Effects of changing the supply of nitrogen and phosphorus on growth and interactions between *Eucalyptus globulus* and *Acacia mearnsii* in a pot trial

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Abstract

Significant increases in aboveground biomass production have been observed in mixed plantations of *Eucalyptus globulus* and *Acacia mearnsii* when compared to monocultures. However, this positive growth response may be enhanced or lost with changes in resource availability. Therefore this study examined the effect of the commonly limiting resources soil N, P and moisture on the growth of *E. globulus* and *A. mearnsii* mixtures in a pot trial. Pots containing two *E. globulus* plants, two *A. mearnsii* plants or one of each species were treated with high and low levels of N and P fertiliser. After 50 weeks, *E. globulus* plants grew more aboveground biomass in mixtures than monocultures. *A. mearnsii* were larger in mixtures only at low N, where both species were similar in size and the combined aboveground biomass of both species in mixture was greater than that of monocultures. At high N and both high and low levels of P fertiliser *E. globulus* appeared to dominate and suppress *A. mearnsii*. In these treatments, the faster growth of *E. globulus* in mixture did not compensate the reduced growth of *A. mearnsii*, so mixtures were less productive than (or not significantly different from) *E. globulus* monocultures. The greater competitiveness of *E. globulus* in these situations may have resulted from its higher N and P use efficiency and greater growth response to N and P fertilisers compared to *A. mearnsii*. This trial indicates that the complex interactions between species in mixtures, and thus the success of mixed plantations, can be strongly influenced by site factors such as the availability of N and P.

Introduction

The aboveground biomass or volume of different species combinations have been compared with monocultures in many studies, and mixtures have been found to have higher, lower or the same

productivity compared with monocultures (Cannell et al., 1992; FAO, 1992). Site factors such as soil N, P and moisture availability can also influence the growth of mixtures relative to monocultures, so mixtures are likely to be more productive than monocultures on some sites but not on others (Assmann, 1970; DeBell et al., 1987; FAO, 1992; Fredericksen et al., 1993; West, 1991).

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Facilitation interactions between species in mixtures generally predominate where resources are in low supply, under high-physical stress or high-consumer pressure (Bertness and Callaway, 1994; Holmgren et al., 1997). The dominance of competition increases towards the more productive end of the environmental gradient, where physical stress or consumer pressures are low (Bertness and Callaway, 1994; Holmgren et al., 1997). Thus facilitative interactions in mixed stands will only increase yields if there is a limited supply of the resource for which acquisition was facilitated or competition was reduced, such that the effect of these positive interactions outweighs the negative, competitive effects. The influence of site factors such as soil N and P availability on the productivity and the interactions and processes in mixtures of tree species is not well understood. However, this information is vital when establishing mixed species plantations.

It would be very costly and time consuming to examine the performance of species in mixtures under the full range of field site conditions. Here, a pot trial was used to examine the effect of soil N, P and moisture on the growth of *E. globulus* and *A. mearnsii* mixtures. These factors often limit the growth of plantations in Australia.

Materials and methods

Species selection and seed source

E. globulus and *A. mearnsii* were used because mixtures of these species have produced significantly higher quantities of aboveground biomass than monocultures in a field trial (Bauhus et al., 2004; Forrester et al., 2004). The seed of both species was purchased from the Australian Tree Seed Centre of CSIRO Forestry and Forest Products in Canberra (Seedlot numbers 18891 and 16265 for *E. globulus* and *A. mearnsii*, respectively).

Experimental design and treatments

The experiment consisted of a $3 \times 2 \times 2 \times 3$ factorial design containing 3 species combinations, 2 levels of P fertiliser, 2 levels of N fertiliser and 3 levels of watering. These treatment

combinations were replicated twice in two blocks and the spatial distribution of pots within the replicates was randomised. The effective replication within this factorial design for each species combination, nutrient level and water level was 36, 24 and 36, respectively. The interactions between the factors were of particular interest and thus a factorial design provided an efficient way of studying this in a single experiment. The factorial design (see Cochran and Cox, 1957; Williams et al., 2002) allowed the examination of all interactions between factors, as opposed to separate experiments that would not have allowed such an examination.

