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NITROGEN RESPONSE AND ^{15}N-LABELLED FERTILISER RECOVERY BY HOOP PINE SEEDLINGS GROWN UNDER GLASSHOUSE CONDITIONS

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BUBB, K. A., XU, Z. H., SIMPSON, J. A. & SAFFIGNA, P. G. 2001. Nitrogen response and ^{15}N-labelled fertiliser recovery by hoop pine seedlings grown under glasshouse conditions. This study was undertaken in response to the need to determine whether hoop pine (*Araucaria cunninghamii*) seedlings established on second rotation (2R) soils will benefit from nitrogen (N) fertilisation if applied in combination with a range of soil surface cover conditions. A trial was established under glasshouse conditions testing in factorial arrangement, 2 contrasting soils (low and average site productivity), 4 soil cover treatments (litter cover, cover crop consisting of kikuyu, ash cover and bare soil), and 6 N-fertiliser treatments (5 rates of inorganic-N fertiliser and a N-fixing legume). The treatments were replicated 3 times and laid out in a completely randomised factorial with 5 rates of inorganic-N fertiliser applied as ammonium sulphate at 0, 167, 333, 500 and 667 mg N kg^{-1} dry soil. The 333 mg N kg^{-1} treatment received ^{15}N-labelled ammonium sulphate. The N-fixing legume was Wynn's cassia (*Cassia rotundifolia*). The seedling growth response for the experimental period of 7 months was significantly higher on the average site productivity plantation soil than that on the low site productivity soil. There was a significant difference between the cover treatments in height and diameter at ground level (DGL) with the order of response being litter > ash = bare > cover crop. No significant growth responses by the hoop pine seedlings to the inorganic-N fertiliser treatments were observed. There was no evidence that the legume treatment had increased the soil-N status. Significant differences between the

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cover treatments with respect to the total recovery of the ^{15}N-labelled fertiliser were noted. The litter cover and bare soil treatments had the highest ^{15}N recovery from the soil–plant system of 96%, compared with 84% for the cover-crop treatment and 63% for the ash treatment. Both the legume and the kikuyu cover crop severely restricted the growth of the hoop pine seedlings. The implications of applying N fertilisers to young hoop pine seedlings at plantation establishment and potential N loss mechanisms are discussed.

Key words: *Araucaria cunninghamii* - seedling growth - nitrogen fertiliser - ^{15}N recovery - surface cover

BUBB, K. A., XU, Z. H., SIMPSON, J. A. & SAFFIGNA, P. G. 2001. Tindak balas nitrogen dan pemulihan baja berlabel ^{15}N oleh anak benih pain bergelang yang ditanam di bawah keadaan rumah kaca. Kajian ini dijalankan sebagai tindak balas kepada keperluan untuk menentukan sama ada anak benih pine bergelang (*Araucaria cunninghamii*) yang ditubuhkan di atas tanah kitaran yang kedua (2R) akan mendapat manfaat daripada penggunaan baja nitrogen (N) sekiranya ia digabungkan dengan keadaan penutup permukaan tanah. Satu percubaan ditubuhkan di bawah keadaan rumah kaca yang diuji dalam kedudukan faktoran, 2 tanah yang kontras (produktiviti tapak yang rendah dan sederhana), 4 rawatan penutup tanah (penutup sarap, tanaman tutup bumi yang mengandungi kikuyu, penutup abu dan tanah dedah) dan rawatan baja 6N (5 kadar baja tak organik N dan kekacang berikat N). Rawatan diulang sebanyak 3 kali dan diletakkan di dalam faktoran rawakan penuh dengan 5 kadar baja tak organik N yang digunakan sebagai ammonium sulfat pada 0, 167, 333, 500 dan 667 mg N kg^{-1} tanah kering. Rawatan 333 mg N kg^{1} menerima ammonium sulfat berlabel ^{15}N. Kekacang berikat-N ialah cassia Wynn (*Cassia rotundifolia*). Tindak balas pertumbuhan anak benih bagi tempoh ujian selama 7 bulan adalah lebih tinggi dengan bererti terhadap tanah ladang di tapak produktiviti sederhana berbanding dengan tanah di tapak tanah produktiviti rendah. Terdapat perbezaan yang bererti antara rawatan penutup dalam ketinggian dan garis pusat pada aras tanah (DGL) dengan turutan tindak balas iaitu sarap > debu= tanah dedah > tanaman tutup bumi. Daripada cerapan yang dibuat, tiada tindak balas pertumbuhan yang bererti oleh anak benih pain bergelang terhadap rawatan baja tak organik N. Tiada bukti bahawa rawatan kekacang telah meningkatkan status -N tanah. Perbezaan yang bererti antara rawatan penutup dengan pemulihan penuh baja berlabel ^{15}N dicatatkan. Rawatan penutup sarap dan rawatan tanah dedah mempunyai pemulihan ^{15}N yang tertinggi daripada sistem tumbuhan sebanyak 96%, berbanding dengan 84% bagi rawatan tanaman tutup bumi dan 63% bagi rawatan debu. Kedua-dua tanaman tutup bumi kekacang dan kikuyu sangat membataskan pertumbuhan anak benih pain bergelang. Implikasi penggunaan baja N terhadap anak muda pain bergelang dalam penubuhan ladang dan mekanisme kehilangan potensi N juga dibincangkan.

