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ORIGINAL PAPER

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# Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems

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**Abstract** Accurate prediction of soil N availability requires a sound understanding of the effects of environmental conditions and management practices on the microbial activities involved in N mineralization. We determined the effects of soil temperature and moisture content and substrate type and quality (resulting from long-term pasture management) on soluble organic C content, microbial biomass C and N contents, and the gross and net rates of soil N mineralization and nitrification. Soil samples were collected at 0–10 cm from two radiata pine (*Pinus radiata* D. Don) silvopastoral treatments (with an understorey pasture of lucerne, *Medicago sativa* L., or ryegrass, *Lolium perenne* L.) and bare ground (control) in an agroforestry field experiment and were incubated under three moisture contents (100, 75, 50% field capacity) and three temperatures (5, 25, 40 °C) in the laboratory. The amount of soluble organic C released at 40 °C was 2.6- and 2.7-fold higher than the amounts released at 25 °C and 5 °C, respectively, indicating an enhanced substrate decomposition rate at elevated temperature. Microbial biomass C:N ratios varied from 4.6 to 13.0 and generally increased with decreasing water content. Gross N mineralization rates were significantly higher at 40 °C (12.9 $\mu$g) than at 25 °C (3.9 $\mu$g) and 5 °C (1.5 $\mu$g $g^{-1}$ soil $day^{-1}$); and net N mineralization rates were also higher at 40 °C than at 25 °C and 5 °C. The former was 7.5-, 34-, and 29-fold higher than the latter at the corresponding temperature treatments. Gross nitrification rates among the temperature treatments were in the order 25 °C >40 °C >5 °C, whilst net nitrification rates were little affected by temperature. Temperature and substrate type appeared to be the most critical factors affecting the gross rates of N mineralization and nitrification, soluble organic C, and microbial biomass C and N contents. Soils from the lucerne and ryegrass plots mostly had significantly higher gross and net mineralization and nitrification rates, soluble organic C, and microbial biomass C and N contents than those from the bare ground, because of the higher soil C and N status in the pasture soils. Strong positive correlations were obtained between gross and net rates of N mineralization, between soluble organic C content and the net and gross N mineralization rates, and between microbial biomass N and C contents.

**Keywords** Gross mineralization rate · Nitrification · Microbial biomass · Pasture management · Radiata pine

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## Introduction

In New Zealand, silvopastoral systems with understories such as lucerne (*Medicago sativa* L.), clovers (*Trifolium* spp), cocksfoot (*Dactylis glomerata* L.), and ryegrass (*Lolium perenne* L.) planted under radiata pine (*Pinus radiata* D. Don) are commonly practiced (Mead 1995). Understories such as ryegrass and cocksfoot can enrich soil with C through the return of their root residues and aboveground litter (Chang et al. 2002; Haggar et al. 1993), while others like white clover (*T. repens* L.) and lucerne can also fix N (Goh et al. 1996) and thus benefit tree growth by providing plant nutrients, especially N, through the release of N-rich root exudates and the decomposition of plant residues. Long-term practice of different silvopastoral or crop rotation systems certainly leads to changes in soil substrate quality, such as soil C and N contents and soil C:N ratios (Blair and Crocker 2000; Chang et al. 2002).

Most of the N input in such silvopastoral systems is in the form of plant residues (above- and belowground) which are of organic origin (Miltner and Zech 1999; Verburg et al. 1999); and the availability of organic N is determined by the rate at which organic N is mineralized by various groups of soil microorganisms. Recent research on N cycling paid particular attention to the role of gross N mineralization rates in belowground processes (Barraclough 1995; Davidson et al. 1991; Fisk et al. 1998; Stottlemyer and Toczydlowski 1999; Zaman et al. 1999a, 1999b). This surge of new interest on gross rates of N mineralization and nitrification is the result of some of the limitations of the net N mineralization measurement, as the net rate does not provide information on the rate of N immobilization which occurs concurrent to N mineralization or information on the total microbial activities (Hart et al. 1994). In contrast, gross N mineralization rates measure the total rate of N mineralization, accounting for the concurrent N immobilization process. The gross rates provide information about total microbial activity and may be a better indicator of potential nutrient availability if the plants can compete effectively with the microbes for the mineralized nutrients, while the net mineralization rate may be a better measure of the actual N availability of the soil.

The activities of microorganisms involved in N mineralization are affected by many factors, both biotic and abiotic (Clarhom and Rosswall 1980; Sarathchandra et al. 1989), and by management practices (Chang and Juma 1996; Gill et al. 1995; Zaman et al. 2002). Despite the availability of techniques such as $^{15}N$ isotope tracers (Barraclough 1995; Davidson et al. 1991) and flow cytometric analysis of microbial populations (Yentsch and Yentsch 1989), the relationships between N mineralization and microorganisms as affected by different environmental factors and management practices are still not well understood (Zaman et al. 1999a, 1999b). The effects of temperature and moisture content on soil N cycling have widely been studied, often with conflicting results, while little is known about the influence of understorey management and its interactions with soil temperature and moisture content on N mineralization and nitrification processes. For example, Sarathchandra et al. (1989) and Stottlemyer and Toczydlowski (1999), both using forest sites, reported that microbial biomass and their activities vary with changing soil temperature, moisture, and substrate type, while Holmes and Zak (1994) and Sierra and Marban (2000) did not observe such responses in hardwood forests. Similarly, Arnold et al. (1999) and Joergensen et al. (1990) reported that temperature rather than moisture appeared to be the critical factor affecting microbial biomass and their activities in northern forest soils and agricultural soils, while Fisk et al. (1998) found that it was moisture that accounted for most of the variation in N mineralization and microbial biomass in alpine tundra systems.

In agroforestry systems, both biotic and abiotic environments can be modified by the different crop/pasture–tree configurations (Chang et al. 2002; Yunusa et al. 1995). Thus, soil N dynamics in silvopastoral systems often show large temporal and spatial variations (Amatya et al. 2002). A better understanding of the effect of temperature and moisture content on soil N cycling processes in different silvopastoral systems will improve our ability to interpret and predict management effects on soil N availability and plant growth performance. Therefore, this study was undertaken with the following specific objectives: (1) to quantify the effects of soil temperature and moisture content on N mineralization and nitrification rates, particularly to elucidate the role N immobilization plays in soil N availability, by measuring gross N mineralization and nitrification rates, and (2) to investigate the effect of different substrate quality that resulted from long-term pasture management on soil microbial biomass C and N and the N mineralization and nitrification rates.

## Materials and methods

### The site, the field experiment, and soil sampling

The site (43 ° 38′ S, 172 ° 30′ E, 11 m altitude) is located 2 km from Lincoln University, on Canterbury Plains, New Zealand. The site has a temperate and subhumid climate. The long-term average temperature is 11.4 °C and average rainfall is 660 mm. The rainfall is distributed more or less evenly throughout the year. Summer evapotranspiration usually exceeds summer precipitation by 2- to 3-fold; and therefore summer droughts are common. The soil is classified as a Templeton silt loam in the New Zealand soil classification system (Udic Haplustepts in the U.S. soil taxonomy system) and is free-draining with moderate water-holding capacity. The soil consists of 1–2 m of fine alluvial sediments over gravels. The top 0.2 m contains about 2.8% C and 0.24% N and has a pH of 5.8 (Chang et al. 2002).