Each pot contained two plants: two *E. globulus* (EE), two *A. mearnsii* (AA) or one of each species (EA). Phosphorus was supplied as triple superphosphate at initial low and high rates of 20 mg P kg⁻¹ soil (low P) and 200 mg P kg⁻¹ soil (high P). Nitrogen was supplied as ammonium nitrate at initial low and high rates of 60 mg N kg⁻¹ soil (low N) and 600 mg N kg⁻¹ (high N). These rates were determined after testing these species in this particular soil, to ensure that the high rates were not toxic nor induced salt stress. Additional fertiliser applications were required to maintain the high N and P availability and to prevent deaths in the low N treatment. Final application rates were 210 and 900 mg N kg⁻¹ soil in the low and high N treatments respectively, and 20 and 267 mg P kg⁻¹ soil in the low and high P treatments respectively. Other nutrients and micronutrients were added to all treatments at the following rates (all in mg kg⁻¹ soil): 365 K, 148 S, 21 Mg, 22 Fe, 8 Cu, 8 Zn, 2 B and 1 Mo. The initial fertiliser was applied by mixing into the potting medium when preparing the medium (2 : 1 coarse river sand and subsoil). Subsoil was obtained from the glasshouse facility site at The Australian National University, Canberra, Australia, and was sieved (6 mm mesh) before mixing. This substrate had a low total N (80 mg kg⁻¹) and P availability (Bray 1 P 17 mg kg⁻¹). More detail about soil nutrition is provided in Forrester (2004).

Water was applied at three levels: high, medium and low. Plants receiving the high and medium treatment were watered three times and once a week, respectively. Those receiving the low treatment were only watered when one of

the plants showed signs of water stress (wilting). On the specified days water was applied until it started to seep out of the bottom of the pot.

Seed propagation and seedling establishment

Seedlings were grown in 11 cm diameter pots (12 cm deep) containing the 2 : 1 coarse river sand and subsoil medium until the experiment began. *A. mearnsii* seedlings were inoculated with a soil slurry to provide Rhizobia spores. The slurry was made from soil taken from a 5-year-old *A. mearnsii* tree in Canberra, ACT, and a 10-year-old *A. mearnsii* tree near Ulladulla, NSW, and consisted of about 250 g of soil in 9 liters of water. After 5 months seedlings (up to 40 cm tall) were transplanted into 75-l pots ("Easy lift" planter bags; 45 cm diameter and 50 cm deep). At this stage all *A. mearnsii* seedlings were nodulated.

Plants were grown in a glasshouse until week 14, when they reached a height that required them to be moved outside. The spatial pattern was maintained outside but the spacing between pots was increased to reduce shading so that there was 50 cm between pots within rows and 1.5 m between rows. Plastic weed mats and plastic collars were used to prevent any root growth into the soil beneath the pots (an additional nutrient source), and to prevent rain from watering the plants, respectively.

Data collection

Stem diameter and plant heights were measured fortnightly from the day after the seedlings were transplanted into the pots until the plants were harvested at the end of the experiment (50 weeks later). Diameters were measured at a point 10 cm above the soil surface (D_{10}) to avoid the swelling at the root collar of *E. globulus*.

To estimate aboveground biomass of plants prior to the destructive sampling at week 50, allometric biomass equations were developed between aboveground biomass and diameter (D_{10} ; in mm) at the end of the experiment. These equations were used to estimate aboveground biomass from fortnightly diameter measurements. There was no significant treatment effect on the relationship between biomass and diameter for *E. globulus* but for *A. mearnsii* there was a signif-

icant P effect. Including height as an explanatory variable did not improve the models.

