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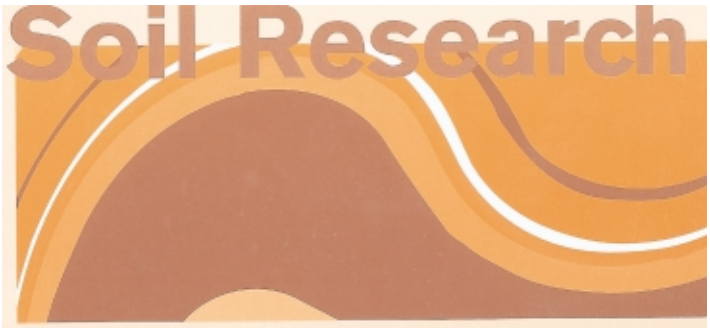

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Soil microbial biomass, metabolic quotient, and carbon and nitrogen mineralisation in 25-year-old *Pinus radiata* agroforestry regimes

S. Saggar[A], *C. B. Hedley, and G. J. Salt*

Landcare Research, Private Bag 11052, Palmerston North, New Zealand.
[A]Corresponding author; e-mail: saggars@Landcare.cri.nz

Abstract

To understand the effects of agroforestry on soil biological processes we assessed the conditions in *Pinus radiata* plantations of 50, 100, 200, and 400 stems/ha after 25 years of growth, and in a grassland. Agroforestry resulted in a 15–25% decline in soil organic C and N compared with grassland, and had a significant negative influence on soil microbial biomass. There was less microbial C and N in soils under 50–400 stems/ha of *P. radiata* than in soils under grassland (0 stems/ha). Soil carbon decomposition and microbial activity were measured by trapping the carbon dioxide produced by incubating soils over a 60-week period. The results showed that soil C decomposition rates were ~1.5 times as much (*c.* 15 mg CO_2-C/kg soil) in soil from grassland as in that from plots with 50 or100 stems/ha (*c.* 10 mg CO_2-C/kg soil), and were further reduced to one half (*c.* 5.5 mg CO_2-C/kg soil) in the plots with 200 or 400 stems/ha. The soils under *P. radiata* gave off less carbon dioxide per unit of biomass (the metabolic quotient) than soils under grassland. These shifts in microbial biomass and its metabolic quotients appear to be associated with differences in the quantity and 'quality' of inputs and soil organic matter decomposition rates, and to reflect the land use change from grassland to forest. Given the general ability of soil microbial biomass to recolonise depopulated areas after tree harvest, we see no problem in restoring populations of these soil organisms vital in controlling nutrient cycling after tree felling, provided adequate adjustments to soil pH are made.

Additional keywords: grassland, land use, microbial biomass C and N, organic C and N mineralisation.

Introduction

In New Zealand, the profitability of agroforestry varies as relative returns from livestock farming and forestry alter. Additionally, agroforestry seems to improve the stability of hill country. Establishment of widely spaced *Pinus radiata* (D. Don) plantations in improved ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) pasture has been embraced because stock grazing during the initial stages of 25-year production forestry could provide a financial return (Hawke and Knowles 1997). Research by several groups has shown that agroforestry has the potential to alter microclimate and acidify soils (Turner and Lambert 1988; Hawke and O'Connor 1993; Hawke and Wedderburn 1994; Giddens *et al*. 1997; Yeates *et al*. 1997). The extent of the soil change is indicated by the change in pH. Depending on the age of the trees and stocking density, soils under *P. radiata* can be as much as 1 pH unit more acid than adjacent grassland soils.

The background of this study is described by Yeates *et al*. (2000), who measured the changes in soil fauna and soil conditions under *P. radiata* planted in a grazed grassland with different tree stocking rates (0, 50, 100, 200, and 400 final stems/ha) during a 25-year period. They observed a shift in the composition of the nematode fauna with increasing tree stocking rate. Whereas bacterial-feeding nematodes dominated under grassland, the proportion of fungal-feeding nematodes doubled as tree stocking rate increased from 0 to 400 stems/ha. Yeates *et al*. (2000) studied neither soil microbial biomass nor C and N

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mineralisation. There is considerable evidence that microbial biomass C, N, and P measurements can be used to evaluate the influence of land-use change on soils (Yeates *et al*. 1997; Yeates and Saggar 1998), and organic matter turnover controls the fluxes of nutrients. Microbial biomass measurements combined with soil respiration (metabolic quotient, qCO_2) have frequently been used as an index of soil development or degradation (Insam and Domsch 1988; Insam *et al*. 1989) and to assess the quality of organic matter input (Anderson and Domsch 1990, 1993). Because *P. radiata* causes changes in the chemical soil properties and soil microfauna and macrofauna (Yeates *et al*. 2000), it is relevant to study its effects on changes in microbial biomass dynamics. Information on changes in microbial biomass, qCO_2, and organic C turnover may help in interpreting the effects of agroforestry on soil organic matter dynamics and assessing its sustainability.

