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Effects of nitrogen fertilization on soil nitrogen pools and microbial properties in a hoop pine (*Araucaria cunninghamii*) plantation in southeast Queensland, Australia

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Abstract A field study was conducted to investigate the effects of N fertilization on soil N pools and associated microbial properties in a 13-year-old hoop pine (*Araucaria cunninghamii*) plantation of southeast Queensland, Australia. The treatments included: (1) control (without N application); (2) 300 kg N ha^{-1} applied as NH_4NO_3; and (3) 600 kg N ha^{-1} as NH_4NO_3. The experiment employed a randomized complete block design with four replicates. Soil samples were taken approximately 5 years after the N application. The results showed that application of 600 kg N ha^{-1} significantly increased concentrations of NH_4^+-N in 0–10 cm soil compared with the control and application of 300 kg N ha^{-1}. Concentrations of NO_3^--N in soil (both 0–10 cm and 10–20 cm) with an application rate of 600 kg N ha^{-1} were significantly higher compared with the control. Application of 600 kg N ha^{-1} significantly increased gross N mineralization and immobilization rates (0–10 cm soil) determined by ^{15}N isotope dilution techniques under anaerobic incubation, compared with the control. However, N application did not significantly affect the concentrations of soil total C and total N. N application appeared to decrease microbial biomass C and N and respiration, and to increase the metabolic quotient (qCO_2) in 0–10 cm soil, but these effects were not statistically significant. The lack of statistical significance in these microbial properties between the treatments might have been associated with large spatial variability between the replicate plots at this experimental site. Spatial variability in soil microbial biomass C and N was found to relate to soil moisture, total C and total N.

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Introduction

Hoop pine (*Araucaria cunninghamii*) is a native rainforest species of southeast Queensland, Australia, with a long-standing reputation for the quality of its timber. Hoop pine plantations cover 50,000 ha, accounting for one-quarter of the total plantation area in Queensland (DPI Forestry 1998). Current forest management practices in these plantations can have significant impacts on soil fertility and plantation sustainability (Xu et al. 1999; Mathers et al. 2002). For example, burning of debris after clearcut harvesting of hoop pine plantation leads to the loss of soil organic matter and associated nutrients (e.g. N), and then soil degradation. Hoop pine is a highly N-demanding species and N fertilizer is normally required for successful establishment of the second rotation plantation. It has been found that N fertilization significantly improved stand N nutrition and enhanced tree growth of hoop pine (Xu et al. 2000). However, there is little information available on the effects of N fertilization on soil N pools and associated microbial processes in hoop pine plantations, particularly on the second rotation sites. Soil microorganisms play a critical role in soil processes such as nutrient cycling and decomposition of organic matter that influence soil quality and plant productivity. Soil microbial biomass comprises 1–5% of total soil organic matter (e.g. Smith and Paul 1990), but represents the most labile pools of nutrients in soil. Soil microbial biomass and microbial activity have been proposed to be potential indicators of effects of forest management on soil organic matter quality (Bauhus et al. 1998). The main objective of this study was to investigate the effects of N application rates on soil N availability, N mineralization and immobilization processes, and the associated microbial properties.

Table 1 Selected soil properties at the Imbil site, southeast Queensland, Australia (means of data of four soil profiles, with one soil profile sampled from each of the four experimental blocks). Data *in parentheses* are SEMs (n=4)