E. globulus

$$\begin{aligned} \text{Ln aboveground biomass(g)} \\ = -1.646 + 2.4124\text{Ln}(D_{10}; \text{mm}) \\ (P < 0.001, \text{Adj } R^2 = 0.903) \end{aligned}$$

A. mearnsii

$$\begin{aligned} \text{low P Ln aboveground biomass (g)} \\ = -0.457 + 2.114\text{Ln}(D_{10}; \text{mm}) \\ \text{high P Ln aboveground biomass (g)} \\ = -3.137 + 2.863\text{Ln}(D_{10}; \text{mm}) \\ (P < 0.001, \text{Adj } R^2 = 0.825) \end{aligned}$$

At the end of the growing period, each plant was harvested and the oven dry weight (60°C for 2 weeks) of the following components was recorded: stem, branch, foliage, fine-roots (< 2 mm), medium roots (> 2 mm and < 5 mm) and coarse roots (> 5 mm). All roots were washed from the soil and detached fine-roots were collected by flotation.

Nitrogen and P concentrations were determined for each plant part except coarse roots. Coarse root and stems were assumed to have the same N and P concentrations so only stem samples were analysed. Soil was sampled from the middle of each pot. To determine N and P concentrations, ground and dried plant or soil material was digested in acid (potassium sulphate dissolved in concentrated sulphuric acid) and hydrogen peroxide (modified from Heffernan, 1985). For soil samples, 1 ml of a copper sulphate solution catalyst was added to the soil for 12 h prior to the digestion. Total N and P were determined simultaneously in a continuous flow system (Technicon® TRAACS 800™ or Technicon Auto-Analyser II) using an ascorbic acid/ammonium molybdate method.

Calculations and statistical techniques

Many indices have been used to describe the growth outcome of mixed-species systems and all have advantages and disadvantages (Jolliffe, 2000; Williams and McCarthy, 2001). A frequently used index and the only one used in this study is the relative yield (RY) of aboveground biomass Harper (1977):

$$\text{RY of species A} = \frac{\text{yield of species A in mixture}}{\text{yield of species A in monoculture}} \quad (\text{Harper 1977})$$

Regression relationships between diameter at 10 cm and aboveground biomass were fitted using GenStat™ (VSN International Ltd, Hemel Hemstead, U.K.). Due to an uneven distribution of residuals both variables were transformed using natural logarithms.

Treatment effects were tested using an Analysis of Variance (ANOVA) in GenStat™ (VSN International Ltd, Hemel Hemstead, U.K.). The Standard Errors of Difference (S.E.D.s) for comparison of treatment means are provided. Natural logarithmic or square root transformations were required for some variables to remove heteroskedasticity. In these cases, 95% confidence intervals are provided. The tables contain the S.E.D.'s for the main effects and for interactions between the factors in the given table.

Comparisons between species and the effect of different species proportions (EE, EA and AA) were examined using the factors "monoculture", "mixmono" and "species": "monoculture" compares EE to AA, "mixmono" compares EA to EE or AA and "species" compares the *E. globulus* and *A. mearnsii* in EA.

Due to the large number of effects and variables examined, the following sections focus on the most important effects. These include interactions between N, species proportions and species. Other important interactions include lower order interactions between two of these factors and the effects of N, P and species proportion alone.

Results

Biomass

All treatments had a significant effect on plant biomass after 50 weeks. For detail on all the significant effects see Forrester (2004). In most cases, water effects were only significant when interacting with other factors, so to reduce the complexity of the discussion they are not described further. Different components of biomass were similarly affected by the treatments.

At low N, both species grew more aboveground biomass in the mixed EA than EE or AA, and total pot aboveground biomass was higher in EA than EE or AA (Table 1). At high N *E. globulus* still grew larger in EA than EE but *A. mearnsii* were smaller in EA than AA and total pot aboveground biomass was higher in EE and EA than in AA. *E. globulus* aboveground biomass was greater in EA than EE at high P, but *A. mearnsii* growth was not significantly different in EA to AA at either level of P. At both levels of P, total pot aboveground biomass was not significantly different between EA and EE but both were higher than AA. At high N and P, *A. mearnsii* was smaller than *E. globulus* and the increased growth of *E. globulus* in EA did not compensate for the smaller size of *A. mearnsii*.

Species proportion and fertiliser significantly influenced aboveground biomass as early as week 20. For *E. globulus*, these early effects followed the same trends as those at 50 weeks. However, for *A. mearnsii*, the effects of N on the species proportion were reversed, such that *A. mearnsii* were larger in terms of aboveground biomass in EA than AA at high N, but smaller at low N. This increase in growth in EA compared to EE or AA was shown using the RY of aboveground biomass in Figure 1.