The primary aim of the present study was to determine the patterns of microbial biomass C and N and metabolic quotient in the soils after 25 years under different agroforestry regimes and adjacent grassland. A secondary aim was to examine the decomposition of organic matter in soils from these regimes to determine the effects of agroforestry on organic matter turnover. This information could potentially be used to determine whether a particular agroforestry regime is correlated with the 'quality' of organic matter inputs.

Methods

Site

Soil samples were obtained from the 93-ha Tikitere agroforestry site located 15 km north-east of Rotorua, New Zealand, at 38° 04′S, 176° 20′E. Full descriptions of the site and soils used in this study are presented elsewhere (Percival *et al*. 1984; Hawke and Knowles 1997; Yeates *et al*. 2000). Briefly, the site on volcanic soils at 350 m asl receives 1490 mm annual precipitation and has a mean daily air temperature of 12.7°C (7.3° in July–17.8°C in February). Soils at the site are developed on patchy Rotomahana Mud on Kaharoa Tephra overlaying older rhyolitic tephra. The soils are classified in the NZ Soil Classification (Hewitt 1992) as Typic Orthic Pumice and in Soil Taxonomy (USDA 1994) as Typic Udivitrand. The site had a 50–60-year history of pastoral land use before *P. radiata* seedlings were planted in 1973 at 4 tree stocking rates that were ultimately thinned to 50, 100, 200, or 400 stems/ha. The unplanted grassland (0 stems/ha) represents regularly fertilised grazed pastures of introduced grasses and legumes. There were 4 replicate plots of each treatment, each plot having a 27-m-wide planted buffer. Information about grazing and fertiliser application is given in Hawke and O'Connor (1993) and Hawke and Knowles (1997). The trees were clear-felled in 1999.

Litter sampling

Sampling was restrained to the topographically 'easy' replicate of the trial used by Yeates *et al*. (2000); within that plot, 4 random locations were chosen and are hereafter regarded as field replicates. The forest floor samples were collected from 2 random positions in each replicate and then pooled into 1 sample for each of the 4 replicates per treatment. The samples were collected from areas without understorey vegetation but with needles. Each individual sample was from a 625-cm^2 quadrat. In all of the plots, forest floor separated easily and completely from the mineral soil, and there was little soil contamination. The forest floor from the 50 stems/ha plot was largely composed of recently fallen needles (L), whereas the forest floors from 100–400 stems/ha plots also contained fermentation (F) and humus (H) layers. The field-moist samples were weighed, and a weighed subsample was oven-dried at 70°C to calculate the dry weight of the composite sample for each plot.

Soil sampling

The soil sampling was also restricted to the contiguous plots of the same replicate used by Yeates *et al*. (2000) in which the flat to easy rolling land was most uniform and the soil least variable. Each treatment covered a 2-ha block, surrounded by a 27-m buffer zone. Four random locations *c.* 50 m^2 were established (pseudoreplicates) in each treatment. Twenty-five cores (25-mm diam., 0–10 and 10–20 cm depths) were taken at approximately even spacings for each location. The cores were pooled, field moist soils were sieved

(<2 mm) soon after collection, and sieved soils were stored at 4°C. These 'fresh' field-moist, sieved samples were used for soil microbial biomass C and N analysis and the incubation study.

Laboratory incubation

Field-moist subsamples (equivalent to 25 g oven-dry soil) of each treatment replicate were placed in beakers and then incubated in sealed 1.8-L Agee jars containing vials with 10 mL CO_2-free water, avoiding any loss of moisture during incubation. Respiration was measured as CO_2 production at weekly or 2-weekly intervals for 60 weeks. On each measurement occasion, a 1-mL headspace sample was collected through a septum in the lid from each jar, and the CO_2 concentration was measured by gas chromatography (GC 8700, Thermal conductivity Detector, Carle Instruments, USA). The jars were flushed with ambient air and resealed for the next measurement. A set of controls (jars without soil) was used as a background reference.

Analysis

Air-dried samples were used for the total C, N, and P analyses. Total C in soils was analysed by a combustion method (Induction Furnace, Leco, St Joseph, MI). Total soil N was determined using a semi-micro Kjeldahl digestion and by measuring NH_4^+-N concentrations in the digests (salicylate/nitroprusside) on a Technicon AutoAnalyzer II system (Blakemore *et al*. 1987). Total P was measured on the Kjeldahl digest.

Soil microbial biomass C was determined by a fumigation-extraction method (Vance *et al*. 1987). Fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 for 30 min (1 : 5 soil : extractant ratio), filtered, and then an aliquot was analysed for C using a TOC 5000 (Shimadzu, Kyoto) analyser. The oxidisable C obtained from the fumigated samples minus that from the non-fumigated samples was taken to represent the microbial-C flush and converted to microbial-biomass C using the relationship: microbial C = C flush/0.41 (Wu *et al*. 1990).

Soil microbial biomass N was measured following the method described by Ross (1992). The K_2SO_4 extracts from fumigated and non-fumigated samples were digested in 0.165 M $K_2S_2O_8$ for 30 min at 121°C, and NO_3^--N and NH_4^+-N were measured by autoanalyser procedures. Ammonium-N concentrations (salicylate/nitroprusside) and NO_3-N concentrations (diazotisation following hydrazine reduction) were measured on a Technicon AutoAnalyzer II system (Blakemore *et al*. 1987). Microbial N was estimated using the relationship: microbial N = N flush/0.45 (Jenkinson 1988).