Soil properties	Soil depth (cm)	
	0–10	10–20
pH (1:5 H_2O)	6.69 (0.14)	6.16 (0.17)
Organic C (%)	5.48 (2.71)	2.69 (1.16)
Total N (%)	0.304 (0.101)	0.180 (0.020)
Total P (mg kg^{-1})	556 (64)	434 (27)
Total K (mg kg^{-1})	4190 (1510)	3885 (1376)
Available P (mg kg^{-1})	15.5 (15.5)	11.6 (9.9)
Exchangeable K (cmol kg^{-1})	0.54 (0.10)	0.44 (0.13)
Exchangeable Ca (cmol kg^{-1})	7.03 (3.12)	3.45 (1.21)
Exchangeable Mg (cmol kg^{-1})	2.13 (0.02)	1.42 (0.29)
Exchangeable Mn (cmol kg^{-1})	0.085 (0.017)	0.075 (0.035)
Cation exchange capacity (cmol kg^{-1})	33.2 (7.4)	23.2 (3.2)
EDTA extractable Cu (mg kg^{-1})	7.7 (4.6)	7.4 (4.9)
EDTA extractable Zn (mg kg^{-1})	15.3 (8.7)	8.1 (7.3)
Electrical conductivity (dS m^{-1})	0.134 (0.052)	0.079 (0.039)
Sand (%)	63.0 (4.1)	57.0 (0.8)
Silt (%)	28.8 (3.3)	32.8 (1.7)
Clay (%)	8.2 (2.1)	10.2 (1.5)

Materials and methods

Experimental site and soil sampling

The experimental site was located in a second rotation hoop pine plantation, at Imbil of southeast Queensland (26°28′S, 152°37′E), Australia, and established in December 1988 at a stocking density of 821 stems ha^{-1}. The soil is a yellow earth (Lithosol, FAO) and its major chemical and physical properties are presented in Table 1. The experiment was originally intended to investigate the impacts of N application on stand N nutrition and tree growth, and employed a randomized complete block design with four replicates for each of five treatments: (1) control (without any fertilizer applied); (2) 100 kg N ha^{-1}; (3) 300 kg N ha^{-1}; (4) 300 kg N ha^{-1}+60 kg P ha^{-1}+50 kg K ha^{-1}; and (5) 600 kg N ha^{-1}. Each gross plot consisted of 112 trees (8 rows×14 trees), occupying 0.141 ha (42 m×33.6 m). N fertilizer (NH_4NO_3) was broadcast in November 1996 through gross plots to simulate the aerial application. For the purpose of this study, only three treatments (1, 3 and 5) were selected for soil sampling. Five soil cores (6 cm in diameter) at the depths of 0–10 and 10–20 cm were randomly taken from each plot in July 2001 and bulked. Field moist soil samples were passed through a 2-mm sieve and stored at 4°C prior to analysis. A subsample of each soil was air-dried and ground (<150 μm) prior to determination of total C and total N. Analyses of all microbial properties and exchangeable NH_4^+-N, NO_3^--N and NO_2^--N concentrations were carried out on the fresh soils and results were expressed on an oven-dry soil basis.

Soil analyses

Soil total C and total N were analysed using an isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000). The concentrations of exchangeable NH_4^+-N, NO_3^--N and NO_2^--N were measured using a Lachat Quickchem automated ion analyser (QuikChem method 10–107–064-D for NH_4^+ and 10107–04–1-H for NO_3^-/NO_2^-). Total mineral N was defined as the sum of the amount of exchangeable NH_4^+-N, NO_3^--N and NO_2^--N in soil.

Soil net N mineralization in 0–10 cm soil was determined by a 7-day anaerobic incubation under laboratory conditions as described by Waring and Bremner (1964). In brief, four portions of the field moist soils (5 g dry-weight equivalent) were weighed into 50-ml polypropylene falcon tubes to each of which 25 ml of ^{15}N-labelled $(NH_4)_2SO_4$ solution (50.9 μg N with 10.3 atom% ^{15}N) was added. Two of these tubes were swirled and the soil extracted with 25 ml of 4 M KCl 0.5 h after the ^{15}N addition, shaken for 1 h, centrifuged at 2,950 *g* for 10 min and filtered through Whatman no. 42 filter paper. The remaining two tubes were swirled, capped and sealed tightly, and then incubated at 40°C for 7 days. After 7 days of incubation, the soil-solution mixture was also extracted with 25 ml of 4 M KCl, as described above. Exchangeable NH_4^+-N in extracts (from both before and after incubation) was determined using a Lachat Quickchem automated ion analyser (see above). Soil gross N mineralization and immobilization rates in the 0–10 cm soil were measured using ^{15}N isotope dilution techniques (Hart et al. 1994). For determination of $^{15}NH_4$-N, the extracts were spiked with a known NH_4^+-N standard solution and steam-distilled (Keeney and Nelson 1982). The distillates were transferred into tin capsules and dried at 65°C, and $^{15}NH_4^+$-N was determined using an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000). The equations of Kirkham and Bartholomew (1954) were used to calculate the gross N mineralization and immobilization rates.