The response of *E. globulus* to N and P fertiliser was greater than the response of *A. mearnsii* (Table 1). For example in EE, *E. globulus* aboveground biomass more than doubled, from 363 g

Table 1. Aboveground biomass (g) of *E. globulus* and *A. mearnsii* in mixtures and monocultures at high and low levels of N and P fertiliser

Fertiliser	Species proportion	Aboveground biomass	
		<i>A. mearnsii</i>	<i>E. globulus</i>
Low P	Monoculture	486	591
	Mixture	495	687
High P	Monoculture	236	668
	Mixture	243	1140
Low N	Monoculture	348	363
	Mixture	461	498
High N	Monoculture	375	896
	Mixture	277	1329

Standard error of difference = 72 (not for N versus P comparisons).

Figure 1. Relative yield (RY) of aboveground biomass for (a) *E. globulus* at low and high levels of N, (b) *A. mearnsii* at low and high levels of N, (c) *E. globulus* at low and high levels of P and (d) *A. mearnsii* at low and high levels of P. A RY of 1 indicates that plants were the same size in mixture as in monoculture. RY < 1 indicates that plants were smaller in mixture than in monoculture and RY > 1 indicates that plants were larger in mixture than in monoculture.

Table 2. Root: shoot ratio of *Eucalyptus globulus* and *Acacia mearnsii* in mixtures and monocultures at high and low levels of N and P fertiliser

Fertiliser	Species proportion	<i>A. mearnsii</i>	<i>E. globulus</i>
Low P	Monoculture	0.38	0.48
	Mixture	0.35	0.49
High P	Monoculture	0.37	0.51
	Mixture	0.22	0.54
Low N	Monoculture	0.29	0.62
	Mixture	0.26	0.65
High N	Monoculture	0.46	0.38
	Mixture	0.31	0.37

Standard error of difference = 0.033 (not for N versus P comparisons).

Table 3. The total soil N and P (g pot⁻¹) in mixtures and monocultures at high and low levels of N and P fertiliser at the end of the experiment

Fertiliser	AA	EA	EE
Total soil N			
Low P	5.7–10.4	7.7–13.0	7.2–12.3
High P	3.0–6.5	4.2–8.2	4.3–8.4
Low N	3.6–7.5	5.0–9.4	5.5–10.0
High N	4.8–9.1	6.7–11.6	5.9–10.5
Total soil P			
Low P	17.6–22.4	17.3–22.0	19.6–24.6
High P	26.2–31.9	27.8–33.8	26.9–32.7
Low N	21.8–27.0	22.0–27.2	24.8–30.5
High N	21.6–26.8	22.6–27.9	21.4–26.7

Ranges are 95% confidence intervals. EE = *E. globulus* monoculture, EA = mixture, AA = *A. mearnsii* monoculture.

at low N to 896 g at high N. In AA, *A. mearnsii* aboveground biomass increased from 348 g at low N to 375 g at high N.

The root : shoot (R : S) ratio was higher for *E. globulus* than *A. mearnsii* (Table 2). The R : S ratio was affected by an interaction between N, species proportion and species and an interaction between P, species proportion and species. The effect of N and P on the R : S ratio was greater than the effect of species proportion. The effect of species proportion was only significant for *A. mearnsii* at high N and high P where the R : S ratio was lower in EA than AA. High N reduced the R : S ratio of *E. globulus* compared to low N. For *A. mearnsii*, the R : S ratio declined at high P compared to low P.

Table 4. Nitrogen in aboveground biomass (g) of *Eucalyptus globulus* and *Acacia mearnsii* in mixtures and monocultures after 50 weeks at high and low levels of N and P fertiliser

Fertiliser	Species proportion	<i>A. mearnsii</i>	<i>E. globulus</i>
Low P	Monoculture	6.64	4.20
	Mixture	6.91	4.74
High P	Monoculture	2.68	4.83
	Mixture	3.20	8.45
Low N	Monoculture	4.85	1.17
	Mixture	7.00	1.76
High N	Monoculture	4.48	7.86
	Mixture	3.11	11.43

Standard error of difference = 0.96 (not for N versus P comparisons).