Introduction

The growth and development of hoop pine seedlings following plantation establishment is primarily controlled by temperature and the availability of light, water and nutrients. It is possible to minimise some of the constraints to early growth through a range of silvicultural practices such as weed control, fertilisation (organic and inorganic) and soil cover management. For instance, the practice of residue retention in southern Australian *Pinus radiata* plantations has been found beneficial to seedling growth by suppressing weed growth through reducing light, water and nutrient competition (Squire *et al.* 1985, Lehane 1995). These benefits are largely brought about through providing a physical barrier to weeds, increased infiltration rates, reduced rates of evaporation, and decreased losses of the soil nutrient reserve by erosion, as well as supplying a water and nutrient reserve and a favourable habitat for soil biota (Radwan 1992, Karlen *et al.* 1994).

Limited research has been carried out on the development of slash-retention systems suitable for routine operations in hoop pine plantations in subtropical Australia. In the past, both the physical nature of slash and the steep terrain have hindered the development of such systems. Consequently the burning of slash has been a common practice during site preparation. Primarily because of the lack of a effective slash-retention system, substantial soil erosion risks occur during the site-preparation and early-establishment phases of the hoop pine plantation. To minimise these erosion risks, cereals such as oats (*Avena sativa*), Japanese millet (*Echinochloa utilis*) and the perennial grass kikuyu (*Pennisetum clandestinum*) are sown immediately following burning (Costantini 1989). Apart from reducing soil erosion, grass cover crops established in the inter-row of young hoop pine plantations have also been found to effectively control weed development and species composition (Costantini 1989). However, a cover crop (particularly kikuyu) is highly competitive for water and nutrients. Therefore to ensure adequate seedling growth, a weed-free area is maintained along the planting zone for around two years after establishment of the hoop pine plantation. Little research has been conducted to investigate the response by hoop pine seedlings to different cover treatments such as slash retention, ash cover and cover crops. Furthermore, the aggressive nature of kikuyu suggests that it may be useful as a catch crop to reduce off-site losses either from N fertilisation or the elevated mineral-N concentrations associated with site preparation (Matson *et al.* 1987). Whilst this strategy has been widely used in agricultural production systems (Thorup-Kristensen 1994), there is a need to examine its potential use in hoop pine plantations.