Soil microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation-extraction method using an *E*C factor of 2.64 (Vance et al 1987) and an *E*N factor of 2.22 (Brookes et al. 1985; Jenkinson 1988). Soluble organic C in the fumigated and unfumigated soil samples was determined using a wet oxidation TOC analyser (model 1010; OI Analytical, Tex.). Total soluble N in the fumigated and unfumigated soil samples was converted into NO_3^- using the persulphate oxidation method (Cabrera and Beare 1993) and determined by a Lachat Quickchem automated ion analyser (see above). Soil respiration was measured using the methods described by Bartha and Pramer (1965). The field moist soils (20 g oven-dry equivalent) were aerobically incubated at 22°C in a 1-l sealed glass jar and CO_2 evolved from soil was trapped in 0.1 M NaOH after 1 h, 5 h, 24 h, 3 days, 7 days, 21 days and 28 days of incubation; the residual NaOH was titrated with 0.05 M HCl to the phenolphthalein endpoint. CO_2 evolved was calculated from the difference in normality between NaOH blanks and samples. The metabolic quotient (qCO_2) was calculated as the ratio of microbial respired C (μg g^{-1} over the 28 days) to MBC. The analytical methods for other soil properties were reported by Xu et al. (1995).

Statistical analysis

One-way ANOVA with blocking was carried out for all data on soil properties in Statistix for Windows version 2.2 and least significant difference (P<0.05) was used to separate the means when differences were significant. Pearson linear correlations between the soil properties, and regression analyses between the N application rate and total soil mineral N were also conducted in Statistix for Windows version 2.2.

Table 2 Effects of N fertilization on soil pH, total C and N pools in a hoop pine plantation at the Imbil site, southeast Queensland. Data are means (n=4); data *in parentheses* are SEMs. Means *within a column* followed by the *same letter* are not different at the 5% level of significance

N rate (kg ha^{-1})	pH	Total C (%)	Total N (%)	NH_4^+-N (μg g^{-1})	NO_3^--N (μg g^{-1})
	0–10 cm				
0	6.53a (0.10)	4.54a (0.36)	0.293a (0.018)	0.56b (0.08)	5.05b (0.93)
300	5.98b (0.20)	4.55a (0.58)	0.277a (0.034)	0.89b (0.29)	6.89ab (1.33)
600	6.14ab (0.24)	4.94a (0.36)	0.301a (0.028)	2.98a (0.81)	15.36a (4.51)
	10–20 cm				
0	5.95a (0.07)	2.70a (0.40)	0.183a (0.041)	5.08a (0.86)	2.79b (0.55)
300	5.65a (0.27)	2.97a (0.20)	0.188a (0.033)	5.54a (0.48)	5.10ab (1.50)
600	6.04a (0.35)	3.65a (0.59)	0.224a (0.050)	7.08a (1.21)	10.42a (3.15)

Results

Soil pH, total C and N pools

The soil pH value was significantly lower in 0–10 cm soil with an N application rate of 300 kg N ha^{-1} compared with the control (Table 2), while no significant differences were observed in pH values in 10–20 cm soil between the N application rates. There were no significant differences in soil total C and total N contents between the N application rates (Table 2). However, compared with the control and application of 300 kg N ha^{-1}, application of 600 kg N ha^{-1} significantly increased concentrations of NH_4^+-N in 0–10 cm soil; the same trend in concentrations of NH_4^+-N was observed in 10–20 cm soil although differences were not significant (Table 2). Concentrations of NO_3^--N of both 0–10 cm and 10–20 cm soils with an application rate of 600 kg N ha^{-1} were significantly higher than those of the control. The amounts of NO_2^--N (data not presented here) were low and not measurable while NO_3^--N was the predominant form of mineral N in 0–10 cm soil (Table 2). Concentrations of total mineral N (the sum of NH_4^+-N, NO_3^--N and NO_2^--N) increased significantly with N application rate (Fig. 1). Interestingly, in this study concentrations of NH_4^+-N were lower in topsoil (0–10 cm) than those in subsoil (10–20 cm) while concentrations of NO_3^--N were higher in topsoil than those in subsoil (Table 2).