The agroforestry experiment, established to study tree/pasture interactions, includes six understorey pasture treatments, replicated in three blocks. A fourth block was established to allow destructive sampling of trees. Together, the treatments were replicated four times. Each plot was 42.0×46.2 m (0.194 ha) with trees planted at a spacing of 7 m (between rows) by 1.4 m (within a row). This gave an initial stocking of 1,000 stems $ha^{-1}$. Tree rows were planted in an east–west direction. Strips 1 m wide centered on tree rows were sprayed with hexazinone at 2.5 kg active ingredient $ha^{-1}$ in the Spring of 1990 and 1991 to ensure good tree establishment. Proper buffer rows were established. Trees in the plots were thinned four times, in 1992, 1993, 1994, and 1996. Each time, 200 stems $ha^{-1}$ were removed. Several prunings were carried out, starting in 1994. Pruning was to an 11-cm caliper (i.e., the diameter of the highest clear bole after pruning) and was done to control the diameter-over-stubs size and to maintain a consistent green crown length. Residues from the pruning operations were carried off-site to prevent adverse effects of the residues on pasture growth. As a silvopastoral experiment, the pasture was to be grazed by sheep. However, the pasture was cut for silage four times in the first two growing seasons, because the trees were too small and could be damaged by grazing sheep. The plots were fenced and rotational grazing commenced in Spring 1993. Throughout the experiment, no fertilizer or irrigation was used. At the time of this study, the stands had been thinned to 200 trees $ha^{-1}$ and pruned to a height of about 4.5 m.

Three understorey treatments (lucerne, ryegrass, bare ground) were selected from the above agroforestry trial for sampling. Five soil cores (0-10 cm depth) were randomly collected from each of the 12 plots (three treatments × four replicates) to form one composite soil sample for each plot. The samples were picked free

**Table 1** Chemical properties (mean ±SE) of soil samples collected from the Lincoln University agroforestry experiment, Canterbury Plains, New Zealand ($n$=4)

| Property | Bare ground | Ryegrass | Lucerne |
|---|---|---|---|
| pH | 5.20±0.025 | 5.82±0.018 | 5.71±0.013 |
| Total N (g $kg^{-1}$ soil) | 2.0±0.09 | 2.4±0.04 | 2.6±0.08 |
| Soluble C ($\mu$g C $g^{-1}$ soil) | 71.2±1.08 | 106.2±1.70 | 75.2±0.94 |
| Total organic C (g $kg^{-1}$ soil) | 23.0±0.14 | 28.1±0.16 | 29.6±0.19 |

of visible roots and litter material and were passed through a 2-mm sieve. These samples were analyzed for different physical and chemical properties, as shown in Table 1.

### Incubation and analytical procedures

The composite samples were first adjusted to three moisture levels, namely 100, 75, and 50% field capacity (FC), using a pressure plate. A non-leaching technique described by Zaman et al. (1999a) was used to study gross and net rates of N mineralization and nitrification. To measure the gross N mineralization rate, 72 soil samples (eight for each moisture content/substrate type combination) of 25 g each (oven-dry basis) were placed in 120-ml ventilated containers and incubated at 5 °C. The same number of soil samples was incubated at 25 °C and 40 °C. The samples were incubated for 30 days to minimize the potential artifacts caused by the packing and handling of the soil before $^{15}N$ labeling commenced. After the 30-day incubation period, the soil samples were injected with 1 ml of $^{15}N$-labeled $(NH_4)_2SO_4$ (60% enrichment) solution, giving 2 $\mu$g $^{15}N$ $g^{-1}$ soil. This application rate minimized the stimulation of microbial activity by the added water and the alteration of the ambient $NH_4^+$ pool size but still provided sufficient amount of $^{15}N$ to raise the isotope composition to a level significantly above the natural $^{15}N$ abundance, to allow the samples to be accurately analyzed on a mass spectrometer. Fifteen minutes after $^{15}N$ was added, four containers were withdrawn from each temperature, moisture content, and substrate type combination, immediately extracted with 50 ml of 2 M KCl, and analyzed for $NH_4^+$, using a flow-injection analyzer (FIA; Tecator, Sweden). The remaining samples in each incubator were extracted with 50 ml of 2 M KCl after a further 24-h incubation period. To measure gross nitrification rates, the above procedures were repeated. However, instead of adding $^{15}N$-labeled $(NH_4)_2SO_4$, the soil samples were uniformly labeled with 1 ml of $^{15}N$-labeled $KNO_3$ (60% enrichment) solution, also giving 2 $\mu$g $^{15}N$ $g^{-1}$ soil. The samples were extracted in a similar way and analyzed for $NO_3^-$. The $^{15}N$ content of the inorganic N in the KCl extracts was determined using the diffusion method described by Stark and Hart (1996) and an isotope ratio mass spectrometer (Europa Scientific, UK). The gross rates of N mineralization (or nitrification) were calculated from the rate at which $^{15}N$ abundance in the labeled $NH_4^+$ (or $NO_3^-$) pool declined over time and the change in size of the $NH_4^+$ (or $NO_3^-$) pool, as described by Davidson et al. (1991):

$$m=\frac{M0-M1}{t}\frac{\log(H0M1/H1M0)}{\log(M0/M1)},$$

where $m$ is the gross N mineralization or nitrification rate ($\mu$g N $g^{-1}$ soil $day^{-1}$), $M_0$ is the initial $^{14+15}N$ pool of $NH_4^+$ or $NO_3^-$ ($\mu$g N $g^{-1}$ dry soil), $M_1$ is the post-incubation $^{14+15}N$ pool of $NH_4^+$ or $NO_3^-$ ($\mu$g N $g^{-1}$ dry soil), $H_0$ is the initial $^{15}N$ pool ($\mu$g N $g^{-1}$ dry soil), $H_1$ is the post-incubation $^{15}N$ pool ($\mu$g N $g^{-1}$ dry soil), and $t$ is the incubation time (1 day).

To measure net N mineralization and nitrification rates, four sub-samples from each temperature, water content, and substrate type combination were extracted with 50 ml of 2 M KCl and analyzed for pre-incubation $NH_4^+$ and $NO_3^-$ concentrations, using the FIA. Then, 36 (four for each water content/substrate type combination) subsamples of 100 g each (oven-dry basis) were placed in 250-ml ventilated containers and incubated under the three temperature regimes. Soil water contents during incubation were maintained by weighing the containers every 4 days and adding deionized water if there was a reduction in soil water content. After 30 days of incubation, 25 g (oven-dry basis) of soil were removed from each container and extracted with 50 ml of 2 M KCl to determine post-incubation $NH_4^+$ and $NO_3^-$ concentrations, using the FIA. Net N mineralization rates were calculated by subtracting pre-incubation $NH_4^+$ and $NO_3^-$ concentrations from post-incubation $NH_4^+$ and $NO_3^-$ concentrations divided by the number of incubation days. Net nitrification rates were determined by subtracting the pre-incubation $NO_3^-$ concentrations from post-incubation $NO_3^-$ concentrations and then dividing by the number of incubation days.

Soil microbial biomass C and N were determined using the fumigation extraction method described by Vance et al. (1987) and Brookes et al. (1985), respectively. Water-soluble organic C was determined by extracting 10 g of soil with 30 ml of deionized water for 30 min (Burford and Bremner 1975), followed by analysis on a total organic C analyzer. These measurements were carried out on samples after the 30-day conditioning period.