Hoop pine plantations in Australia have been established on a range of soils, and consequently the physicochemical characteristics of these soils may vary widely. There is also some evidence to suggest that declines in soil fertility (particularly N) have occurred during site preparation and the early plantation establishment period (Holt & Spain 1986). There is therefore a need to investigate whether second rotation (2R) hoop pine plantations may benefit from N fertilisation at plantation establishment. As an alternative to inorganic-N fertilisers, leguminous crops have been trialed over a wide range of forestry systems (Koch 1987). Richards and Bevege (1967) found that perennial legumes stimulated the growth of a young hoop pine plantation grown on N-deficient soils. Similarly, in a study of a 2R *Pinus radiata* plantation in southern Australia established with lupins, it was found that by age 3–5 y biomass accumulation was up to double that of the control (Lehane 1995). There is clearly a need to investigate whether hoop pine seedlings grown on a range of 2R-plantation soils (particularly between marginal and typical sites) will benefit from N fertilisation. Furthermore, there is a need to investigate the interaction between surface conditions and fertiliser treatments as this interaction is often ignored by forest researchers (Radwan 1992).

A glasshouse trial was chosen for this study because it avoided the range of compounding variables associated with field trials (e.g. climatic stress and predation). The use of ^{15}N-labelled fertiliser to investigate the fate and interaction of N fertiliser applied to hoop pine seedlings under a number of surface cover treatments has not been previously reported. The objectives of this study were (1) to investigate the responses by 2-y-old hoop pine seedlings grown in soils of contrasting site productivity to a range of soil cover and N-fertiliser treatments, and (2) to determine the recovery of ^{15}N-labelled fertiliser applied to 2-y-old hoop pine seedlings on contrasting soils under different soil-cover treatments.

Materials and methods

The glasshouse trial was conducted over a period of 7 months. The glasshouse was maintained at a constant temperature of 25 °C; humidity was not controlled. Closed pots consisting of standard horticultural polythene pots (19-cm diameter, 18-cm height) with accompanying drainage dishes were used. Two-year-old nursery hoop pine seedlings which are routinely used to establish hoop pine plantations were used for this experiment. They were chosen at random from 2-y-old genetically improved stock (seed-orchard grade seed) representing a batch of the median height class (32.5 cm).

Experimental design

The experiment was a complete factorial design testing 2 contrasting soils, 4 soil cover treatments and 6 N-fertiliser treatments with 3 replicates, to give a total of 144 pots. In conjunction with one N-fertiliser treatment, ^{15}N-labelled fertiliser was used in order to estimate fertiliser N recovery. Two contrasting soils, namely a Lithosol (FAO) from a plantation site with low site productivity, and a Ferralsol from a plantation site with an average site productivity, were obtained from Brooloo State Forest (26° 31'S, 152° 36'E) approximately 150 km northwest of Brisbane, Queensland, Australia. Soil was collected from points along a 300-m transect down a typical slope in each hoop pine plantation; soil samples were taken from a depth of 10 cm and then sieved (2-mm mesh). Subsamples were taken to determine moisture content and a range of chemical characteristics (Table 1). Pots were filled with an equivalent of 3 kg of oven-dry (105 °C) soil and a tubed seedling (with the original tubing soil removed) was planted in the centre of each plot.

Table 1. Chemical properties of hoop pine seedling potting soils

Soil type	Total N (%)	Total P (mg kg^{-1})	Total K (mg kg^{-1})	Organic C (%)	pH	CEC (cmol kg^{-1})
Ferralsol	0.33	704	5133	3.8	6.8	28.4
Lithosol	0.17	479	1953	2.2	5.5	16.1