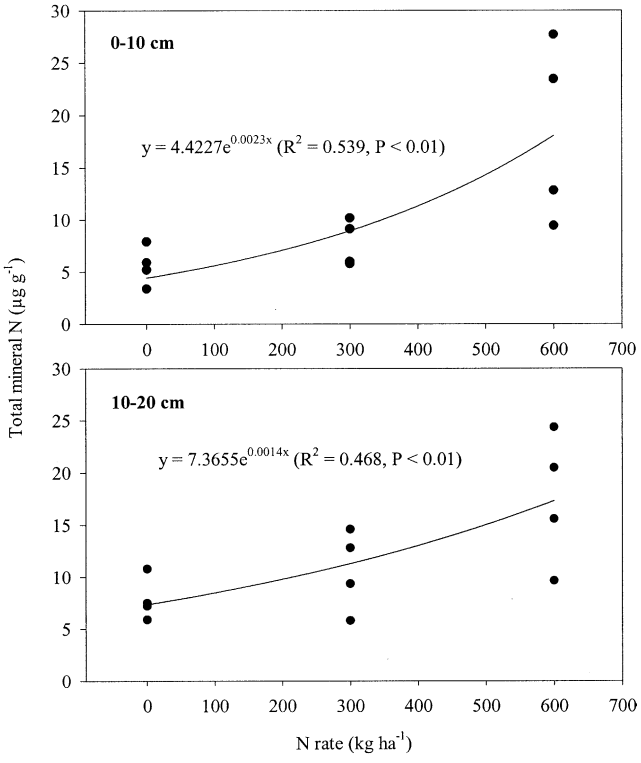

Fig. 1 Effects of N fertilization on soil total mineral N 5 years after N application in a hoop pine plantation at the Imbil site, southeast Queensland

Soil N mineralization

N application did not significantly affect net N mineralization in 0–10 cm soil although the general trend of net N mineralization rates appeared to increase with N application rate (Table 3). Net N mineralization consisted of 2.32–2.76% of total soil N (0–10 cm soil). On the other hand, gross N mineralization significantly increased with N application rates, while gross N immobilization rates were also significantly greater in the soil with an application rate of 600 kg N ha^{-1} compared with the control and the application rate of 300 kg N ha^{-1} (Table 3). The gross N mineralization rate accounted for >3% of total soil N while the gross N immobilization rate represented <1% of total soil N (Table 3).

MBC and MBN

Across the different N application treatments (and replicates), the MBC content ranged from 454 to 1,163 μg g^{-1} (0–10 cm soil) and from 214 to 852 μg g^{-1} (10–20 cm soil). Likewise, the MBN content ranged from 58 to 198 μg g^{-1} (0–10 cm soil) and from 37 to 100 μg g^{-1} (10–20 cm soil). MBC accounted for

Table 3 Effects of N fertilization on soil N mineralization and immobilization rates (0–10 cm) in a hoop pine plantation at the Imbil site, southeast Queensland. Data are means (n=4); data *in parentheses* are SEMs. Means *within a column* followed by the *same letter* are not different at the 5% level of significance. m_n Net N mineralization rate, m_g gross N mineralization rate, i_g gross N immobilization rate