Potentially mineralisable-N was determined by the anaerobic incubation method of Keeney (1982) and is referred to as N_{pot}. Mineral-N (NO_3^--N plus NH_4^+-N) was determined in 2 M KCl (1 : 10, w/v) before and after the incubation, by autoanalyser procedures outlined above.

The 0.5 M K_2SO_4 extracts from the non-fumigated samples were used to determine mineral-N (NO_3^--N plus NH_4^+-N) by autoanalyser procedures. Net N mineralised was the difference between 60-week incubated and 0-day mineral-N values of each soil.

The metabolic quotient (qCO_2) or specific respiration rate was calculated as the ratio between the CO_2-C produced/h averaged over the first 2-week incubation period and the microbial biomass C measured at the beginning of incubation.

Data analysis

Unless otherwise stated, soil results are expressed on the basis of the oven-dry (105°C) weight of the material. The experiment was 'psuedo-replicated' with respect to treatments only. Means (n = 4) and standard errors of the means were calculated for forest floor C, N, and P. Soil property data (Tables 2 and 3) were analysed by split-plot analysis of variance (ANOVA) in SYSTAT 7 (Wilkinson 1996). The main-unit factor was treatment, whereas depth was considered a split-unit factor as the 2 depth measurements were subsampled from each main-unit (replicate). Minimum significant differences (m.s.d.s) for pairwise comparisons of means were computed using Bonferroni adjusted t-values and standard errors that allowed for the split-plot design (Mead1988). For the decomposition, the incubation period was divided into 3 phases, during which changes in respired C were approximately linear: 0–5 weeks, 5–30 weeks, and from 30 weeks onwards. For the 3 phases (Table 4), linear regression was used to estimate the mean daily rate of increase for each replicate at each depth. Then separately for each phase, split-plot ANOVA was used to test for differences in these rates between treatments and depths. For pairwise comparisons of means, m.s.d.s were calculated at P = 0.05 as described previously.

Table 1. Amount and C, N, and P contents of forest floor materials (L + H horizons) from plots under *Pinus radiata* agroforestry regimes
Values are ± standard error; $n = 4$

Stems/ha	Forest floor (t/ha)	Forest floor C (t/ha)	Forest floor N (kg/ha)	Forest floor P (kg/ha)	Forest floor C:N	Forest floor C:P
0 (grassland)	–	–	–	–	–	–
50	8.1±2.7	3.7±1.2	82±27	8.0±2.6	45	463
100	21.3±1.7	8.7±0.7	315±25	24.8±2.0	28	353
200	29.0±1.1	12.0±0.5	368±14	32.7±1.3	33	369
400	19.7±1.6	9.0±0.7	254±20	17.5±1.4	35	506

Results

Forest floor composition

In the 50 stems/ha plots, forest floor was sparse and largely composed of recently fallen needles compared with the forest floors from 100–400 stems/ha plots, which contained fermentation (F) and humus (H) layers. The nutrient (C, N, and P) concentration of the forest floor from plots of different tree densities did not differ significantly (data not shown). As expected, the amounts of C, N, and P in the forest floor differed, being smallest in the 50 stems/ha plots and greatest in the 200 stems/ha plots (Table 1). Forest floor C:N and C:P ratios were within the ranges reported by Scott *et al*. (1998) and Saggar *et al*. (1998). In these soils, as in New Zealand forest soils in general, P deficiency has been corrected by application of superphosphate. This appears to have resulted in forest floor C:P ratios of <550, critical for net P mineralisation (Saggar *et al.* 1998).

Soil organic C, total N, and Olsen P

Soils under *P. radiata* tended to be more acidic than soils under grassland, and in the 0–10 cm depth ($F_{1,15} = 165.9$, $P < 0.001$) the level of acidity increased with increasing tree density ($F_{4,15} = 47.0$, $P < 0.001$) (Table 2). Although organic C contents of the 0–10 cm mineral soil were higher under grassland than under trees ($F_{4,15} = 8.8$, $P < 0.001$), the content of 10–20 cm mineral soil did not differ between grassland and forest. The trends in total N were similar to those of organic C. Olsen P values were low in grassland and in the 50 stems/ha plots, and high in the other treatments. The C:N ratio, which indicates the rough decomposability of material in the soil, was slightly greater under *P. radiata* than under grassland (Table 3). In every case the ratio was greater in 10–20 cm soil, and the highest value (17.5) was found under 400 stems/ha. In 0–10 cm the value was least (12.6) under grassland (Table 3).

Microbial biomass C and N contents were significantly related to tree density and showed variations similar to soil organic matter (Table 2). However, the differences in microbial biomass C between grassland and forest soils were slightly more pronounced than in organic C, resulting in significant changes in microbial C:organic C values (Table 3). At 0–10 cm, these values were highest (13.6 mg/g) under grassland, and declined with increasing number of *P. radiata*, to be lowest (7.8 mg/g) under 400 stems/ha (Table 3). The microbial C:organic C values were lower in 10–20 cm soil than in 0–10 cm soil and did not differ significantly between the grassland and forest. Both these measures of C and N availability to microflora appear to separate the soils on the basis of current vegetation.