A number of cover treatments which simulated routine site conditions were applied to the surfaces of the appropriate pots. These were litter, ash and bare cover, and a cover crop. The litter treatment consisted of 60 g (oven-dry equivalent) of hoop pine litter which was placed evenly over the pot surface about the seedling. The fresh litter (consisting of final-order branches together with attached needles) was obtained from a mature 62-y-old hoop pine plantation adjacent to the area from which the potting soils were obtained. This litter mass was representative of the density found in the mature hoop pine plantation. Hoop pine foliage litter was used in preference to other forms of litter (e.g. branches, bark, stem wood and understorey material) because of its homogeneous nature and its importance in nutrient cycling. Thus, any nutrient contribution or immobilisation from subsequent litter decomposition was assumed to be constant across the treatment. The ash treatment consisted of 100 g of ash which was evenly spread across the surface of the pot (approximately 2 cm depth) about the seedling. The ash was collected from a 2R plantation area which was recently burnt for site preparation. Previous chemical analysis of ash (unpublished) revealed the following nutrient concentrations (%): total N (0.15); total P (0.77); total K (5.24); Ca (22.4); Mg (2.74); Mn (0.36); B (0.0016); Zn (0.04); Cu (0.0066). The pasture grass kikuyu (*Pennisetum clandestinum*) was established as the cover crop. Ten kikuyu seeds were planted one month prior to commencement of the study in each of the cover-crop pots and generally good germination occurred. Although there was some variation in the number of grass seedlings this was not reflected in the overall biomass of the grass sward. The fourth cover treatment was bare cover, where a seedling was transplanted into a pot containing only soil. During the course of the study the bare soil surface was maintained by regular manual weed tending.

The 6 N treatments consisted of 5 rates of ammonium sulphate (0, 167, 333, 500 and 667 mg N kg^{-1} dry soil) and an organic N treatment. Ammonium sulphate was applied in a 100 mL aqueous solution as 2 equal dressings at commencement of (October 1993), and midway through (January 1994) the experiment. The treatment with 333 mg N kg^{-1} dry soil received ammonium sulphate with 10.1 atom % ^{15}N excess to determine N fertiliser recovery at completion of the experiment. The ^{15}N-labelled fertiliser was first dissolved in 100 mL of distilled water and then applied evenly across the surface of the pot using a graduated pipette. The N-fixing legume Wynn's cassia (*Cassia rotundifolia*) was used as the organic N-fertiliser treatment. This legume species is widely used by local pastoralists for improving soil N fertility. Prior to planting, legume seeds were treated with a commercial inoculant. Following this, and one month prior to commencement of the study, 10 seeds were planted in each of the organic-N-fertiliser pots. Good germination occurred and no thinning was required.

There is evidence to suggest that applications of N-only fertiliser may cause deficiencies in the availability of other essential elements (Binkley 1986). In order to safeguard against this, all pots received a single basal dressing of P, K, Cu, Zn, B and Mo at rates equivalent to 113, 47, 4.7, 4.7, 4.7 and 0.1 mg kg^{-1} dry soil respectively. The P fertiliser was applied as triple superphosphate (solid), while all other elements were applied as a single aqueous solution derived from KCl, $CuSO_4.H_2O$, $ZnSO_4.5H_2O$, H_3BO_3, and $Na_2MoO_4.H_2O$.

Maintenance

The pots were arranged within the glasshouse on 1 m high benches with separate areas allocated for each block; within each block the treatments were completely randomised. Adequate spacing was provided to the pots to prevent shading effects. The spacing distance was increased progressively in line with plant growth. Glasshouses have a degree of spatial variability with respect to light intensity, temperature and humidity. Consequently, the blocks were routinely rotated (monthly) within the glasshouse to minimise these effects. A watering regime was designed to ensure that during the course of the experiment the moisture content of the potting soil remained between 50 and 100% of the available water range. Prescriptions were amended regularly to respond to variations in water demand caused by changes in seasonal and climatic conditions as well as plant growth. Pots were generally watered (with de-ionised water) every 2–3 days, except in mid-summer when watering was carried out daily. Excess drainage water which collected in the dish beneath each pot was recirculated so that N losses due to leaching could be assumed to be zero.