N rate (kg ha^{-1})	m_n (mg kg^{-1})	m_n:total N ratio (%)	m_g (mg kg^{-1})	m_g:total N ratio (%)	i_g (mg kg^{-1})	i_g:total N ratio (%)
0	68.5a (6.0)	2.32a (0.07)	89.8b (4.1)	3.06b (0.07)	22.2b (1.0)	0.76b (0.02)
300	76.8a (14.8)	2.76a (0.34)	95.8ab (17.1)	3.44ab (0.34)	19.5b (2.4)	0.71b (0.02)
600	77.1a (5.5)	2.57a (0.13)	114.5a (4.3)	3.86a (0.30)	27.2a (3.1)	0.90a (0.04)

Table 4 Effects of N fertilization on soil microbial properties in a hoop pine plantation at the Imbil site, southeast Queensland. Data are means (n=4); data *in parentheses* are SEMs. Means *within a column* followed by the *same letter* are not different at the 5% level of significance. *MBC* Microbial biomass C, *MBN* microbial biomass N

N rate (kg ha^{-1})	MBC (μg g^{-1})	MBN (μg g^{-1})	Microbial C:N ratio	MBC:total C ratio (%)	MBN:total N ratio (%)	Respiration (μg CO_2-C g^{-1} h^{-1})	Metabolic quotient (μg CO_2-C mg^{-1} microbial C h^{-1})
	0–10 cm						
0	924.5a (71.4)	155.7a (27.6)	6.58a (1.25)	2.05a (0.14)	5.21a (0.78)	0.520a (0.048)	0.575a (0.048)
300	718.4a (148.6)	97.5a (20.4)	7.40a (0.29)	1.54a (0.17)	3.42a (0.29)	0.425a (0.048)	0.675a (0.155)
600	724.9a (153.3)	118.1a (21.4)	6.28a (0.89)	1.44a (0.22)	3.81a (0.34)	0.450a (0.065)	0.675a (0.206)
	10–20 cm						
0	374.7a (67.9)	74.6a (10.3)	5.03a (0.54)	1.42a (0.19)	4.13a (0.57)	0.375ab (0.048)	1.050a (0.096)
300	375.6a (87.1)	50.8a (8.3)	7.53a (1.30)	1.24a (0.25)	2.69a (0.29)	0.275b (0.025)	0.900a (0.242)
600	535.5a (116.4)	77.8a (8.9)	6.68a 0.85	1.42a 0.21	3.45a (0.21)	0.425a (0.025)	0.825a (0.165)

1.10–2.43% (0–10 cm soil) and 0.81–2.01% (10–20 cm) of total soil C, while MBN comprised 2.89–6.79% (0–10 cm) and 1.92–5.20% (10–20 cm) of total soil N (Table 4).

No significant differences were observed in the MBC and MBN contents, microbial C:N ratios and ratios of MBC to total C and MBN to total N in soils of either depth among the N application rates (Table 4), while the MBC and MBN contents and ratios of MBC to total C and MBN to total N in 0–10 cm soil in the control appeared to be greater than in soils treated with N fertilizer although the differences were not significant (Table 4).

Soil respiration

Soil respiration tended to be lower in 0–10 cm soils with N application compared with the control although the differences were not significant (Table 4). The respiration rate in 10–20 cm soil with the application of 600 kg N ha^{-1} was higher than that with the application of 300 kg N ha^{-1} (Table 4). The trends for the cumulative CO_2 respiration were the same (data not shown). In addition, there were no significant differences found in qCO_2 between the treatments, although qCO_2 appeared to be higher in 0–10 cm soils with N application compared with the control (Table 4).