Table 2. Some properties of soils under grassland and *P. radiata* forest regimes

m.s.d.$_{(T1\ v.\ T2)}$ compares treatment means averaged over both depths; m.s.d.$_{(D1\ v.\ D2)}$ compares depth means averaged over all treatments; m.s.d.$_{(D1T1\ v.\ D2T1)}$ compares depth means for one treatment; m.s.d.$_{(D1T1\ v.\ D1T2)}$ compares treatment means for each depth and at different depths

Stems/ha	Soil pH (1:2.5)			Total C (g C/kg soil)			Total N (g N/kg soil)			Olsen P (mg P/kg soil)		
	0– 10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean
0 (grassland)	5.42	5.45	5.44	55.6	27.7	41.7	4.41	1.85	3.13	29	22	26
50	5.37	5.47	5.42	45.3	25.2	35.3	3.13	1.54	2.34	26	10	18
100	5.25	5.35	5.30	46.4	27.4	36.9	3.31	1.8	2.56	64	31	48
200	4.98	5.13	5.06	39.5	29.3	34.4	2.58	1.86	2.22	57	29	43
400	4.92	5.51	5.22	42.1	24.5	33.3	2.61	1.4	2.01	68	19	44
Depth mean	5.19	5.38		45.8	26.8		3.21	1.69		49	22	
m.s.d.$_{(T1\ v.\ T2)}$ ($P = 0.05$)	0.15			5.4			0.52			30		
m.s.d.$_{(D1\ v.\ D2)}$ ($P = 0.05$)	0.03			2.0			0.21			5		
m.s.d.$_{(D1T1\ v.\ D2T1)}$ ($P = 0.05$)	0.09			6.3			0.66			15		
m.s.d.$_{(D1T1\ v.\ D1T2)}$ ($P = 0.05$)	0.18			8.1			0.81			36		

Stems/ha	Biomass C (mg C/kg soil)			Biomass N (mg N/kg soil)			Mineral N (mg N/kg soil)			Pot. mineralisable N (mg N/kg soil)		
	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean	0–10cm	10–20 cm	Treatment mean
0 (grassland)	758	178	468	96	36	66	4.0	1.1	2.6	55	10	33
50	511	211	361	79	29	54	4.1	0.8	2.5	39	7	23
100	476	177	327	63	18	41	3.7	1.1	2.4	35	9	22
200	314	203	258	40	23	31	1.6	1.0	1.3	26	11	19
400	330	156	243	36	19	27	2.8	1.0	1.9	26	9	18
Depth mean	478	185		63	25		3.2	1.0		36	9	
m.s.d.$_{(T1\ v.\ T2)}$ ($P = 0.05$)	71			17			1.1			6		
m.s.d.$_{(D1\ v.\ D2)}$ ($P = 0.05$)	40			78			0.3			4		
m.s.d.$_{(D1T1\ v.\ D2T1)}$ ($P = 0.05$)	123			24			1.0			11		
m.s.d.$_{(D1T1\ v.\ D1T2)}$ ($P = 0.05$)	132			28			1.5			12		

Table 3. Soil C:N ratio, microbial C:organic C ratio (mg/g), and metabolic quotient (mg CO_2-C/g biomass C.h) in grassland and *P. radiata* forest soils

$m.s.d._{(T1\ v.\ T2)}$ compares treatment means averaged over both depths; $m.s.d._{(D1\ v.\ D2)}$ compares depth means averaged over all treatments; $m.s.d._{(DIT1\ v.\ D2D1)}$ compares depth means for one treatment; $m.s.d._{(DITI\ v.\ D1T2)}$ compares treatment means for each depth and at different depths

Stems/ha	Soil C:N ratio			Microbial:organic C			Metabolic quotient		
	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean
0 (grassland)	12.6	15.0	13.8	13.6	6.4	10.0	1.88	1.11	1.49
50	14.5	16.4	15.5	11.2	8.3	9.8	1.54	0.82	1.18
100	14.0	15.2	14.6	10.3	6.5	8.4	1.50	0.99	1.24
200	15.4	15.9	15.7	7.9	6.9	7.4	1.50	0.90	1.20
400	16.2	17.5	16.9	7.8	6.4	7.1	1.49	0.87	1.18
Depth mean	14.5	16.0		10.2	6.9		1.58	0.94	
$m.s.d._{(T1\ v.\ T2)}$ ($P = 0.05$)	1.8			2.4			0.24		
$m.s.d._{(D1\ v.\ D2)}$ ($P = 0.05$)	0.3			0.4			0.05		
$m.s.d._{(D1T1\ v.\ D2T1)}$ ($P = 0.05$)	1.1			1.5			0.15		
$m.s.d._{(D1T1\ v.\ D1T2)}$ ($P = 0.05$)	2.2			3.2			0.32		