Growth measurements and harvest techniques

Hoop pine seedling height was measured from the cotyledon scar, and diameter at ground level (DGL) immediately above this mark. The cotyledon scar was used as a reference to avoid any discrepancies that might have occurred in the event of soil expansion or compaction, or planting depth differences. Both the height and DGL increments were determined from measurements taken at the start and completion of the experiment. At completion of the experiment, the plants in each pot were destructively harvested. Biomass of hoop pine seedlings was determined for foliage (branches and needles), stem and roots. These components were subsequently oven-dried and a dry mass (70 °C) determined. The above- and below-ground biomass (70 °C) was also determined for the cover and legume crops. During the period of the experiment foliage litterfall was collected, frozen stored and the mass reconciled with the corresponding pots. A representative sample (300 g) of the potting soil (^{15}N-treatment only) was taken, sieved to remove roots and air-dried prior to chemical analysis. Both the plant biomass and soil material used to determine the background ^{15}N abundance were obtained from the nil N-treatment pots. The plant biomass samples required for chemical analysis were first ground in a rotary mill (0.5-mm-mesh sieve) followed by a planetary cylinder mill.

Chemical analysis

Soil and plant analysis for total N and ^{15}N enrichment was carried out a Europa Scientific Tracermass (9001) mass spectrometer coupled with a Roboprep Sampler Convertor (7001). Soil total K, pH (1:5 H_2O) and cation exchange capacity (CEC) were determined by the methods of Rayment and Higginson (1992). Soil total P was determined colorimetrically by the molybdenum blue method following extraction with boiling HCl (20 %, w/w) as described previously by Xu *et al.* (1995). Soil organic C was determined by the method of Walkley and Black (1934).

Results

Hoop pine seedling growth

Statistical analysis using ANOVA revealed significant treatment effects from soil, cover, N-fertiliser and the interaction between cover and N-fertiliser (Tables 2 and 3). These results indicated that growth response was significantly higher on the Ferralsol soil than on the Lithosol. With respect to height and DGL increments, the order of response to cover treatments was litter > ash = bare > kikuyu. However there were no significant differences in total biomass between the litter, ash and bare cover treatments. With the cover crop of kikuyu there were no significant responses by hoop pine seedlings to the inorganic-N fertilisers. As with the kikuyu cover crop, the legume treatment severely restricted hoop pine seedling growth and development. Furthermore, the significant interaction between the cover and N-fertiliser treatments was due largely to the competitive effects of the kikuyu and legume treatments (Table 4).

Table 2. Analysis of variance for hoop pine seedling growth seven months after planting

		Mean square			*F*-value		
Source of variation†	df	Height increment	DGL increment	Total biomass	Height increment‡	DGL increment	Total biomass
Rep	2	0.0183	0.902	170.53	3.16 *	2.97 ns	0.73 ns
Soil	1	0.0798	11.839	1649.71	13.78 **	38.93 **	7.10 **
Cover	3	0.3976	26.304	9691.94	68.64 **	86.49 **	41.70 **
Fertiliser	5	0.1482	16.416	3791.84	25.59 **	53.98 **	16.31 **
S × C	3	0.0148	0.144	1253.05	2.55 ns	0.47 ns	5.39 ns
S × F	5	0.0109	0.724	448.64	1.88 ns	2.38 *	1.93 ns
C × F	15	0.0295	2.265	687.99	5.10 **	7.45 **	2.96 **
S × C × F	15	0.0035	0.412	170.95	0.61 ns	1.36 ns	0.74 ns
Error	94	0.0058	0.304	232.44			
Total	143						

† S = soil treatment. C = cover treatment. F = N fertiliser treatment.

‡ "**", "*", and "ns" indicate significance at the 0.01 and 0.05 levels of probability and not significant at the 0.05 level of probability respectively.