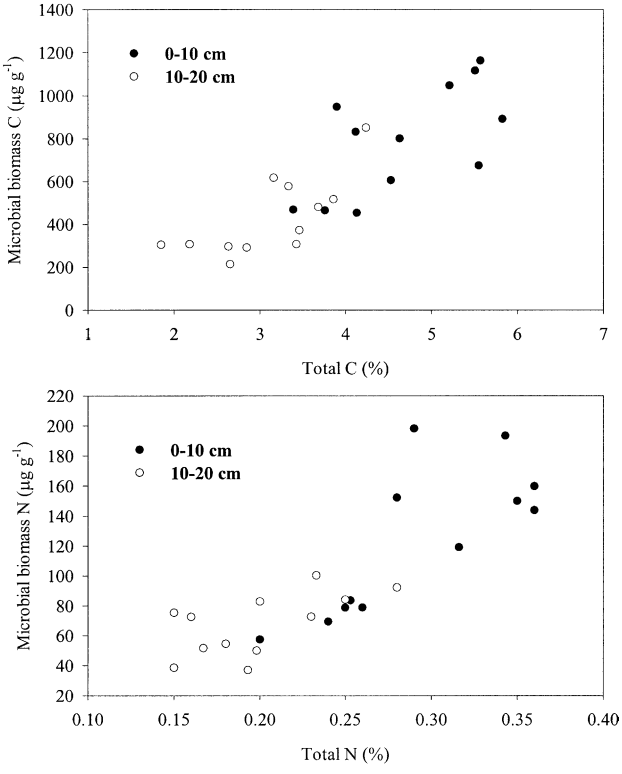

Fig. 2 Relationships between soil total C and microbial biomass C (MBC) and between total N and microbial biomass N (MBN) in a hoop pine plantation at the Imbil site, southeast Queensland

Table 5 Correlation coefficients (r) between soil properties (0–10 cm depth, n=12). See Tables 3 and 4 for abbreviations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Total C														
2. Total N	0.874**													
3. NH_4-N	0.073	–0.124												
4. NO_3-N	0.562	0.628*	0.372											
5. MBC	0.658*	0.791**	–0.310	0.324										
6. MBN	0.791**	0.777**	–0.264	0.336	0.730**									
7. m_n	0.839**	0.687*	–0.098	0.403	0.446	0.378								
8. m_n:total N ratio	0.278	–0.057	–0.011	–0.067	–0.200	–0.281	0.684*							
9. m_g	0.777**	0.657*	0.219	0.561	0.297	0.349	0.875**	0.553						
10. m_g:total N ratio	0.261	–0.035	0.216	0.140	–0.277	–0.229	0.614*	0.876**	0.691*					
11. i_g	0.708**	0.813**	0.224	0.802**	0.410	0.618*	0.565	–0.032	0.747**	0.190				
12. i_g:total N ratio	0.250	0.360	0.147	0.642*	0.034	0.359	0.190	–0.118	0.368	0.181	0.093			
13. Respiration	0.455	0.455	–0.140	0.297	0.490	0.797**	0.3245	–0.111	0.174	–0.168	0.440	0.368		
14. qCO_2	–0.455	–0.470	0.217	–0.132	–0.795**	–0.382	–0.268	0.140	–0.256	0.113	–0.213	0.093	0.068	

*P=0.05, **P=0.01

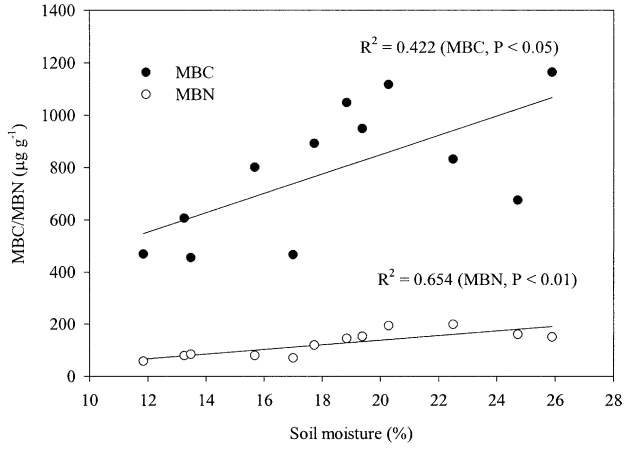

Fig. 3 Relationships between soil moisture content and MBC and MBN in a hoop pine plantation at the Imbil site, southeast Queensland. For abbreviations, see Fig. 2

Relationships between the soil parameters

Simple linear correlation analysis was carried out between all variables (0–10 cm) across treatments (Table 5). The MBC and MBN were significantly correlated with total soil C (r=0.658, P<0.05) and with total soil N (r=0.777, P<0.01), respectively (Fig. 2, Table 5). Meanwhile, concentrations of soil MBC and MBN were significantly related (r=0.730, P<0.01), and MBN was also significantly correlated with soil respiration rate (r=0.797, P<0.01). In addition, it has been found in this study that concentrations of MBC and MBN were significantly correlated with soil moisture (Fig. 3). Soil net and gross N mineralization rates were significantly correlated with concentrations of total soil C and total soil N (Table 5). Gross N immobilization rates were also highly related to the concentrations of total soil C and total soil N contents, and with the concentrations of soil NO_3^--N (Table 5).