### Statistical analysis

Only the main treatment effect (i.e., no replication) for temperature was included in the ANOVA model for a 3×3×3 factorial experiment, because temperature was not truly replicated as the incubation was carried out in three incubators. Further analyses were performed to look at the effects of substrate type and moisture content at each temperature level. Least significant difference (LSD) values at $\alpha$=0.05 were calculated when the treatment or interaction effects were significant, to perform multiple comparison of the means. The LSD values were used to test the significance of the differences between the means at the proper interaction level. When no significant interactions were present, the main effects were compared using the same approach. Pearson correlation coefficients were calculated to assess the inter-relationships between the different parameters measured. All analyses were performed using SYSTAT (1994).

## Results

### Soluble organic C, microbial C and N, and microbial C:N ratio

The amount of soluble organic C was generally greater in the ryegrass and lucerne than in the bare ground treatment. However, the two pasture soils were significantly different from each other only at 40 °C and 100% FC and at 40 °C and 75% FC (Fig. 1; ANOVA data not shown). At 40 °C, soil from the ryegrass and lucerne plots on average contained 384 and 354 $\mu$g C $g^{-1}$ soil of soluble organic C, respectively. The corresponding soluble organic C contents in the ryegrass and lucerne soils, respectively, were 151 and 136 $\mu$g C $g^{-1}$ soil at 25 °C and were 142 and 126 $\mu$g C $g^{-1}$ soil at 5 °C. On average, the highest soluble C content was found in the ryegrass soil and the lowest in the bare ground soil; however, differences between the substrate types were relatively small as compared with that caused by the temperature

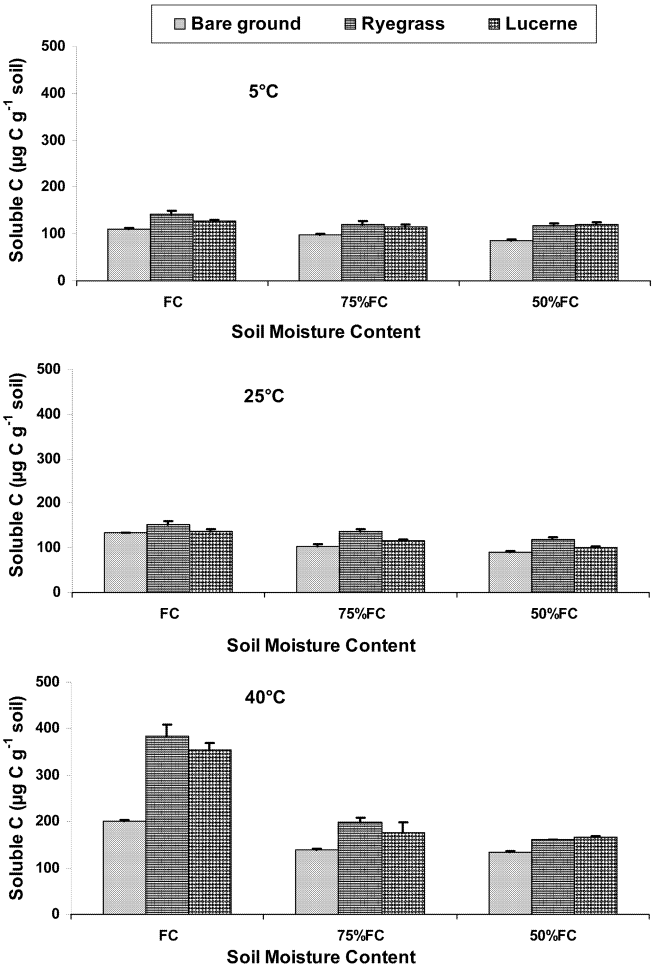


**Fig. 1** Effects of substrate type, temperature, and moisture content on soluble organic C content in a 30-day incubation experiment. *FC* Full field capacity. *Vertical bars* indicate standard errors

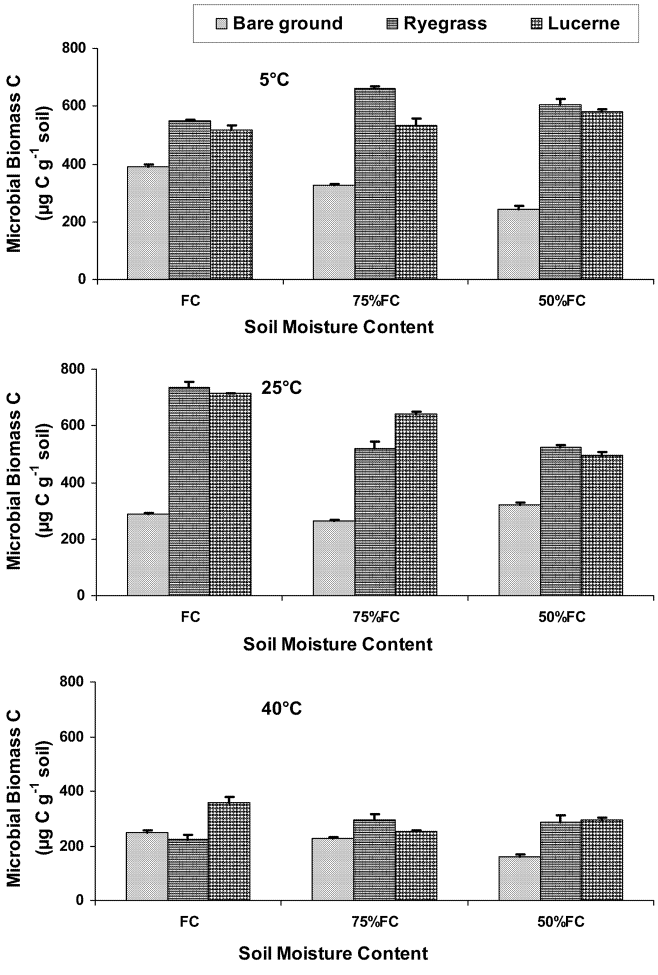


**Fig. 2** Effects of substrate type, temperature, and moisture content on microbial biomass C in a 30-day incubation experiment

treatment (Fig. 1). Differences in soluble C among the moisture treatments were small at 5 and 25 °C incubation temperatures. At 40 °C, the amount of soluble organic C released was significantly affected by moisture content and was in the order: 100% FC> 75% FC >50% FC.

Microbial biomass C was dramatically affected by substrate type and temperature, but less so by moisture content (Fig. 2). Microbial biomass C was significantly higher in the ryegrass and lucerne than in the bare ground soil, except at 40 °C and 100% FC. In terms of the overall temperature effect, microbial biomass C was high at 25 °C (736 $\mu$g) and 5 °C (660 $\mu$g), but decreased dramatically at 40 °C (356 $\mu$g C $g^{-1}$ soil), indicating that the highest incubation temperature used in this experiment was outside the optimum temperature range for microbial growth. Moisture content also influenced microbial biomass C content (Fig. 2), although to a lesser degree and the effect depended on temperature. For example, in the two pasture soils, microbial biomass C at the 25 °C incubation temperature was high at 100% FC and decreased with decreasing water content.

Microbial biomass N exhibited a similar trend to that of microbial biomass C (Fig. 3). Microbial biomass N was generally not different between the two pasture soils, except at 5 °C and 50% FC and at 40 °C and 100% FC. Microbial biomass N was affected by incubation temperature in the following order: 25 °C (135 $\mu$g N $g^{-1}$ soil) >5 °C (107 $\mu$g N $g^{-1}$ soil) >40 °C (79 $\mu$g N $g^{-1}$ soil). Microbial biomass N at 25 and 40 °C was affected by soil moisture content in the following order: 100% FC >75% FC >50% FC, except for the bare ground soil incubated at 25 °C. At 5 °C, microbial biomass N content in the bare ground soil also decreased as soil moisture content decreased.