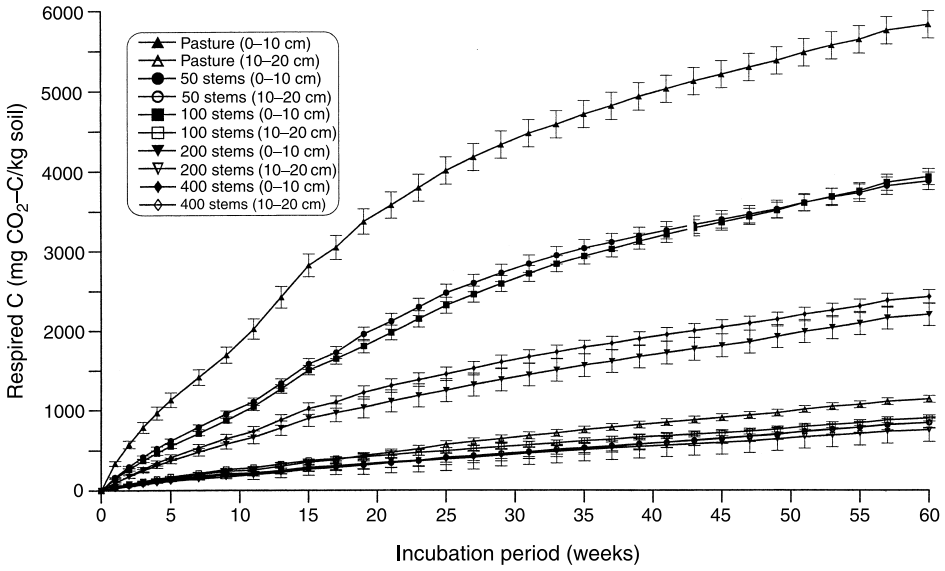

Fig. 1. Cumulative evolution of CO_2-C in grassland and *P. radiata* soils during 60-week incubation. Each value represents the mean of 4 replicates with the standard error shown by vertical bars.

With very low values in mineral N in both 0–10 cm (1.6–4.1 mg N/kg) and 10–20 cm (0.8–1.1 mg N/kg) soil depths, mineral N did not differ significantly between grassland and *P. radiata* (Table 2). However, 25 years of plantation growth had a significant effect on potentially mineralisable N (N_{pot}) in the 0–10 cm soil of grassland and trees at different stocking rates ($F_{4,15} = 10.2$, $P < 0.001$). Grassland soil had higher N_{pot}, and 200 and 400 stems/ha soil had lower N_{pot}, than 50 and 100 stems/ha soil (Table 2), indicating that a well-established legume-based grassland leads specifically to increase in N_{pot} but may lead to higher total N levels as well.

Soil respiration

The CO_2-C respired from the 0–10 cm and 10–20 cm soil during the 60-week incubation amounted to 5.83 and 1.14 g/kg soil for the grassland soil, and 2.43–3.88 and 0.75–0.90 g/kg soil, for soils under *P. radiata*, respectively (Fig. 1). This comprised 2.6 and 10.5% of the initial soil C. The grassland soil had the greatest rate of CO_2 respiration, and the *P. radiata* 400 stems/ha soil the lowest, throughout the incubation. Trends in soil respiration were similar in all soils, and for each the rate of CO_2 respired declined with time (Table 4).

There were 3 phases apparent during the incubation (Table 4). During the first 5 weeks (Phase I) of the incubation, respiration rate was rapid (28.2 mg CO_2-C/kg soil.day for the 0–10 cm grassland soil and 9.8–16.3 mg CO_2-C/kg soil.day for the *P. radiata* soils). This initial active decomposition was followed by a slower secondary metabolism (Phase II, weeks 5–30) with respiration rate dropping to 5.5–18.2 mg CO_2-C/kg soil. day. For Phase III (weeks 30–60) the respiration rate was much slower (3.7–6.6 mg CO_2-C/kg soil. day) in the respective soils. The respiration rates for the 10–20 cm soil were 2.8–4.4 mg CO_2-C/kg soil. day during Phase I, dropping to 1.6–2.8 mg CO_2-C/kg soil.day during Phases II and III.

Table 4. Carbon mineralisation rates (mg CO_2-C respired/kg soil) and net N mineralisation (mg N/g soil N) in grassland and *P. radiata* forest soils during 60-week incubation

$m.s.d._{(T1\ v.\ T2)}$ compares treatment means averaged over both depths; $m.s.d._{(D1\ v.\ D2)}$ compares depth means averaged over all treatments; $m.s.d._{(D1T1\ v.\ D2T1)}$ compares depth means for one treatment; $m.s.d._{(D1T1\ v.\ D1T2)}$ compares treatment means for each depth and at different depths