Discussion

This study was carried out approximately 5 years after the N application during which the N applied was subject to tree uptake and leaching loss; therefore any changes in soil N pools and the associated microbial properties should be indicative of long-term effects of N application. It has been reported that N fertilization increased soil N availability in forest soils (e.g. Strader and Binkley 1989; Priha and Smolander 1995). Results from this study showed that N application increased concentrations of labile N pools (NH_4^+-N and NO_3-N), but did not affect total soil N (Table 2). This is consistent with the study of Priha and Smolander (1995), in which it was found that concentrations of mineral N (the sum of NH_4^+-N and NO_3^--N) increased after 30 years of repeated N applications (total 900 kg N ha^{-1}) in soils under Norway spruce forests. It has been also reported that N fertilization enhanced the accumulation of mineral N in other soils (Fisk and Schmidt 1996). Some other studies showed that N applied was either immobilized in forest soils at sites poor in N (Feger 1992; Prescott et al. 1993) or converted into NO_3^--N at a site rich in N and leached (Feger 1992). Apparently the extent of the impact of N fertilization on soil N pools depends on the amounts and forms of N added, original soil N levels and the sampling time after N addition. In addition, predominance of NO_3^--N in soil mineral N in 0–10 cm soil in all treatments indicated the high nitrifier activity, low denitrification rates and low microbial NO_3^- uptake in the topsoils. Stark and Hart (1997) also found high gross nitrification rate soils under undisturbed coniferous forest ecosystems.

Net soil N mineralization rate measured by anaerobic incubation has been considered to be a reliable N availability index (Bundy and Meisinger 1994). In this study, the N application appeared to enhance net N mineralization in 0–10 cm soil although this effect was not statistically significant (Table 3). Soil gross N mineralization

rates significantly increased with the N application rate (Table 3). Gross N mineralization rates were approximately 31.3%, 24.1% and 48.5% higher than net N mineralization rates in the control, 300 kg N ha^{-1} and 600 kg N ha^{-1}, respectively. Priha and Smolandrer (1995) also found that N fertilization (900 kg N ha^{-1}) increased the net mineralization in a soil under Norway spruce forests measured by the aerobic incubation method. Also, Connell et al. (1995) reported that N fertilization increased net N mineralization (measured by the aerobic incubation of undisturbed soil columns) in forest soils. Increased net and gross N mineralization rates by N fertilization were also reported by Ledgard et al. (1998). The enhanced N mineralization in N fertilized soils compared with the control may reflect a greater input or quality (low C:N ratio) of substrate (plant litters) for mineralization (Fisk and Schmidt 1996; Ledgard et al. 1998). Moreover, higher gross N mineralization and immobilization rates in soils with N application compared with the control also indicated faster N turnover and tighter N cycling in N fertilized soils. It is worthwhile to note the limitations of the anaerobic incubation method in the measurement of gross N mineralization and immobilization using ^{15}N. The incubation under the anaerobic condition depresses the aerobic microbial activity and the results of gross N mineralization and immobilization under the anaerobic condition may only represent part of soil N dynamics. However, the ^{15}N labelling of soil is easier and relatively more uniform under the anaerobic condition than aerobic incubation. Moreover, results of N dynamics from the anaerobic incubation method were very consistent and reliable (Bundy and Meisinger 1994). More recently, Wang et al. (2001) compared gross N mineralization using ^{15}N under aerobic and anaerobic conditions using 20 soils with a clay content ranging from 30 to 720 μg g^{-1}, and found a significant relationship (R^2=0.86, P<0.01) between gross N mineralization rates under the aerobic condition and those under the anaerobic condition. Therefore, the gross N transformation rates measured under the anaerobic condition should reasonably be indicative of soil N dynamics for relative comparison between treatments in this study.

