection, and the landholders for allowing us access to sites. Kim Pullen provided assistance in field. We thank Celeste Linde (Australian National University), Elizabeth Lindsay and Mike Hodda (CSIRO Entomology) for methodological advice and information on various groups of soil organisms. Financial support for this research was provided by the CSIRO Water for a Healthy Country Flagship.

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