Table 5. Phosphorus in aboveground biomass (g) of *Eucalyptus globulus* and *Acacia mearnsii* in mixtures and monocultures at high and low levels of N and P fertiliser

Fertiliser	Species proportion	<i>A. mearnsii</i>	<i>E. globulus</i>
Low P	Monoculture	0.28–0.52	0.25–0.46
	Mixture	0.21–0.49	0.28–0.66
High P	Monoculture	0.43–0.78	0.46–0.85
	Mixture	0.38–0.91	0.70–1.78
Low N	Monoculture	0.33–0.61	0.22–0.41
	Mixture	0.40–0.95	0.31–0.74
High N	Monoculture	0.35–0.66	0.51–0.94
	Mixture	0.20–0.47	0.62–1.58

Ranges are 95% confidence intervals.

Nitrogen and phosphorus in soils and plants

There were no measurable differences in soil N contents between the two N-fertilisation levels after 50 weeks (Table 3). In contrast, soil P was higher at high P. Soil N was lower at high P than low P. There were no other significant treatment effects on soil N or P contents.

The total amount of N in aboveground biomass was affected by an interaction between N, species proportion and species (Table 4). Nitrogen in *A. mearnsii* aboveground biomass was higher in EA than AA at low N but not significantly different at high N. However, N in *E. globulus* aboveground biomass was higher in EA than EE at high N but not significantly different at low N. Aboveground P increased with P fertiliser but there were no interactions between P, species proportion and species (Table 5). Nitrogen and P

Table 6. Nitrogen and phosphorus (g) in plant biomass (above- and belowground biomass of both plants in a pot), and the percent of total system N or P (N or P in soil and plant biomass) in plant biomass in mixtures and monocultures at high and low levels of N and P fertiliser

Nitrogen and phosphorus (g) in plant biomass						
Fertiliser	Nitrogen (SED = 1.0)			Phosphorus (SED = 0.17)		
	AA	EA	EE	AA	EA	EE
Low P	17.4	15.1	10.7	1.25	1.23	1.14
High P	8.2	13.9	13.3	3.01	3.08	2.45
Low N	13.4	11.2	3.6	1.81	2.07	1.31
High N	12.2	17.3	20.5	2.45	2.24	2.28
Percent of total system N or P in plant biomass						
Fertiliser	Nitrogen (SED = 4.81)			Phosphorus (SED = 0.592)		
	AA	EA	EE	AA	EA	EE
Low P	69.6	61.5	47.9	5.97	5.95	5.51
High P	63.7	67.2	58.1	9.19	9.02	7.70
Low N	66.3	63.0	36.5	6.50	7.15	4.66
High N	67.0	65.7	69.5	8.67	7.82	8.55

SED = Standard error of difference (not for N versus P comparisons). EE = *E. globulus* monoculture, EA = mixture, AA = *A. mearnsii* monoculture.

Table 7. Nitrogen use efficiency (aboveground biomass (g)/nitrogen in aboveground plant biomass (g)) of *Eucalyptus globulus* and *Acacia mearnsii* in mixtures and monocultures at high and low levels of N and P fertiliser. Standard error of difference = 12.9 (not for N versus P comparisons).

Fertiliser	Species proportion	<i>A. mearnsii</i>	<i>E. globulus</i>
Low P	Monoculture	77	222
	Mixture	78	210
High P	Monoculture	120	210
	Mixture	104	202
Low N	Monoculture	99	316
	Mixture	80	294
High N	Monoculture	98	117
	Mixture	102	119

Standard error of difference = 12.9 (not for N versus P comparisons).

interacted such that aboveground N was higher at high P than low P for *E. globulus* and vice versa for *A. mearnsii* (Tables 4 and 5). Nutrient contents were provided instead of nutrient concentrations so that uptake could be compared. Concentrations can be misleading when comparing plants of different sizes when large plants take up more nutrient but have lower foliar nutrient concentrations due to a dilution effect.

