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NITROGEN IN ECTOMYCORRHIZAL MAT AND NON-MAT SOILS OF DIFFERENT-AGE DOUGLAS-FIR FORESTS

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Summary—In an attempt to determine how stand age and mat-forming fungi affect N chemistry in forest soils, soils with or without ectomycorrhizal mats were collected from five Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] forest stands, age 2–450 yr, in the Oregon Cascade Mountains. A number of chemical and biological variables were measured in these soils, including: soil organic matter (SOM), pH, total N, inorganic N, extractable NH_4^+ and NO_3^-, fungal hyphal length, numbers of bacteria, and labile C and N. Total soil N and labile N both were significantly greater in old-growth soils than in other soils, suggesting that forest soils become enriched in organic N with age. Labile C concentrations were higher in mat than in non-mat soils of all age classes but were significantly higher only in the older stands. This finding suggests that ectomycorrhizal mat communities affect soil N with age both by selectively removing organic N and by producing compounds with high C:N ratios.

INTRODUCTION

It is generally accepted that disturbance alters both the composition and quantity of soil organic matter (SOM) in forest soils and that, as secondary succession proceeds, these changes may be reversed (Vitousek *et al.*, 1989). For example, in a study of northern hardwoods, Aber *et al.* (1978) found that both N availability and the amount of forest floor organic matter declined for 15–30 yr after cutting and that system recovery to pre-cut amounts took 60–80 yr. The degree to which SOM changes after a disturbance is influenced by (1) the characteristics of the detritus both present at the time of the disturbance and subsequently added, (2) climate, (3) soil N and P availability, (4) the type and severity of the disturbance and (5) stand successional stage at the time of disturbance (Vitousek *et al.*, 1988).

We examined differences in the SOM both of different-age forest stands and of mat and non-mat soils within the same stands in order to test two hypotheses: first, that stand age can affect qualitative and quantitative characteristics of soil N in both mat and non-mat soils, and second, that stand age has an effect on observed differences between mat and non-mat soil chemistry.

Our first objective was to determine how SOM, total N, labile (mineralizable) N, extractable NH_4^+ and NO_3^-, and microbial biomass in both mat and non-mat soils of Douglas-fir-dominated stands change with stand age. Research of this nature has been rare for coniferous forests of the Pacific Northwest (Edmonds *et al.*, 1989). The second objective was to determine how ectomycorrhizal mat communities alter labile C and N concentrations in colonized soils of different-age Douglas-fir stands. This determination is of particular interest because it has been hypothesized that mat communities can selectively remove organic N from forest soils (Griffiths *et al.*, 1990, 1991).

Gautieria sp. mats were examined in this study both because this ectomycorrhizal fungus forms easily identifiable white-grayish mats in mineral soil (Griffiths *et al.*, 1991) and so can be studied in contrast with soils not obviously colonized by fungal material, and because this species occurs frequently in Douglas-fir forests (Luoma, 1991).

MATERIALS AND METHODS

Site description

The five stands were located in the H. J. Andrews Research Forest, Blue River, Ore. All sites had similar aspects (southwest), elevations (850–975 m) and slopes (20%), but they differed in both age and disturbance history as follows. One site had been shelterwood-harvested 2 yr and slash-burned 1 yr prior to sampling, leaving 20 old-growth trees ha^{-1} or *ca* 33% of the original tree concentration; no new trees had been planted at this site. Two sites had been clear-cut, burned and replanted, one site 11 yr and the other 36 yr prior to sampling. The remaining two sites consisted of a mature stand (age 130 yr), created by natural fire disturbance, and an old-growth stand (age 450 yr).

Sample collection and preparation

At all sites except one, five replicate soil samples with adjacent *Gautieria* mat and non-mat soils were

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collected. The exception was the 11 yr old stand from which only two replicate samples were collected because of the limited number of ectomycorrhizal mats present at the site. In the 2 yr old shelterwood, mat soil was found only near the rooting zone of the remaining old-growth trees on this site.

Mineral soil with the litter horizon removed was sampled to a depth of 10 cm. Samples were placed in plastic bags and transported in an ice chest to the analytical laboratory where the samples were stored at 5°C until they were processed for analysis. Subsamples for the total N and the NO_3^- and SOM measurements were air dried and sieved (2 mm) prior to analysis. Bacteria counts and fungal hyphal measurements were made on non-sieved field-moist soils. Labile C and N and extractable NH_4^+ measurements were made on sieved field-moist soils.