Table 3. The main effects of soil, surface cover and N fertiliser treatments on the growth of hoop pine seedlings seven months after planting

Treatment		Height increment (m)	DGL increment (mm)	Total biomass (g)
Soil:	Ferralsol	0.20a	2.72a	49.0a
	Lithosol	0.16b	2.14b	42.2b
Cover:	Litter	0.28a	3.15a	53.6a
	Kikuyu	0.03c	1.19c	21.1b
	Ash	0.19b	2.72b	51.6a
	Bare	0.22b	2.66b	56.0a
N-fertiliser:	Legume	0.02b	0.78c	21.4b
	N_0	0.19a	2.65b	48.8a
	N_{167}	0.23a	2.90ab	53.8a
	N_{333}	0.23a	3.00a	56.8a
	N_{500}	0.21a	2.71ab	46.3a
	N_{667}	0.20a	2.55b	46.4a
LSD ($p < 0.05$)				
Soil treatment		0.03	0.19	6.1
Cover treatment		0.04	0.26	8.7
N-fertiliser treatment		0.04	0.32	10.6

Note: Means in each treatment followed by the same letter are not significantly different from each other, ($p < 0.05$) by Duncan's multiple range test.

Table 4. The interaction between surface cover and N-fertiliser treatments on the growth of hoop pine seedlings seven months after planting

	Cover			
Fertiliser	Litter	Kikuyu	Ash	Bare
		Height inc. (m)		
Legume	0.00	0.03	0.03	0.02
N_0	0.31	0.05	0.22	0.19
N_{167}	0.39	0.02	0.20	0.30
N_{333}	0.40	0.03	0.31	0.20
N_{500}	0.31	0.02	0.30	0.19
N_{667}	0.27	0.05	0.25	0.26
		LSD ($p<0.05$) = 0.13		
		DGL inc. (mm)		
Legume	0.31	1.08	0.85	0.87
N_0	3.22	1.61	2.91	2.86
N_{167}	4.01	1.43	2.83	3.31
N_{333}	4.11	1.38	3.33	3.17
N_{500}	3.88	0.76	3.34	2.86
N_{667}	3.37	0.89	3.06	2.86
		LSD ($p<0.05$) = 0.92		
		Total biomass (g)		
Legume	20.9	24.4	22.6	17.8
N_0	63.4	23.7	53.3	54.6
N_{167}	62.1	21.1	57.7	74.4
N_{333}	71.5	20.7	60.5	74.6
N_{500}	49.3	16.5	56.4	63.2
N_{667}	54.5	20.4	59.1	51.5
		LSD ($p<0.05$) = 21.2		

Fate of ^{15}N-labelled fertiliser

Statistical analysis using ANOVA revealed several significant relationships between the ^{15}N recovery and the soil and cover treatments (Table 5). Whilst the total ^{15}N recovered in the soil treatments were similar, the ^{15}N-labelled fertiliser recovered by the hoop pine seedlings was significantly higher on the Lithosol (mean 42.2%) than on the Ferralsol (mean 33.8%). The effect of the cover treatments was significant with the highest ^{15}N recovery occurring in the litter and bare cover (means 96.0% and 96.6% respectively), followed by kikuyu (mean 82.4%) and ash (mean 62.6%). The ^{15}N recovered in the plant biomass of the cover crop treatment was principally partitioned into the kikuyu.

Table 5. ^{15}N recovery (%) in the plants and potting soils from applications of ^{15}N-labelled ammonium sulphate at 333 mg N kg^{-1} dry soil

Treatment		Hoop pine biomass	Kikuyu biomass	Soil	Total recovery	Deficit (%)	Suspected loss mechanism
Soil	Cover						
Ferralsol							
	Litter	56.1	-	39.2	95.3 (3.4)	4.7	
	Kikuyu	2.9	62.6	15.1	80.6 (5.3)	19.4	Denitrification
	Ash	36.6	-	25.5	62.1 (10.5)	37.9	Ammonia volatilisation
	Bare	39.5	-	57.9	97.4 (5.4)	2.6	
Lithosol							
	Litter	73.6	-	23.0	96.6 (3.9)	3.4	
	Kikuyu	5.1	58.8	20.2	84.1 (10.3)	25.9	Denitrification
	Ash	41.4	-	21.7	63.1 (7.2)	36.9	Ammonia volatilisation
	Bare	48.8	-	46.9	95.7 (3.0)	4.3	
LSD ($p < 0.05$)							
Soil		*6.7*		*5.9*	*ns*		
Cover		*9.5*		*8.4*	*12.5*		

Note: Coefficient of variation (%) in parentheses; "ns" indicates not significant.