Stems/ha	Carbon mineralisation rate per day during									Net N mineralised		
	0–5 week (Phase I)			5–30 week (Phase II)			30–60 week (Phase III)			0–10 cm	10–20 cm	Treatment mean
	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean	0–10 cm	10–20 cm	Treatment mean			
0 (Grassland)	28.2	4.0	16.1	18.2	2.8	10.5	6.6	2.2	4.4	477	92	285
50	16.3	3.4	9.9	12.3	1.8	7.1	5.0	1.8	3.4	327	79	203
100	14.4	4.4	9.4	11.7	2.0	6.9	5.9	1.6	3.8	309	91	200
200	9.8	3.6	6.7	5.5	1.6	3.6	3.8	1.4	2.6	175	86	131
400	11.5	2.8	7.2	6.6	1.8	4.2	3.7	1.9	2.8	196	85	141
Depth mean	16.0	3.6		8.5	1.8		5.0	1.8		297	87	
$m.s.d._{(T1\ v.\ T2)}$ ($P = 0.05$)	3.25			1.52			0.88			42.8		
$m.s.d._{(D1\ v.\ D2)}$ ($P = 0.05$)	1.37			0.69			0.46			22.3		
$m.s.d._{(D1T1\ v.\ D2T1)}$ ($P = 0.05$)	4.24			2.13			1.42			69.8		
$m.s.d._{(D1T1\ v.\ D1T2)}$ ($P = 0.05$)	5.13			2.49			1.56			76.8		

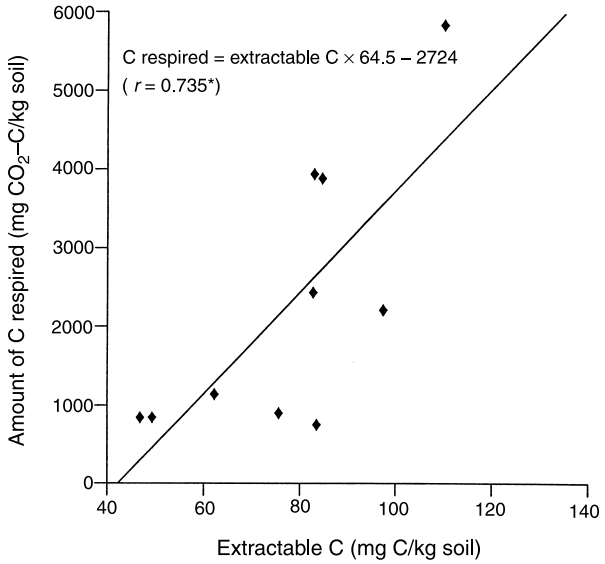

Fig. 2. Relationship between extractable C and CO_2-C respired in grassland and agroforestry soils during 60-week incubation period.

For all 3 phases there was very strong evidence for treatment × depth interactions ($F_{4,15}$ = 23.9, $P < 0.001$; $F = 42.0$, $P < 0.001$; and $F = 5.59$, $P = 0.006$) (Table 4). Although the m.s.d.s show no evidence for treatment effects at 10–20 cm, there are clear differences at 0–10 cm; grassland had a significantly higher rate of increase of respired C than all other treatments in all 3 phases; in Phase I, 50 stems had a higher mean than 200; in Phase II, 50 and 100 stems had higher rates of increase than 200 and 400 stems; in Phase III, the 100-stem treatment had a significantly higher rate of increase than both 200 and 400 stem treatments. There is no evidence for other treatment differences.

A significant relation was also found between the cumulative C respiration values during the 60-week incubation and K_2SO_4-extractable C from the unfumigated treatment (Fig. 2; $n = 10$, $r = 0.735$, $P < 0.05$).

N mineralisation

Net N mineralised from 0–10 and 10–20 cm soil during the 60-week incubation amounted to 477 and 92 mg/kg for the grassland soil, and 175–327 and 79–91 mg/kg for soils under *P. radiata*, respectively (Table 4). Net N mineralisation was significantly greater for the grassland soil, and decreased with increase in tree density in the 0–10 cm soil samples, whereas no such differences were obtained for the 10–20 cm soil samples. Soils having low C mineralisation rates also showed low N mineralisation rates, and the ratio of C respired to net N mineralised for the 0–10 cm grassland and *P. radiata* soils was 12–13. The relationship between CO_2-C respired and N mineralised was further examined. The cumulative C respiration values during the 60-week incubation were significantly correlated with N mineralised (Fig. 3; $n = 10$, $r = 0.997$, $P < 0.001$).

Metabolic quotient

Metabolic quotient values showed similar trends to those of microbial biomass values. They tended to be the greater for the 0–10 cm soil than for the 10–20 cm soil, and for grassland

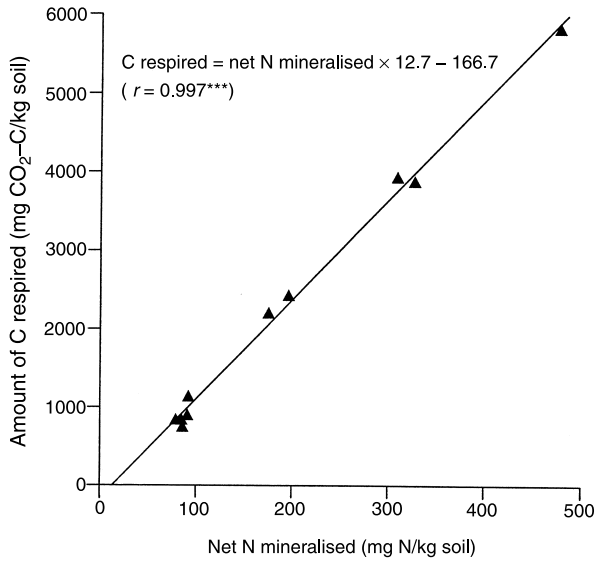

Fig. 3. Relationship between accumulated net N mineralised and CO_2-C respired in grassland and agroforestry soils during 60-week incubation period.