The N or P in the total plant biomass of a given pot (biomass of both plants in a pot above- and belowground) was influenced by interactions

Table 8. Phosphorus use efficiency (aboveground biomass (g)/phosphorus in aboveground plant biomass (g)) of *Eucalyptus globulus* and *Acacia mearnsii* in mixtures and monocultures at high and low levels of N and P fertiliser

Fertiliser	Species proportion	<i>A. mearnsii</i>	<i>E. globulus</i>
Low P	Monoculture	1089–1325	1361–1652
	Mixture	1173–1464	1245–1553
High P	Monoculture	219–266	811–985
	Mixture	230–287	784–991
Low N	Monoculture	486–589	1013–1230
	Mixture	498–621	849–1059
High N	Monoculture	492–598	1090–1323
	Mixture	542–677	1149–1452

Ranges are 95% confidence intervals.

between N and species proportion and between P and species proportion (Table 6). The N in total plant biomass increased with the proportion of *A. mearnsii* (lowest at EE and highest at AA) at low N but increased with the proportion of *E. globulus* at high N. There was no significant difference in the total P in pot biomass between species proportions at low P, but at high P the total P in pot biomass was lower in EE than EA or AA.

The proportion of N in the soil–plant system used by the plants was estimated as the proportion of the N in soil plus biomass (above- and

belowground) that was in the biomass. The proportion of this total system N (plant and soil N) held in the plant biomass was affected by an interaction between N and species proportion such that less than 40% of this N was in plant biomass in EE at low N but in all other species proportions at high and low N over 60% of total system N was in the plant biomass (Table 6). The proportion of total system P in plant biomass increased from low P to high P and from low N to high N (Table 6). There was a general increase in the proportion of total system P in plant biomass from EE to AA.

Resource use efficiency

Nitrogen and phosphorus use efficiency (grams of aboveground biomass per gram of N or P in aboveground biomass) was higher for *E. globulus* than *A. mearnsii* (Tables 7 and 8). Nitrogen use efficiency was higher at low N than at high N for *E. globulus* but not for *A. mearnsii*. Also, P use efficiency was higher at high N for *E. globulus* but not for *A. mearnsii*. Nitrogen use efficiency was higher at low P for *E. globulus* (although not significantly) but higher at high P for *A. mearnsii*. Phosphorus use efficiency was higher for both species at low P than high P (Table 8).

Discussion

The pot experiment showed that N and P supply have a strong influence on the growth of mixtures. *E. globulus* grew more aboveground biomass in EA than EE, whereas *A. mearnsii* was larger in EA than AA in most treatments (low P, high P and low N) but smaller at high N. The positive growth response of *E. globulus* to growing in EA, as shown by the RY, was enhanced at high levels of P due to a reduction in competition from *A. mearnsii*.

Effects of species proportion at low N

At low N, both species grew larger and produced more combined biomass in EA than EE or AA. Higher growth of *E. globulus* may have resulted from a higher N availability through either N fixation by *A. mearnsii* or accelerated N cycling.

Nitrogen in aboveground biomass was highest in AA and lowest in EE, suggesting a higher N availability with increasing proportions of *A. mearnsii*. Similarly, in published pot and field trials, increased growth of *Eucalyptus* species in mixture with N-fixing tree species has resulted (at least in part) from higher N availability (Baker et al., 1994; Bauhus et al., 2004; Binkley et al., 1992a, 2003a; Boydon et al., 2005; Forrester et al., 2004; Khanna, 1997; Vezzani et al., 2001).