Discussion

Despite additions of the essential macro and trace elements, the significantly higher growth response on the Ferralsol suggests that the productivity potential of the low fertility soil during the early establishment phase may be governed by factors other than nutrient deficiencies. Under the experimental conditions the noted differences in the soil properties were organic carbon (OC), CEC and pH. Importantly, OC is a major regulator of nutrient supply through biological mineralisation, and a significant contributor to CEC. The lower soil pH in the Lithosol may also limit nutrient supply through lower rates of nitrification (Bauhus *et al.* 1993). Although both of these factors may have been responsible for the growth responses noted, further research is required to confirm this or alternatively identify other factors which may have been involved. The results suggest that additions of inorganic-N fertilisers at establishment will not directly benefit hoop pine growth on either low- or average-fertility sites, although, the preparation of the potting soils (e.g. sieving) may have stimulated N mineralisation rates above the ambient rates of *in situ* soil profiles. However, these rates in the field situation are also likely to be elevated from the effects of disturbance brought about by site preparation (Smethurst & Nambiar 1990). Because N availability was not measured during this experiment some care is required in interpreting these results.

The significant and positive height and DGL response to litter cover suggests that this treatment is likely to be beneficial to hoop pine seedling growth in relation to the other cover treatments tested. The reasons for these observations are not fully understood; however, it is suggested that the litter cover provided a more constant soil environment which led to a greater nutrient supply. For instance, based on the watering prescriptions formulated throughout the study the trend in plant water use was legume = kikuyu > ash = bare > litter (data not presented). This suggested that the fluctuation in water content may have been lowest for the litter treatment. Shorter wetting and drying cycles have been associated with accelerated nutrient mineralisation (Hallsby 1995). The presence of litter might also have enhanced the supply of nutrients to roots through increased microbial and mycorrhizal activity and indirectly through litter decomposition. However, the latter is unlikely as data presented by Bubb *et al.* (1998a) suggested that the fresh litter was more likely to have immobilised N. Also the insulating effects of the litter cover should have reduced both the diurnal fluctuation in maximum and minimum soil temperature. In contrast to the litter treatment, the lack of a significant response to the ash treatment indicated that surface ash was not beneficial to seedlings during the initial establishment period. However, indirect effects (e.g. soil sterilisation), which were not examined in this study, should not be disregarded when extrapolating these responses to field environments.

The negative seedling growth response to the legume treatment and the increased plant water use of this treatment suggested that the legume was as highly competitive for nutrients and water as kikuyu. Consequently, the use of this legume during the field establishment phase would require that a legume-free zone about the tree be maintained in order to minimise these adverse competition effects. The demonstrated competitive nature of both cover species indicated that they could be useful as catch crops to reduce off-site losses of native or applied mineral-N.

Although the direct contribution to the soil-N reserve by the legume was not determined, there was some qualitative evidence to suggest that it was low. For instance, the pale colour of the nodules reflected poor levels of leghaemoglobin which, according to Galston *et al.* (1980), infers a low level of N-fixing activity. Furthermore, it is widely recognised that abundant levels of mineral-N inhibit nodulation and nodule activity. Soil mineral-N concentrations higher than 10 mg kg^{-1} have been reported to cause a marked reduction in nodulation (MacDicken 1994). The study by Bubb *et al.* (1998b) indicated that the ambient soil mineral-N concentrations in the surface soil (0–10 cm) at typical plantation sites greatly exceeded this level. Subsequent analysis by the natural ^{15}N abundance method also suggested that the N uptake by the legume was solely from the potting soil. The mean natural ^{15}N abundance of the legume foliage grown in the Ferralsol (10.9%) and the Lithosol (9.4%) were not statistically different ($p < 0.05$) from those of the reference foliage (hoop pine seedlings in nil N treatments) at 10.6 and 9.0% respectively. However, this experiment was not designed specifically to accommodate the variability commonly associated with this technique (Shearer & Kohl 1993), and therefore the results are not entirely conclusive. Overall, the preliminary assessment of the N-fixation benefits from the legume crop was discouraging, although it is worth bearing in mind that quantitative data relating to N-fixing plants in forestry are often difficult to secure.