soils (1.88 mg CO_2-C/g biomass C.h) than for *P. radiata* soils (1.49–1.54 mg CO_2-C/g biomass C.h) (Table 3). The differences observed in microbial biomass C in the soils under grassland and *P. radiata* regimes (Table 2) were not parallelled by changes in soil respiration, indicating microbial biomass activities to be different, i.e. qCO_2 differs between grassland and forest.

Discussion

Mineral soils under *P. radiata* were found to contain less microbial biomass C and N than soils under grassland, and the contents of microbial biomass C and N declined with an increase in the number of trees from 50 to 400 stems/ha. These results indicate that pine trees reduce the size of various soil C and N pools, and that soil processes under *P. radiata* are profoundly different from those occurring in grasslands. Soil microbial biomass is integral to the ecological functioning of soils and responds rapidly to management and cultural practices (Yeates and Saggar 1998; Saggar *et al.* 1999*a*). Impacts of land-use or vegetation changes are often detectable in soil microbial biomass before they can be detected in organic matter (Powlson *et al.* 1987). Our results showing the decline in microbial biomass C and N with increasing *P. radiata* density indicate that nutrient inputs to mineral soil under *P. radiata* decreased and were lower than under grassland. This would eventually result in lower total organic C and N levels, as shown in the soils we analysed (Table 2). These values take no account of forest or grassland litter at these sites. The decline in soil organic matter under *P. radiata* may be attributed, to some extent, to the effects of acidic soil pH (Motavalli *et al.* 1995) caused by organic acids and resins released by decomposing needles. Acidic pH is associated with changes in microbial composition and activity (Yeates *et al.* 1997). There were marked differential effects of *P. radiata* on the proportions of total soil organic C immobilised in the microbial biomass (Table 3). The ratio of microbial C to total organic C was higher in the grassland and decreased with

increasing *P. radiata* stocking rate. The reduction in this ratio under *P. radiata* is indicative of a shift in the state of equilibrium of the soil subsystem, and is consistent with Anderson and Domsch's (1989) predictions that microbial C:organic C eventually declines in conditions where organic matter input is low.

Our data on the decomposition of organic matter in these soils showed that throughout the 60-week incubation period, grassland soils had the highest rate of CO_2 respired, and that soils from 200 to 400 stems/ha *P. radiata* had the lowest rate. Rates of CO_2 respired declined rapidly after 5 weeks (Fig. 1 and Table 4) in all soils. Higher CO_2 production during this period (Phase I) may be due partly to the disturbance of the soil during sampling, sieving, and processing, resulting in greater availability of metabolically accessible compounds, and partly to incubating under laboratory conditions. Grassland soils with more labile organic matter inputs resulted in greater availability of metabolically accessible compounds during this phase. The proportional decline in C mineralisation rate was greater in the grassland soil than in the *P. radiata* soils during Phases II and III, suggesting the presence of more labile C in grassland soil. We found overall rates of C mineralisation from grassland soil to be about 1.5–2 times those from *P. radiata* soils. The organic matter from grassland soils had slightly lower C:N ratio than that from *P. radiata* soils. The higher rate of mineralisation in grassland soil is probably related to the greater inputs and consequently higher proportions of relatively fresh, easily decomposable plant material (grass roots and above-ground litter) in grassland soil, which presumably contained substrates that were readily metabolised, leading to the greater concentration of microbial biomass.

The metabolic quotient (qCO_2) evaluates the efficiency of soil microbial biomass in using the organic C compounds (Anderson and Domsch 1989). The qCO_2 data may provide means of determining whether microbial biomass in grassland soil was metabolically more active. The greater qCO_2 values in grassland soil (Table 3) could reflect an increase in the ratio of active:dormant components of the biomass. This ratio may have been modified by the difference in the available substrate (more root exudates and fine roots under grassland compared with those under *P. radiata*). A high microbial quotient generally implies a ready supply of fresh organic residues (Anderson and Domsch 1989). The high qCO_2 values in soil under grassland also suggest that during mineralisation of organic matter, microbes divert more C to respiration than to new microbial biomass, causing more C loss than from microbes in *P. radiata* soils. A decline in qCO_2 with *P. radiata* forest and increasing soil depth may indicate either the presence of microbial populations, which are more efficient in incorporating C compounds, or availability of relatively less labile organic residues. Although qCO_2 undoubtedly indicates microbial efficiency, several factors such as low pH, qualitative changes within microbial population (e.g. increase in the proportion of fungi), and prevalence of zymogenous over autochthonous microbiota may explain the differences in qCO_2 between grassland and *P. radiata* forest. Whereas our data suggest an enhancement in the metabolic efficiency (i.e. lower qCO_2) of microbial biomass resulting from planting of *P. radiata* on farmland as agroforestry, this quotient may not reflect ecosystem development (Wardle and Ghani 1995). Although the ultimate interpretation of this ecophysiological index remains uncertain, our results are consistent with those of Insam and Haselwandter (1989) and Anderson and Domsch (1990), which indicate that high qCO_2 values, under grassland, reflect younger microbial communities that rapidly turnover.