Differences in resource availability can be associated with differences in biomass allocation, as plants allocate more biomass to obtain the resources that are limiting. Allocation belowground may decrease if aboveground competition for light increases, however an increase in competition for below ground resources (such as N) could have the opposite effect (Bloom et al., 1985). In this experiment, *E. globulus* allocated a higher proportion of biomass belowground (higher R : S ratio) at low N than high N. However, there were no significant differences in allocation (no change in R : S ratio) between species proportions for either species at low N. While *E. globulus* had significantly higher leaf areas than *A. mearnsii* (2.9 and 0.9 m² respectively; Forrester 2004), *E. globulus* may have experienced less competition for light in EA than EE. In addition, the N supply for *E. globulus* appeared to be higher in EA than EE since N in aboveground biomass increased from EE to EA. The lack of change in the R:S ratio may be a combined effect of these two factors. *A. mearnsii* may have been larger in EA than AA due to reductions in competition for soil resources as evidenced by the higher N content of aboveground biomass of *A. mearnsii* in EA than AA.

Interspecific competition can be lower in mixtures than intraspecific competition in monocultures, when there is some differentiation of resource use (Kelty and Cameron, 1995; Vandermeer, 1989). Resource differentiation may have reduced the competition between *A. mearnsii* and *E. globulus* in EA such that a higher proportion of the nutrient might have been utilised in EA than AA or EE. This was quantified by measuring the total N or P in plant biomass and soil, and calculating the proportion of the sum (nutrient in plant biomass + nutrient in soil) that was in plant biomass. While not significant, the proportion of N and P in plant

biomass was higher in treatments with acacias in most cases (except at high N), and more accurate measurements (a higher number of samples per pot) may have shown significant treatment effects. This finding is supported by other studies that have shown that N-fixing species cannot only increase N resources but also increase the overall P availability. For example, soil P concentrations declined with increasing proportions of *Facaltaria moluccana* in mixture with *E. saligna* at 17 years (Binkley et al., 2000; Kaye et al., 2000) however, more P was cycled through litterfall in stands with *F. moluccana* (Binkley et al., 1992a) at age 6 years. Here, the N-fixer appeared to be capable of using P that was unavailable to *Eucalyptus* (Binkley and Ryan, 1998; Binkley et al., 2000).

Effects of species proportion at high N

At high N, *E. globulus* growth was greater in EA compared to EE, but growth of *A. mearnsii* was lower in EA than AA. As a result, EE was more productive than EA because the greater *E. globulus* growth in EA did not make up for the reduction in *A. mearnsii* growth. Also, the amount of N in total pot biomass was highest in EE and lowest in AA. So N uptake increased with aboveground biomass rather than with the proportion of *A. mearnsii*. *E. globulus* had a higher N use efficiency and showed a greater growth response to N than *A. mearnsii*. Thus *E. globulus* appeared to have a competitive advantage at high N and was able to suppress *A. mearnsii*. However, this greater competitiveness of *E. globulus* only became apparent later in the experiment. Similar patterns were found by Boyden et al. (2005) who examined the inter- and intraspecific interactions between *E. saligna* and *F. moluccana* neighbours along gradients of N and P availability in a field trial. In that experiment the growth of the N-fixing *F. moluccana* was higher with *E. saligna* neighbours at low N, but lower at high N, where the competitive influence of *E. saligna* was higher.

Effects of species proportion at high and low P

The growth response to P was less pronounced than the response to N. *A. mearnsii* growth was not significantly higher in EA than AA at either high or low P, however *E. globulus* grew larger in EA than EE at both levels of P. Furthermore the

increase in *E. globulus* growth in EA compared to EE (as shown by RY of aboveground biomass) was higher at high P than low P. Similarly, Boyden et al. (2005) found that the growth of *E. saligna* trees increased with *F. moluccana* at high P but declined at low P. Similarly, Boyden et al. (2005) found that the growth of *E. Saligna* trees increased with *F. moluccana* increased with P addition in a pot trial (Binkley et al., 2003b). Phosphorus uptake in aboveground biomass was similar in EE, EA and AA, suggesting no limitation of P in any treatment. Similarly, the proportion of plant + soil P in plant biomass did not change in EE, EA or AA showing no evidence of resource differentiation for P. Since *E. globulus* had a higher P use efficiency and a greater response to P fertiliser than *A. mearnsii*, *E. globulus* may have experienced less competition in EA than EE, especially at high P where *A. mearnsii* biomass production was lowest and *E. globulus* RY was highest.