Microbial biomass C:N ratios varied from 4.6 to 13.0 among the substrate type, temperature, and moisture content combinations (Table 2). At 100% FC, there were little changes in the microbial C:N ratios of the two pasture treatments at any of the temperature regimes. At 75% FC, microbial C:N ratios in all treatments were higher than those at 100% FC, except for the ryegrass and lucerne treatments at 25 °C; and a further increase in microbial C:N ratio generally occurred from 75% FC to

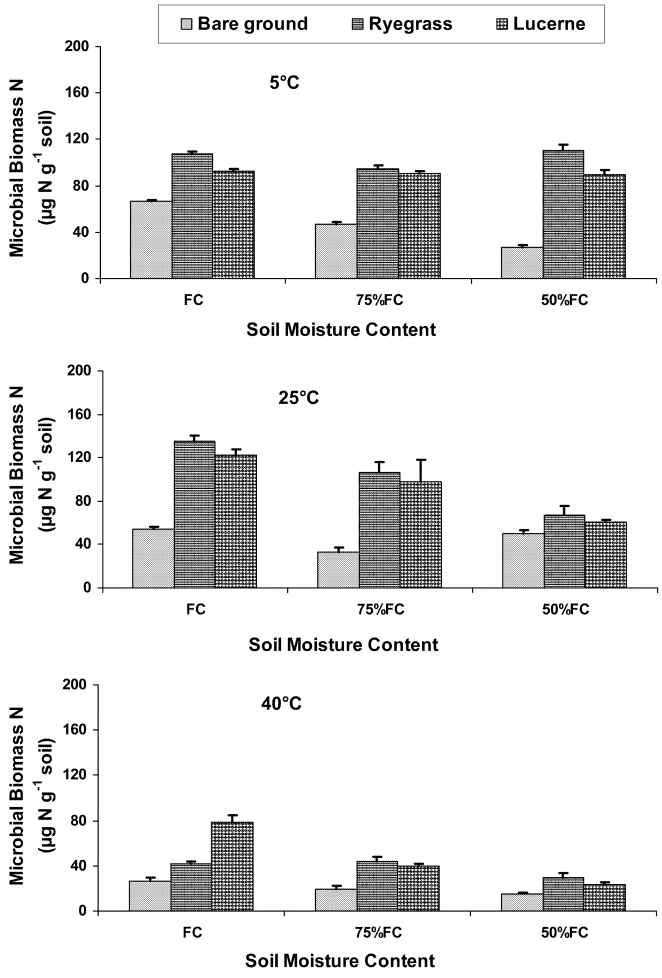


**Fig. 3** Effects of substrate type, temperature, and moisture content on microbial biomass N in a 30-day incubation experiment

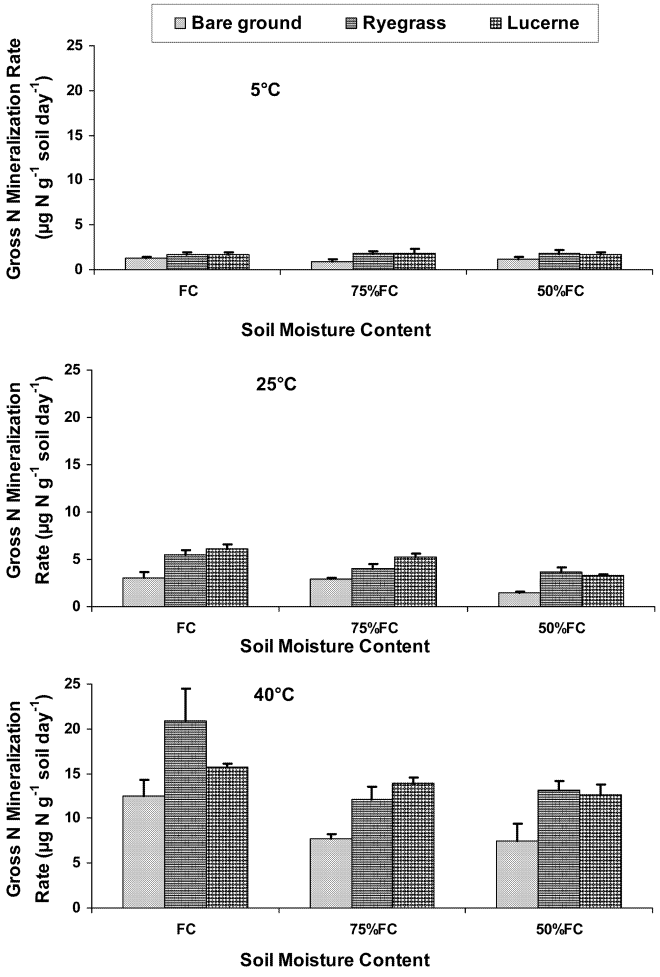


**Fig. 4** Effects of substrate type, temperature, and moisture content on gross N mineralization rates

50% FC. Microbial C:N ratios in the two pasture treatments were in the range of 4.6–5.8 at 100% FC, 5.0–7.0 at 75% FC, and 5.5–13.0 at 50% FC.

## Gross and net N mineralization rates

Substrate type and soil temperature and moisture content affected gross N mineralization rates (Fig. 4; $P<0.001$, ANOVA data not shown). The gross N mineralization rates of the two pasture (ryegrass, lucerne) soils were not significantly different from each other except at 40 °C and 100% FC. At 25 and 40 °C, the rates in the two understorey soils were significantly higher than those in the bare ground soil. When the samples were incubated under 5 °C, there was no significant difference in gross N mineralization rate among the substrate types. In terms of the overall temperature effect, the rates were significantly higher at 40 °C than at 25 and 5 °C, regardless of the substrate type or moisture content. For example, at 100% FC, gross N mineralization rates in the ryegrass soil were 20.9, 5.5, and 1.6 $\mu$g N g$^{-1}$ soil day$^{-1}$ for the 40, 25, and 5 °C treatments, respectively. Soil moisture content had no effect on gross N mineralization rates at 5 °C. At 40 °C, soil moisture content significantly changed gross N mineralization rates and the rates were higher at 100% FC than at 75% FC and 50% FC. The effect of soil moisture

**Table 2** Effects of substrate type, temperature, and moisture content on microbial biomass C:N ratios (mean ±SE; $n$=4)

| | Bare ground | Lucerne | Ryegrass |
|---|---|---|---|
| Full field capacity | | | |
| 5 °C | 5.9±0.13 | 5.6±0.14 | 5.1±0.15 |
| 25 °C | 5.3±0.17 | 5.8±0.21 | 5.5±0.18 |
| 40 °C | 9.9±1.33 | 4.6±0.43 | 5.4±0.44 |
| 75% field capacity | | | |
| 5 °C | 7.2±0.43 | 5.9±0.26 | 7.0±0.24 |
| 25 °C | 8.1±0.76 | 5.5±0.31 | 5.0±0.29 |
| 40 °C | 12.6±1.59 | 6.4±0.29 | 6.9±0.67 |
| 50% field capacity | | | |
| 5 °C | 9.2±0.49 | 6.5±0.32 | 5.5±0.09 |
| 25 °C | 6.6±0.31 | 8.2±0.18 | 8.0±0.78 |
| 40 °C | 10.5±0.53 | 13.0±1.41 | 10.3±2.12 |