The values for MBC, MBN and soil respiration were within normal ranges in forest soils (Srivastava and Singh 1991; Maxwell and Coleman 1995). Results from this study tended to show that N fertilization reduced microbial biomass and microbial activity in 0–10 cm soil although these effects were not statistically significant (Table 4). Priha and Smolander (1995) reported that N application reduced microbial biomass N in a soil under Norway spruce forests, which was attributed to the decreased immobilization of N under the N application. It was also found that addition of NH_4NO_3 significantly suppressed soil microbial biomass and microbial activity in other coniferous forests (Martikainen et al. 1989; Thirukkumaran and Parkinson 2000). In addition, reduced MBC and MBN were also found in response to N fertilization in pasture soils (Lovell et al. 1995; Ajwa et al. 1999), which was related to the reduction in root biomass caused by high N input and consequently lower microbial biomass. The decreased microbial biomass and activity in the N fertilized soils compared with the control in this study might be attributable to lower availability of C with decreasing soil pH induced by the N application (Thirukkumaran and Parkinson 2000).

The lack of statistically significant differences of microbial properties between N treatments might have been associated with a large spatial variability between the replicate plots at the experimental site. It has been widely recognized that there is a large spatial variability in soil properties, particularly biological ones in forest soils (Bruckner et al. 1999; Morris 1999; Laverman et al. 2000). It should be noted in this study that spatial variability in these microbial parameters was also large. For example, concentrations of MBC ranged from 801 to 1,116 μg g^{-1} in 0–10 cm soil in the control plot [coefficient of variation (CV) 15.5%]; the corresponding values for soils with application of 300 and 600 kg N ha^{-1} were 465–1,047 μg g^{-1} (CV 41.6%) and 454–1,163 μg g^{-1} (CV 42.3%), respectively. In addition, inconsistent responses of microbial properties of 10–20 cm soil (compared with 0–10 cm soil) to N fertilization and even greater CVs (e.g. CV 36–46% for MBC) also reflected the large spatial variability in these microbial parameters at this site (Table 4).

It has been reported that MBC and MBN (Corre et al. 2002) and bacterial and fungal biomass (Morris 1999) were subject to spatial (topographical) variation, particularly to drainage at different positions of the slope. Morris and Boerner (1999) also found that in hardwood forest soils microbial biomass (including bacteria and fungi) varied significantly along gradients of moisture. Görres et al. (1998) also reported high levels of spatial variation in soil biological properties in forest soil which appears to be very susceptible to changes in soil moisture. In this study, we also found that soil MBC and MBN were significantly correlated with soil moisture (Fig. 3), indicating that spatial variability in soil moisture between the replicate plots at the experimental site may have influenced the soil microbial properties. In addition, soil MBC and MBN were also highly correlated with soil total C and total N contents (Fig. 2). Therefore, it is suggested that variation in soil MBC and MBN in this study could largely be explained by a combination of spatial variability in soil moisture and the levels of soil total C and N. These results also indicated that spatial variability in soil microbial properties should be addressed to detect the significant effects of forest management practices (e.g. fertilization) on these properties.

In conclusion, results from this study demonstrated that application of N about 5 years ago in a hoop pine forest increased concentrations of soil labile mineral N (NH_4^+-N and NO_3^--N), particularly at the depth of 0–10 cm and at a rate of 600 kg N ha^{-1}, but did not significantly affect the concentrations of soil total C and total N. N application enhanced soil gross N mineraliza-

tion and immobilization in 0–10 cm soil, with N fertilized soils showing faster N turnover and tighter N cycling compared with those of the control. The lack of statistically significant differences between the microbial properties of the N treatments might have been associated with the large spatial variability between the replicate plots at this experimental site. Spatial variability in soil MBC and MBN was related to soil moisture and soil total C and total N.

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