The results also suggest that P fertiliser may have increased N availability, either by influencing N mineralisation by soil microbes or the P uptake capacity of fine roots. For example, the soil N content was lower at high P than at low P indicating that more N may have been available and used by the plants at high P when compared to low P. Increases in N mineralisation have been measured following the addition of P fertiliser to soils of several *Eucalyptus* forests (Bauhus and Khanna, 1994; Falkiner et al., 1993; Hossain et al., 1995). Alternatively, P availability might influence rates of N-fixation. For example Boyden et al. (2005) suggested that the higher growth of *E. saligna* with *F. moluccana* at high P but not at low P might have resulted from higher rates of N-fixation by *F. moluccana* at high P. Rates of N-fixation for *F. moluccana* increased with P addition in a pot trial (Binkley et al., 2003b).

Implications for mixed-species systems

The results of this experiment show that species interactions differ when there are differences in the availability of limiting resources. At all levels of N and P *E. globulus* was larger in mixture than EE and this advantage (as shown by RY) was maintained or increased from low to high N or P. Thus the positive interactions increased relative to competitive interactions for *E. globulus*

in mixtures. However, for *A. mearnsii*, growth improved in EA when compared to AA at low N only and was unchanged or reduced in EA compared to AA at high N and at both levels of P. Thus increases in N or P appeared to increase competition from *E. globulus*, which outweighed any positive effects of increased resource availability for *A. mearnsii*. Similarly, the success of certain mixed-species combinations in field trials has depended on site factors. For example the facilitative effect of higher N availability appears more likely to increase growth on sites where N is a major limiting resource (Binkley, 1992; Binkley and Green, 1983; DeBell et al., 1987; Khanna, 1997, 1998). On sites with low N availability, the N-fixing species *Facaltaria moluccana*, *Alnus rubra* and *Lupinus arboreus* increased the growth of *Eucalyptus saligna*, *Pseudotsuga menziesii* and *Pinus radiata* respectively (Beets and Madgwick, 1988; Binkley, 1983, 2003; Binkley et al., 1992b, 2003a; DeBell et al., 1987). However, the same species combinations were not successful (similar or lower productivity in mixtures than monocultures) on other sites where N availability was higher (Binkley, 1983; Binkley et al., 1992b) or the relative availability of other resources such as water and P were the major growth limiting resources (DeBell et al., 1987; Smethurst et al., 1986). When the interactions between species reduce the competition for a major growth limiting resource, mixtures are more productive than monocultures. When other factors limit growth, the different species will compete for those resources and competition will be stronger than facilitative or competitive reduction interactions.

These results emphasise the importance of appropriate site selection based on the interactions that are likely to occur in a mixture. Similar conclusions regarding the effect of site characteristics on the success of mixtures have been reached in other studies (Assmann, 1970; Boydon et al., 2005; DeBell et al., 1987; Fredericksen et al., 1993; West, 1991). In addition, it may be possible to increase the relative effect of the positive interactions in a mixture by relieving competition for resources that will not increase as a result of species interactions. For example, N-fixing species may use more P than non-N-fixing species and may compete intensely for P when mixed with a non-N-fixing species (Marschner, 1988). Therefore the competition for

P may reduce the facilitative effect of higher N availability, particularly as the stand ages and more P is stored in the biomass. The addition of P fertiliser to a mixture containing a N-fixer may therefore increase the growth of the mixture relative to a monoculture and maintain this effect as the plantation develops (Binkley et al., 1992a; Khanna, 1998). This pot trial demonstrates how an increase in the biomass production of *E. globulus* in mixture compared to monoculture can be further enhanced by adding P (as shown by RY). The additional P may not only reduce competition for P but may increase rates of N-fixation (Binkley et al., 1992a, 2003b).

It is important to note that while a pot trial enables more control over experimental conditions than field trials, there are significant differences in the temporal and spatial aspects of such studies. Thus the results of this particular species combination cannot be directly extrapolated to field conditions. However, it can be assumed that similar interactions will occur in field situations when the availability of resources changes and, interestingly, the same species combination has been successful in several field trials (Bauhus et al., 2004; Forrester et al., 2004; Khanna, 1997; Samraj et al., 1977).

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