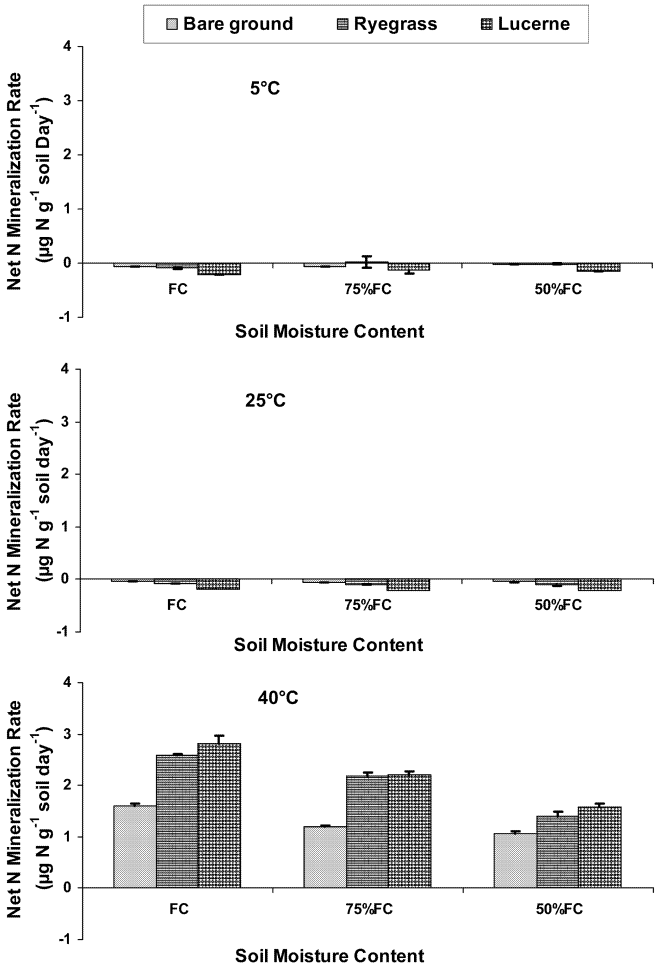


**Fig. 5** Effects of substrate type, temperature, and moisture content on net N mineralization rates in a 30-day incubation experiment

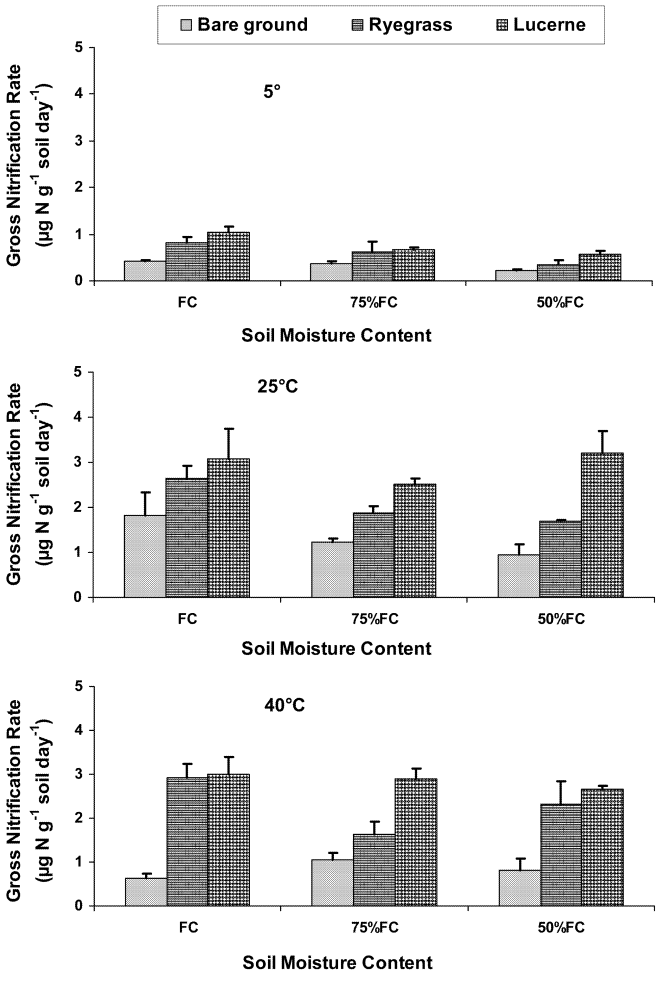


**Fig. 6** Effects of substrate type, temperature, and moisture content on gross nitrification rates

content on gross N mineralization rates was also statistically significant at 25 °C, but at a much smaller scale as compared with incubation at 40 °C.

For net N mineralization rates, differences between the two pasture treatments were significant only when the samples were incubated at 100% FC and 40 °C; and the differences between the two pasture treatments and the bare ground treatment were significant at 40 °C, regardless of soil moisture content (Fig. 5). Incubating the soil samples at 40 °C dramatically increased net N mineralization rates, as compared with those at 25 and 5 °C. For example, at 40 °C, the net N mineralization rate of lucerne at 100% FC was 2.8 $\mu$g N $g^{-1}$ soil $day^{-1}$, compared with –0.18 and –0.21 $\mu$g N $g^{-1}$ soil $day^{-1}$ for the 25 and 5 °C incubation temperatures, respectively. Net N mineralization rates were generally negative (meaning net immobilization) under the 25 and 5 °C incubation conditions. Again, the effects of soil moisture content on net N mineralization rates were more pronounced at 40 °C than at the other two temperatures. Under the highest incubation temperature, net N mineralization rates decreased from 100% FC to 75% FC and also from 75% FC to 50% FC.

## Gross and net nitrification rates

The general trends of substrate type, temperature, and moisture content effects on gross nitrification rates were similar to that of net nitrification rates (Figs. 6, 7). The magnitude of gross vs net nitrification rates was very similar, as compared with that of the gross vs net N mineralization rates, where there was about a 10-fold difference between the gross and net N mineralization rates (Figs. 4, 5) for any particular treatment combination.

Gross nitrification rates were significantly affected by substrate type, temperature, and moisture content (Fig. 6; ANOVA data not shown). The effect of substrate type depended on the incubation temperature. For example, there was no difference between the substrate types at 5 °C, while significant differences were observed at the other two incubation temperatures. Under incubation temperatures of 25 and 40 °C, gross nitrification rates

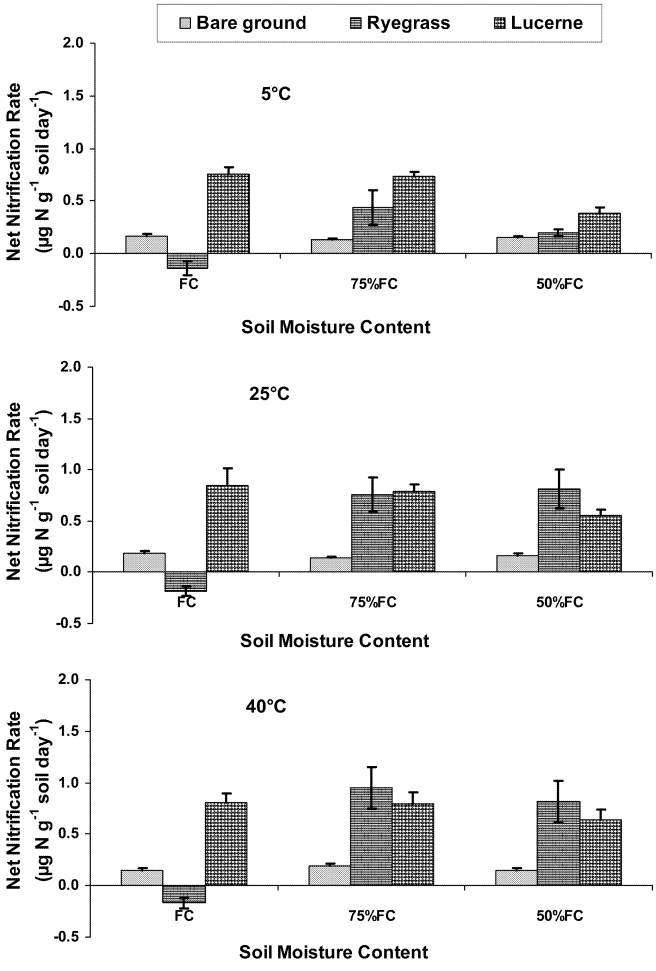


**Fig. 7** Effects of substrate type, temperature, and moisture content on net nitrification rates in a 30-day incubation experiment

were significantly higher in the lucerne than in the ryegrass soils on three occasions: incubation at 25 °C and 75% FC or 50% FC and at 40 °C and 75% FC, respectively. Gross nitrification rates were greater at 25 and 40 °C than at 5 °C, regardless of substrate type and soil moisture content.