Analytical methods

SOM was estimated by loss on ignition (6 hr, 550°C). Total soil N content was measured with Kjeldahl digestion (Bremner and Mulvaney, 1982). The inorganic N species (NH_4^+ and NO_3^-) were extracted by adding 5.1 ml 2M KCl to 1 g soil. Extractable NH_4^+ concentrations were determined after 1 h with a selective ion electrode (Corning ammonium electrode, Medford, Mass.). Extractable NO_3^- concentrations were measured with an Alpkem Rapid Flow Analyzer 300.

Labile C and N were assayed with the following 14 day procedure. On the first day, 10 g samples of field-moist soil were added to 55 ml test tubes, which were then sealed with a serum bottle stopper and microwaved three times at 1400 W for 55 s, with 1-min intervals between exposures. The microwaving technique was based on one developed by Hendricks and Pascoe (1988) to prevent soil overheating. On the second day, 1 ml sterile deionized NH_4^+-free water was injected into each tube after an equal volume of headspace was removed. After 14 days, CO_2 and N_2O production were measured by gas chromatography (Griffiths *et al.*, 1990), and NH_4^+ concentrations in the incubated soils were assayed by using the previously described procedures for measuring extractable NH_4^+ concentrations in the original soil samples. Labile C was calculated from the observed CO_2 concentrations. Labile N was calculated as (NH_4^+ in the incubated soil) + (N_2O produced) − (extractable NH_4^+ and NO_3^- before incubation).

Hyphal lengths of total and active fungi and bacteria counts were measured with the techniques of Ingham *et al.* (1991). For the fungal hyphal length measurements, 1 g field-moist soil was added to 9 ml sterile H_2O and shaken for 5 min in a 0.3 m arc to release organisms from the soil particles. Then, 2 ml 20 μg ml^{-1} fluorescein diacetate solution (pH 6.0) was added to 1 ml of the 1:10 soil slurry. The stained soil suspension was allowed to stand for at least 3 min, and then 1 ml of 1% molten agar containing 0.5 M dibasic potassium phosphate (pH 9.0) was added to it. Hyphal lengths were then measured with a Zeiss epifluorescence-phase contrast microscope (160 × total magnification). For the total bacterial counts, a 1:100 soil solution was stained with 1 ml fluorescein isothiocyanate solution (Babiuk and Paul, 1970) for at least 3 min. The solution was then passed through a 0.2 μm membrane filter followed by 1 ml sodium bicarbonate and 1 ml pyrophosphate solution. All bacteria in 10 fields were counted for each soil sample.

All data were analyzed with Statgraphics (Statistical Graphics Corporation, Rockville, Md) for the personal computer. The data were log-transformed and analyzed for 2-way interactions between stand age and soil type (mat or non-mat). Mean separations were based on Fisher's Protected Least Significant Difference with $P \leq 0.05$. To evaluate the relationship between stand age and total N concentrations, Spearman Rank Correlation was used.

RESULTS AND DISCUSSION

Soil N

Both non-mat and ectomycorrhizal mat-colonized mineral soils in the old-growth stand had significantly higher total N concentrations than did any of the other soil samples on a g dry weight basis [Fig. 1(a)].

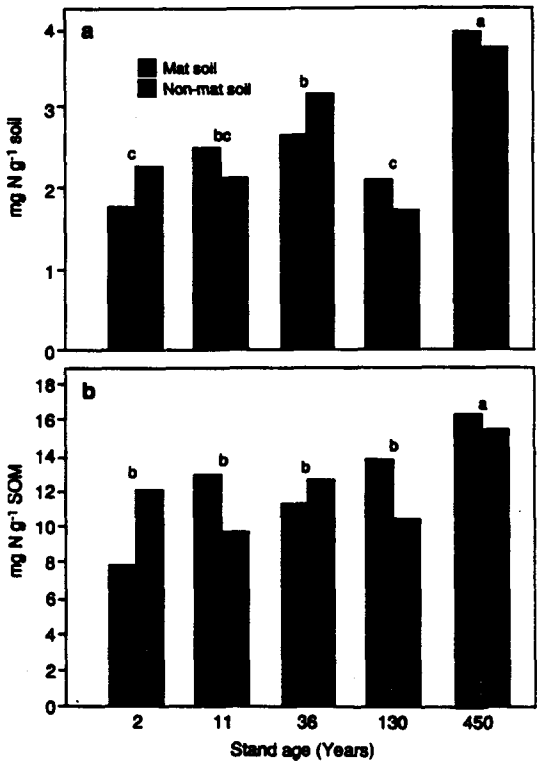

Fig. 1. Total N in mineral soil as: (a) mg N g^{-1} dry wt soil and (b) mg N g^{-1} SOM. There were no significant differences (at $P < 0.05$) observed between mat and non-mat soils of the same age, but there were significant differences among different-age soils, as indicated by the different letters.