The recovery of ^{15}N-labelled fertiliser is considerably higher than that typically reported from field experiments in forestry systems (i.e. < 30%) (Thomas & Mead 1992), although high ^{15}N recoveries (> 75%) such as these have been reported from a number of glasshouse experiments (Binkley 1986). The significant difference between the two soils in uptake of ^{15}N-labelled fertiliser by the hoop pine seedlings is evidence that different levels of added N interaction (pool substitution) existed. Powlson and Barraclough (1993) have described added N interaction as the labelled mineral-N from fertiliser taking the place of unlabelled mineral-N that would otherwise be immobilised. The difference in the rate of added N interaction demonstrates that care must be taken when interpreting the ^{15}N recovery within the individual phases of the plant-soil system. Because no response to inorganic-N fertiliser was observed, a "real" added nitrogen interaction cannot be inferred in this instance.

The reasons for the significant differences in ^{15}N recovery due to cover treatments cannot be directly explained. However, there is some evidence to suggest that the unexplained N losses incurred by the ash-cover and cover-crop treatments may be associated with ammonia volatilisation and denitrification respectively. At the completion of the experiment the ash-cover treatment had increased the soil pH from 6.8 to 8.2 for the Ferralsol and from 5.5 to 8.0 for the Lithosol. The ammonium–ammonia equilibrium constant (K_b), indicates the percentages of ammonia present in aqueous solutions at pH 6, 7, 8 and 9 are approximately 0.1, 1, 10 and 50% respectively (Freney 1980). This suggests that the increased soil pH would have resulted in higher ammonia concentration being present in the soil water. Additionally, the plant water use indicated that a significant difference in vapour pressure existed between the soil water and air. The combination of these factors would have substantially increased the potential for N losses by ammonia volatilisation (Freney *et al.* 1981, Patra *et al.* 1992). Whilst this explanation needs to be confirmed by direct experimental evidence, these results suggest that applying N-fertilisers to hoop pine plantations at establishment, where surface ash is prominent, may lead to significant N losses through ammonia volatilisation.

Although N losses through volatilisation from the large canopy of the kikuyu sward cannot be altogether disregarded, the observed N losses by the cover treatment may have been principally linked with denitrification. Research on forest and agricultural systems has identified anaerobic conditions and the availability of nitrate and soluble C as the major factors affecting denitrification (Ineson *et al.* 1991, Willison & Anderson 1991). The results presented by Bubb *et al.* (1998b) demonstrated that nitrification was an important process which readily occurred in hoop pine plantation soils. Also, the high root density and subsequent fine root turnover from the kikuyu sward were likely to have been a reliable source of C substrate for denitrifying bacteria. Anaerobic conditions may have prevailed on a number of occasions in the pots of cover-crop treatments when over-watering occurred. This would have been brought about when changes to the weather pattern lowered plant water use substantially below that calculated from the preceding period. As a result, potting soils were maintained at field capacity for an extended period. During these periods, conditions probably existed which would have bolstered denitrification. Whilst the extent that anaerobic conditions occur in hoop pine plantations is largely unknown, these results suggest that denitrification should not be ignored as a potential N loss mechanism. Despite the losses of ^{15}N-labelled fertiliser from the cover-crop treatment, the results suggest that kikuyu is a suitable catch crop for retention of N. Furthermore, this type of experiment would be useful in ranking potential candidate catch-crop species.

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