Gross nitrification rates also varied with moisture content, but no consistent trend was found. For example, at 25 °C, gross nitrification rates in the lucerne soil were higher at 50% FC than at 75% FC; and, at 40 °C, the rates in the ryegrass soil were higher at 100% FC than at 75% FC, while no such trend was observed in any other treatments.

Net nitrification rates were not significantly affected by temperature (Fig. 7; ANOVA data not shown). However, substrate type and moisture content had significant impacts on net nitrification rates. Net nitrification rates were higher in the two pasture soils than in the bare ground soil, except at 100% FC, when net nitrification rates in the ryegrass soil were negative. The rates were lower at 100% FC than at 75% FC or 50% FC for the ryegrass soil, but were otherwise unaffected by soil moisture content in the other two substrate types.

## Discussion

### Soluble organic C and microbial biomass C and N

Soluble organic C, mineral N, and other nutrients released from above- and belowground litter are generally first utilized by soil microflora for their maintenance and growth (Holmes and Zak 1994). However, the high amounts of soluble organic C at 40 °C (Fig. 1) might have been caused by enhanced substrate decomposition or microbial mortality (Joergensen et al. 1990). At 25 °C, the amount of soluble organic C released was higher than that at 5 °C, but did not reach the level achieved at 40 °C (Fig. 1). The higher amounts of microbial biomass C at 40 °C than at 5 and 25 °C was consistent with the higher soluble C content at 40 °C (Figs. 1, 2). The high amounts of soluble organic C at elevated temperature could also have been caused by thermal denaturation or changes in the microbial population (Sierra and Marban 2000). The optimum temperature for microbial growth is usually around 25 °C (Paul and Clark 1996). Sierra and Marban (2000) reported that microbial biomass C decreases with increasing temperature up to 50 °C, from its highest level at 20 °C. Joergensen et al. (1990) also showed that increasing incubation temperature from 25 to 35 °C resulted in a higher specific death rate of microorganisms, as measured by $CO_2$ evolution and ATP contents. However, optimum temperatures for microbial activity much lower than 25 °C have also been reported. For example, in an incubation experiment using an acid forest soil, temperature appeared to be more important than moisture content in controlling microbial biomass and dehydrogenase activity; and increasing the temperature between 5 and 25 °C significantly decreased microbial biomass and dehydrogenase activity (Arnold et al. 1999).

In this study, soil moisture content affected microbial biomass C and the highest microbial biomass C was usually obtained at 100% FC, except at the 5 °C incubation temperature (Fig. 2). This is consistent with Sierra and Marban (2000) and Zaman et al. (1999a), who also reported that microbial biomass C was highest at or near 100% FC and decreased with decreasing soil water content. Both lucerne and ryegrass understories had a significant influence on microbial biomass C as compared with the bare ground treatment because, presumably, the higher organic C contents in the two pasture soils (Table 1) stimulated microbial growth (Gaillard et al. 1999; Zaman et al. 1998). The highest microbial biomass N was generally observed at 100% FC, followed by 75% FC; and it decreased markedly from 75% FC to 50% FC, except for the two pasture soils at 5 °C and the bare ground soil at 5 °C (Fig. 3). These results are comparable with those of Zaman et al. (1999a), who also reported highest microbial biomass N at 100% FC.

The generally higher amounts of soluble organic C in the two pasture soils than in the bare ground soil, particularly under the 40 °C incubation condition or in the ryegrass soil, is a reflection of the greater total soil organic C content in the two pasture soils (Table 1). High soluble C concentrations have been found to stimulate microbial activities (Gaillard et al. 1999), because organic substrates are sources of energy for the microbes (Staaf and Berg 1981; Zaman et al. 1998). Therefore, substrate type had a marked impact on microbial biomass C and N at all temperature and moisture content combinations studied in this experiment. This is consistent with the work of Nilsson et al. (1999), who, in testing the effect of plant litter species composition and diversity on boreal forest plant–soil system properties, found that the presence of litter (a source of readily available C) was important in influencing a range of above- and belowground properties and processes.

Incubation conditions in this study represent the range of soil environmental conditions that can typically be found (e.g., soil moisture content from full to 50% FC and soil temperature from 5 to 25 °C) or can be found under somewhat extreme conditions (e.g., soil temperature at 40 °C). The temperature and moisture effects are most strongly seen at 40 °C, relative to 5 and 25 °C (the results were similar under these two incubation temperatures). Therefore, under the range of conditions studied, the soils were responsive to changes in the environmental conditions in their soluble organic C and microbial biomass C and N contents.

## Gross and net N mineralization and nitrification rates

The greater gross N mineralization rates at 40 °C than at the other incubation temperatures contrasts with the reversed pattern in microbial biomass C and N contents (Figs. 2, 3, 4). The greater gross N mineralization rates under the highest temperature treatment could be related to the increased rate of substrate decomposition or the lysis of dead microbial biomass at elevated temperatures, as evident from the release of higher amounts of soluble organic C (Fig. 1; Joergensen et al. 1990; Sierra and Marban 2000; Sims 1995). Joergensen et al. (1990) reported that increasing incubation temperature from 25 to 35 °C resulted in enhanced substrate decomposition due to increased specific death rates of microorganisms. Bonde et al. (1988) also observed a downward drift in microbial biomass at an incubation temperature of 37 °C. Higher gross N mineralization rates with increasing temperature were also reported by Stottlemyer and Toczydlowski (1999).

The net N mineralization rates at 40 °C were higher than at the other incubation temperatures (Fig. 5), which is consistent with the findings of Sierra and Marban (2000), who reported that net N mineralization rates were very slow between 20 and 30 °C at 100% FC and increased with increasing temperature from 40 to 50 °C at the same moisture content. The negative values of net N mineralization rates at 5 and 25 °C (Fig. 5) were due to the active immobilization of N by the microbial pool at that temperature (Figs. 2, 3). An active microbial pool can have both high gross N mineralization and immobilization (Stottlemyer and Toczydlowski 1999), which results in either very small or negative net N mineralization rates, depending on the balance of the two concurrent processes. Net N mineralization rates did increase under high temperature and moisture contents in the two pasture soils but did not increase to the same magnitude as that of the gross N mineralization rates, because it is the balance between mineralization and immobilization that determines the net N mineralization rate (Schmidt et al. 1999). For example, at 100% FC, gross N mineralization rates were 7.5, 34, and 29 times higher than net mineralization rates at 40, 25 and 5 °C, respectively (Figs. 4, 5). These results are comparable with those of Stottlemyer and Toczydlowski (1999), who reported that gross N mineralization rates were 23 times higher than net mineralization rates in a forest soil. Net N mineralization rates therefore do not give a clear picture of the total rate of microbial activities, because they do not provide information on the rate of the microbial immobilization which is concurrent with the N mineralization process. Both net and gross rates of N mineralization were low at 5 and 25 °C incubation conditions, regardless of the substrate type or moisture content, indicating generally low microbial activities.