Table 1. Chemistry of mat and non-mat soils collected from different-age stands

	pH		SOM %		Extractable NH_4^+ ng g^{-1} N		Extractable NO_3^- pg g^{-1} N	
Stand age*	Mat	Non-mat	Mat	Non-mat	Mat	Non-mat	Mat	Non-mat
2	4.44	4.90	24.1	18.9	170	200	65.5	43.2
11	4.50	4.73	20.7	22.0	210	210	1.1	3.3
36	4.28	4.38	25.7	26.3	300	350	2.7	4.4
135	4.18	4.48	15.8	16.8	320	280	45.2	31.3
450	4.33	4.48	25.0	24.9	220	200	NA†	NA†

*Age since last major disturbance.
†Data not available.

When total N was normalized to g SOM, significant differences between total N in old-growth stands and in other stands still existed [Fig. 1(b)]. In addition, there was a significant positive linear correlation ($P < 0.001$, $r = 0.56$) between stand age and total N normalized to g SOM. There was no significant difference, however, between total N concentrations in mat and non-mat soils in the same stand. These results suggest not only that old-growth soils contain more N g^{-1} dry wt, but also that there is an enrichment of SOM N with stand age. The results diverge from those of Gholz *et al.* (1985) who observed that in a slash-pine plantation ecosystem in northern Florida, both SOM and total soil N declined during the first 30 yr after disturbance. The difference in results may be explained by the post-harvest soil treatment practices and relatively short rotation cycle used in the Florida plantations.

In our samples, extractable NO_3^- and NH_4^+ typically comprised less than 0.01% of total N [Table 1, Fig. 1(a)], implying that the soil N was essentially all in organic form. From this we conclude that SOM becomes enriched in organic N over time. Assuming that N:SOM ratios parallel N:C ratios, the shifts observed in the N:SOM ratios suggest a decrease in C relative to N with stand age. Similar C:N ratio decreased over time has been observed for the decay of leaf litter (Berg *et al.*, 1982; Staaf and Berg, 1982; McClaugherty *et al.*, 1985; Blair, 1988; White *et al.*, 1988), small woody debris (Edmonds, 1987), coarse woody debris (Harmon *et al.*, 1986) and forest humus (Henderson, 1985).

It has been suggested that N enrichment in these decaying materials may be due either to the sequestration of N as proteins and peptides into humic acids during decomposition (Suberkropp *et al.*, 1976) or to the immobilization of N in the form of microbial biomass (Staaf and Berg, 1982). It is also thought that because N is often a growth-limiting element in forest soils (Edmonds *et al.*, 1989) and may limit litter decay rates (McClaugherty *et al.*, 1985), N is preferentially retained while C is oxidized to CO_2 (Staaf and Berg, 1982). Microbial biomass as measured by hyphal length and bacterial numbers [Fig. 2(a) and (b)] and labile N (Myrold, 1987) [Fig. 3(b)] did not increase consistently over time. Thus we can eliminate increased stocks of microbial biomass as the cause for the observed gradual enrichment in total N with stand age [Fig. 1(b)].

The apparently higher C:N ratios in the soil of younger stands [Fig. 1(b)] could be due either to the loss of N from SOM or to the input of new SOM with high C:N ratios during disturbance. The many reports of N released from forest soils as the result of fire (Grier, 1975; Boerner, 1982) and other disturbances (Vitousek *et al.*, 1981; Binkley, 1984) support the former hypothesis; all of the disturbed sites in this study had been burned as a part of the disturbance.