Our aim in the present study was to determine the pattern of change in microbial biomass C and N and metabolic quotient in the soils under different agroforestry regimes and grassland, and to determine whether changing to an agroforestry regime was correlated to the 'quality' of organic matter inputs. Although the degree of organic matter

accumulation in soil depends upon the relationship between C inputs and decomposition rates, soil organic C changes slowly with changes in land-use and plant species, and changes are difficult to measure against a large background of soil organic matter content already present. However, more rapid changes occurred in microbial biomass, qCO_2, and microbial C : organic C (Tables 2 and 3) following planting of *P. radiata* on grassland (i.e. changes in inputs and decomposition rates). These significant changes in microbial parameters are clearly associated with the influence of plant species on the quality of organic matter inputs and respiration.

The laboratory incubation showed that higher rates of C mineralisation from grassland soil were probably related to the greater inputs and consequently higher proportions of relatively labile plant materials. The relationship between the amount of C respired during the incubation period and labile C represented by K_2SO_4-extractable C from the unfumigated treatment is significant (Fig. 2; n =1 0, r = 0.735, $P < 0.05$). On average, during a 60-week incubation, *c*. 65 times more C was respired for each unit of extractable C available at the start of incubation. This may be because respiration is a function of extractable C, and the size and activity of microbial biomass. Grassland soils with higher extractable C and greater microbial biomass tend to mineralise C at higher rates than *P. radiata* soils. Our results also show that despite greater C mineralisation in the grassland soil, more C and N are conserved than in the *P. radiata* soils (Table 2). These results imply that C inputs in grassland soils are not only more easily decomposable than those in *P. radiata* soils but are also greater.

Measurements of mineral N showed that all the soils had similar and low levels of mineral N (Table 2), even though total N and microbial levels were in the range for grasslands and *P. radiata* soils elsewhere in New Zealand (Yeates *et al*. 1997; Saggar *et al*. 1999*b*). This suggests that N cycling was very tight. The mineral N is a static measurement of soil labile N status. The anaerobic N mineralisation technique (Waring and Bremner 1964) is considered to be one of the most reliable and sensitive estimates of potentially mineralisable N (N_{pot}) (Binkley and Hart 1989). In our study, the amount of N_{pot} was significantly more in grassland soil, 25-year *P. radiata* resulting in a significant decline in N_{pot} (Table 2). Over the 60-week incubation there was an increase in mineral N (net mineralisation) in all soils. As with C mineralisation, the amount of N mineralised during a 60-week laboratory incubation was greater for grassland soil than for 50–100 stems/ha *P. radiata* soils, which, in turn, was greater than for 200 and 400 stems/ha soils (Table 4), confirming that decomposable organic matter is greater in grassland soil than in soils under *P. radiata*. It can be concluded that a well-established legume-based grassland leads specifically to an increase in total N and N_{pot}, which may also result in higher N supply compared with *P. radiata*. In this study, C mineralisation was significantly correlated with net N mineralisation (r = 0.997***, n = 10) (Fig. 3). On average, throughout the incubation, *c*. 12.7 units of C were respired for each unit of net N mineralised.

Conclusions

This study has highlighted the importance of *P. radiata* agroforestry regimes to changes in microbial biomass, qCO_2, and organic C turnover, which are useful in understanding the effects of agroforestry on soil organic matter dynamics and assessing its sustainability. In New Zealand, planting of *P. radiata* trees on farmland as agroforestry was advocated, principally with the idea that income from stock grazing the grassland would provide some financial return during the initial stages of 25-year production forestry (Hawke and Knowles 1997). During the 25-year agroforestry cycle, soil pH declined, and organic C and

N, and microbial biomass levels were reduced. Soil organic matter decomposition and microbial activity measurements showed a reduction in the C mineralisation rates with one and a half times as much mineralisation in the grassland as in the plots with 50–100 trees/ha, and further reduction to one half in the plots with 200–400 trees/ha. The soils under *P. radiata* gave off less carbon dioxide per unit of biomass (the metabolic quotient) than soils under grassland. Therefore, these deviations in microbial biomass and its metabolic quotients appear to be associated with differences in the quantity and 'quality' of inputs and soil organic matter decomposition rates, and reflect the land use change from grassland to coniferous forest. This study, however, suggests that despite greater decomposable organic matter in grassland soil than in soils under *P. radiata*, the ratio of total C to net N mineralisation did not differ with the change in land use. Given the ability of soil microbial biomass to recolonise depopulated areas after tree harvest, we see no problem in restoring populations of these soil organisms vital in controlling nutrient cycling after tree felling, provided adequate adjustments to soil pH are made.

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