Net N mineralization rates in the two pasture soils were similar when incubated at 40 °C, regardless of moisture content; and the absolute values in the two pasture soils were significantly higher than those in the bare ground soil (Fig. 5). The accumulation of above- and belowground plant residues can affect N mineralization and microbial biomass (Fisk et al. 1998). Therefore, the higher net N mineralization rates in the two pasture soils could be due to the enrichment of soil with organic substrates from above- and belowground residues of ryegrass and lucerne (Table 1).

The effect of soil moisture content (under 25 and 40 °C incubation conditions) on gross N mineralization rates (Fig. 4) was consistent with studies conducted by Zaman et al. (1999a) and Stottlemyer and Toczydlowski (1999), who also reported higher gross N mineralization rates at around 100% FC than in treatments with less soil moisture content. At 100% FC, the condition for microbial activity was at the optimum level because, on the one hand, there was sufficient water available for the metabolism of microbial and other organisms and for the movement of soil organisms to access substrates in the soil and, on the other hand, there was still good soil aeration. The similar gross N mineralization rates among the soil moisture levels under the 5 °C incubation condition appeared to indicate that microbial activities were limited by the low temperature.

Gross nitrification rates were highest at 25 °C and were essentially very similar to the rates at 40 °C, but were very low at 5 °C (Fig. 6), indicating that the activity of nitrifying bacteria is optimal at or around 25 °C. This

**Table 3** Correlation coefficients (*r* values) among gross and net rates of N mineralization (*min.*) and nitrification (*nit.*), soluble organic C, and microbial biomass (*MB*) C and N (*n*=27)

| Variable | Gross min. rate | Net min. rate | Gross nit. rate | Net nit. rate | Soluble C | MB-C | MB-N |
|---|---|---|---|---|---|---|---|
| Gross min. rate | 1.0 | | | | | | |
| Net min. rate | 0.94** | 1.0 | | | | | |
| Gross nit. rate | 0.58** | 0.36 | 1.0 | | | | |
| Net nit. rate | 0.08 | 0.08 | 0.25 | 1.0 | | | |
| Soluble C | 0.86** | 0.83** | 0.43* | –0.04 | 1.0 | | |
| MB-C | –0.46* | –0.60** | 0.09 | 0.27 | –0.28 | 1.0 | |
| MB-N | –0.39* | –0.50** | 0.09 | 0.19 | –0.12 | 0.94** | 1.0 |

* Significant at $P<0.05$
** Significant at $P<0.01$

contrasts with the optimum temperature for gross N mineralization rates. Our results are consistent with other reports that the optimum temperature for nitrification ranges over 25–35 °C, although nitrification may still occur below 5 °C and above 40 °C (Bremner and Blackmer 1981; Sierra and Marban 2000). However, our results do not support the earlier findings of Stottlemyer and Toczydlowski (1999), who observed that both net and gross nitrification rates declined with increasing temperature from 15 to 20 °C. They attributed the lack of positive response in nitrification to increasing temperature to the limited availability of soluble organic C in their soils.

Gross nitrification rates in the two pasture soils were significantly higher than those in the bare ground soil, due to greater $NH_4^+$ production (i.e., greater gross N mineralization rates) in the former. In other studies, gross nitrification rates have been measured within a short period of time [ranging from 24 h (Davidson et al. 1991; Matheson 2001) to 72 hr (Zaman et al. 1999a, 1999b)] after the application of $^{15}N$ labeled $NO_3^-$. However, net nitrification measurements are usually carried out after longer periods [that range from 7 days (Zaman et al. 2002) to 30 days (Stottlemyer and Toczydlowski 1999)], during which changes in microbial community composition could complicate the interpretation of net nitrification rates. Recently, Matheson (2001), using the isotopic dilution technique, observed that a considerable amount of the added $^{15}NO_3^-$ was lost to $NO_3^-$ consumptive processes, such as microbial immobilization, denitrification, and dissimilatory $NO_3^-$ reduction to $NH_4^+$ pools within 10–60 min of the $^{15}N$ addition, indicating that net nitrification rates can underestimate microbial activities in the soil.

Net immobilization of $NO_3^-$ was uncommon during the incubations, regardless of the treatment, with the ryegrass soil incubated at 100% FC as an exception (Fig. 7) for which we do not have an explanation. The positive nitrification rates in the 5 and 25 °C incubations, regardless of substrate type and moisture content (with the exception noted above), contrast with those of the net mineralization rates. Rates for net nitrification were not very different between the temperature treatments, indicating that the optimum temperature for net nitrification may be much lower than that for net mineralization. The net nitrification rates were again very similar between the two pasture soils (except for the ryegrass soil at 100% FC), indicating the similarity between these two substrate types in their influence on microbial activities.

## Inter-relationships

Gross N mineralization rates were positively correlated with net N mineralization rates (Table 3), indicating that similar factors regulated the processes of N consumption (immobilization) and the production (mineralization) of N during the incubation (Burke et al. 1989; Stottlemyer and Toczydlowski 1999). Gross nitrification rates were positively correlated with gross N mineralization rates, indicating that nitrification was favored by increased mineralization rates, as $NH_4^+$ is used as a substrate for nitrification. The lack of any significant correlation between gross and net nitrification rates is consistent with Stottlemyer and Toczydlowski (1999). Soluble organic C showed a positive correlation with gross and net N mineralization rates and with gross nitrification rates. This relationship further suggests that the mineralization rate increases in part because of the increase in available soluble organic C, as the heterotrophic microorganisms need readily available sources of energy. The relationships between soluble organic C and gross nitrification rates indicated that soluble organic C may enhance nitrification indirectly by stimulating mineralization, because autotrophic micro-organisms, which mostly derive their C from inorganic sources,are involved in the nitrification processes. Both microbial biomass C and N were found to be negatively correlated with gross N and net N mineralization rates. The negative correlation between microbial C and N and N mineralization rates indicates that microbial biomass acted as a sink for N (immobilization) in this incubation experiment. The relationship between net mineralization rate and microbial biomass N and C further explains that N availability was directly controlled by microbial activity. These findings contrast with those of Holm and Zak (1994) and Sierra and Marban (2000), who found no relationship between net N mineralization rate and microbial biomass.

## Conclusions

Under the conditions studied, temperature clearly has a dominant influence on soil microbial activities, including the solubilization of organic C, microbial biomass C and N, gross N mineralization and nitrification rates, and net mineralization rates, since the magnitude of changes in the measured parameters was much greater in response to temperature changes (in the studied range of 5–40 °C) than to changes in soil moisture content or substrate type. In the field, periods with high temperature are always associated with greater demands for water for evapotranspiration, which may lead to lower soil moisture contents. Although, in this short-term incubation study, net nitrification rates at 40 °C incubation temperature showed little response to the increased rate of ammonification, we expect nitrification rates to increase if the length of incubation is increased. Amatya et al. (2002) found that soil $NO_3^-$ concentrations increased throughout the summer in the bare ground plots, but not in the ryegrass plots, because $NH_4^+$ accumulated in the bare ground plots due to lack of plant uptake. The two pasture soils showed remarkable similarities, in contrast to the bare ground soil, in all of the parameters measured under most of the treatment combinations. We conclude that the continuous use of land for bare ground or pasture species changed the soil properties in terms of their soluble organic C content, their ability to support microbial populations, and their rates of N mineralization and nitrification.

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## References

Amatya G, Chang SX, Beare M, Mead DJ (2002) Soil properties under a *Pinus radiata*-ryegrass silvopastoral system in New Zealand. Part II. C and N of soil microbial biomass, and soil N dynamics. Agrofor Syst 54:149–160

Arnold SS, Fernandez IJ, Rustad LE, Zibilske LM (1999) Microbial response of an acid forest soil to experimental soil warming. Biol Fertil Soils 30:239–244

Barraclough D (1995) $^{15}N$ isotope dilution techniques to study soil nitrogen transformations and plant uptake. Fert Res 42:185–192

Blair N, Crocker GJ (2000) Crop rotation effects on soil carbon and physical fertility of two Australian soils. Aust J Soil Res 38:71–84

Bonde TA, Schnurer J, Rosswall T (1988) Microbial biomass as a fraction of potential mineralizable nitrogen in soils from long-term field experiments. Soil Biol Biochem 20:447–452

Bremner JM, Blackmer AM (1981) Terrestrial nitrification as a source of atmospheric nitrous oxide. In: Delwiche CC (ed) Denitrification, nitrification and atmospheric nitrous oxide. Wiley, New York, pp 151–170

Brookes PC, Landemann A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 7:837–842

Burford JR, Bremner JM (1975) Relationships between the denitrification capacities of soils and total water soluble and readily decomposable soil organic matter. Soil Biol Biochem 7:389–394

Burke IC, Reiners WA, Schimel DS (1989) Organic matter turnover in a sagebrush steppe landscape. Biogeochemistry 7:11–31

Chang SX, Amatya G, Beare MH, Mead DJ (2002) Soil properties under a *Pinus radiata*–ryegrass silvopastoral system in New Zealand. Part 1. Soil N and moisture availability, soil C, and tree growth. Agrofor Syst 54:137–147

Chang X, Juma NG (1996) Impact of crop rotations on microbial biomass, faunal populations, and plant C and N in a Gray Luvisol (Typic Cryoboralf). Biol Fertil Soils 22:31–39

Clarholm M, Rosswall T (1980) Biomass and turnover of bacteria in a forest soil and a peat. Soil Biol Biochem 12:49–57

Davidson EA, Hart SC, Shanks CA, Firestone MK (1991) Measuring gross N mineralization, immobilization, and nitrification by $^{15}N$ isotopic pool dilution in intact soil cores. J Soil Sci 42:335–349

Fisk MC, Schmidt SK, Seastedt TR (1998) Topographic patterns of above and belowground production and nitrogen cycling in alpine tundra. Ecology 79:2253–2266

Gaillard V, Chenu C, Recous S, Richard G (1999) Carbon, nitrogen and microbial gradients induced by plant residues decomposing in soil. Eur J Soil Sci 50:567–578

Gill K, Jarvis SC, Hatch DJ (1995) Mineralization of nitrogen in long-term pasture soils: effects of management. Plant Soil 172:153–162

Goh KM, Mansur I, Mead DJ, Sweet GB (1996) Biological nitrogen fixing capacity and biomass production of different understorey pastures in a *Pinus radiata*-pasture agroforestry system in New Zealand. Agrofor Syst 34:33–49

Haggar JP, Tanner EVJ, Beer JW, Kass DCL (1993) Nitrogen dynamics of tropical agroforestry and annual cropping systems. Soil Biol Biochem 45:1363–1378

Hart SC, Nason GE, Myrold DD, Perry DA (1994) Dynamics of gross nitrogen transformations in an old forest: the carbon connection. Ecology 75:880–891

Holmes WE, Zak DR (1994) Soil microbial biomass dynamics and net nitrogen mineralization in Northern hardwood ecosystems. Soil Sci Soc Am J 58:238–243

Joergensen RG, Brookes PC, Jenkinson DS (1990) Survival of the soil microbial biomass at elevated temperatures. Soil Biol Biochem 22:1129–1136

Matheson FE (2001) Nitrogen removal and the fate of nitrate in riparian buffer zones. PhD thesis, University of Durham, Durham

Mead DJ (1995) The role of agroforestry in industrialized nations: the southern hemisphere perspective with special emphasis on Australia and New Zealand. Agrofor Syst 31:143–156

Miltner A, Zech W (1999) Microbial degradation and resynthesis of proteins during incubation of beech leaf litter in the presence of mineral phase. Biol Fertil Soils 30:48–51

Nilsson MC, Wardle DA, Dahlberg A (1999) Effects of plant litter species composition and diversity on the boreal forest plant–soil system. Oikos 86:16–26

Paul EA, Clark FE (1996) Soil microbiology and biochemistry, 2nd edn. Academic Press, San Diego

Sarathchandra SU, Perrot KW, Littler RA (1989) Soil microbial biomass: Influence of simulated temperature changes on size, activity, and nutrient content. Soil Biol Biochem 21:987–993

Schmidt IK, Jonasson S, Michelson A (1999) Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. Appl Soil Ecol 11:147–160

Sierra J, Marban L (2000) Nitrogen mineralization pattern of an Oxisol of Guadeloupe, French West Indies. Soil Sci Soc Am J 64:2002–2010

Sims JT (1995) Organic wastes as alternative nitrogen sources. In: Bacon PE (ed) Nitrogen fertilization in the environment. Dekker, New York, pp 487–535

Staaf H, Berg B (1981) Plant litter input to soil. In: Clark FE, Rosswall T (eds) Terrestrial nitrogen cycles: processes,

ecosystem strategies and management impacts. Ecological Bulletin, Stockholm, pp 147–167
Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci Soc Am J 60:1846–1855
Stottlemyer R, Toczydlowski D (1999) Nitrogen mineralization in a mature boreal forest, Isle Royale, Michigan. J Environ Qual 28:709–720
SYSTAT (1994) SYSTAT for Windows, ver 5. SYSTAT, Evanston, Ill.
Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707
Verburg PSJ, Dam DV, Hefting MM, Tietema A (1999) Microbial transformations of C and N in a boreal forest floor as affected by temperature. Plant Soil 208:187–197
Yentsch CM, Yentsch CS (1989) Flow cytometric analysis of microbial populations. In: Hattori T, Ishada Y, Maruyama Y, Morita RY, Uchida A (eds) Recent advances in microbial ecology. Japan Scientific Society, Tokyo, pp 707–711
Yunusa IAM, Mead DJ, Pollock KM, Lucas RJ (1995) Process studies in a *Pinus radiata*–pasture agroforestry systems in a subhumid temperate environment. I. Water use and light interception in the third year. Agrofor Syst 32:163–183
Zaman M, Cameron KC, Di HJ, Noonan MJ (1998) Nitrogen mineralisation rates from soil amended with dairy pond waste. Aust J Soil Res 36:217–230
Zaman M, Di HJ, Cameron KC, Frampton CM (1999a) Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. Biol Fertil Soils 29:178–186
Zaman M, Di HJ, Cameron KC (1999b) A field study of gross rates of N mineralization and nitrification rates and their relationships to microbial biomass and enzyme activities in soils treated with dairy effluent and ammonium fertilizer. Soil Use Manag 15:188–194
Zaman M, Di HJ, Sakamoto K, Goto S, Hayashi H, Inunbushi K (2002) Effect of sewage sludge compost and chemical fertilize application on microbial biomass and N mineralization rates. Soil Sci Plant Nutr 48:195–201