Labile N and C

For all age classes, the mat soil samples contained less labile N than the non-mat soil samples, regardless of whether the amount of labile N was calculated on a dry weight [Fig. 3(a)], unit SOM [Fig. 3(b)], or percent of total N [Fig. 3(c)] basis. The difference was statistically significant only for the old-growth soils, however. As elaborated in the following paragraph,

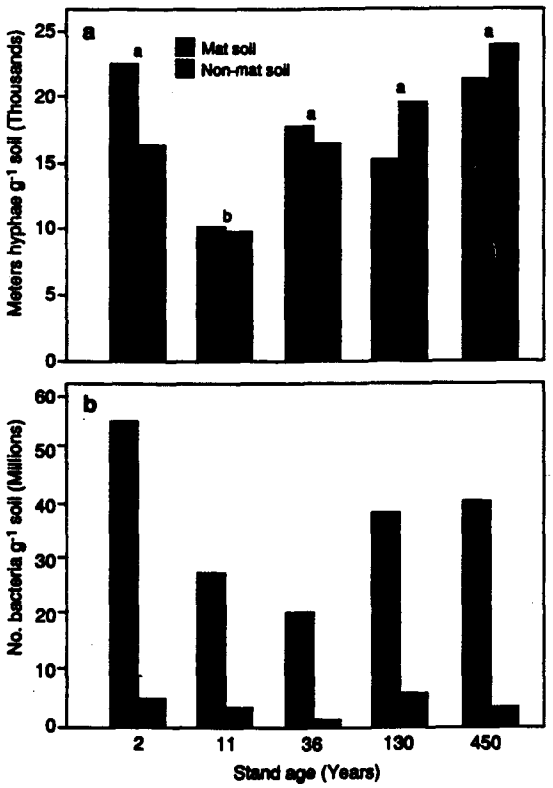

Fig. 2. Microbial biomass as: (a) m fungal hyphae g^{-1} soil (exclusive of fungal rhizomorph material) and (b) bacteria g^{-1} soil. Significant differences (at $P < 0.05$) among different-age soils are indicated by different letters.

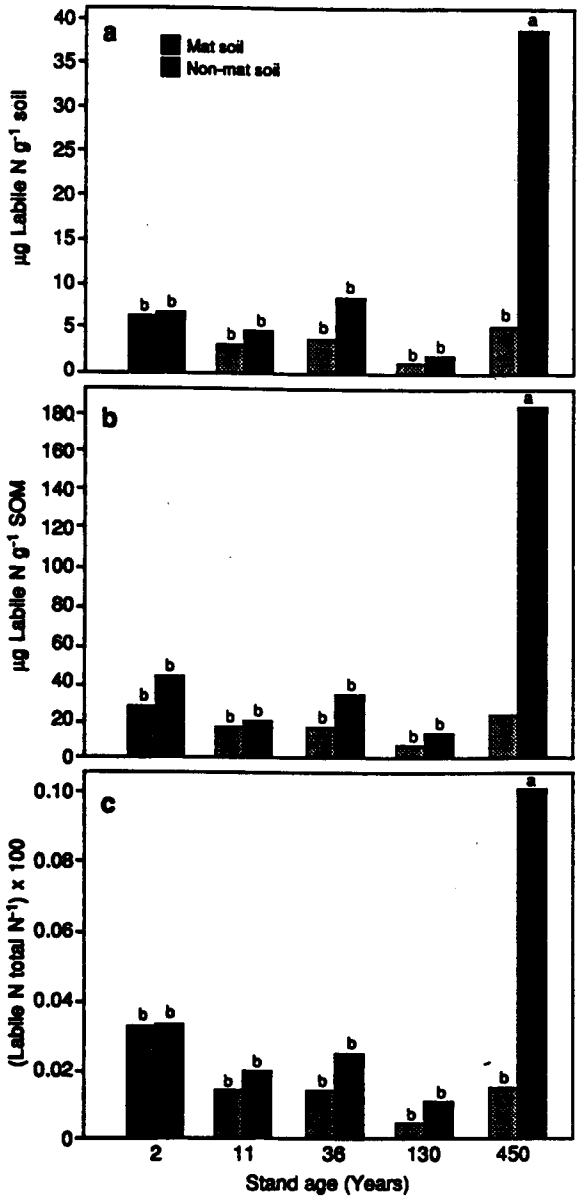

Fig. 3. Labile N in mineral soil as: (a) μg labile N g^{-1} dry wt soil,(b) μg labile N g^{-1} SOM, and (c) labile N total N^{-1} both normalized to g SOM. Significant differences (at $P < 0.05$) among different-age soils are indicated by different letters.

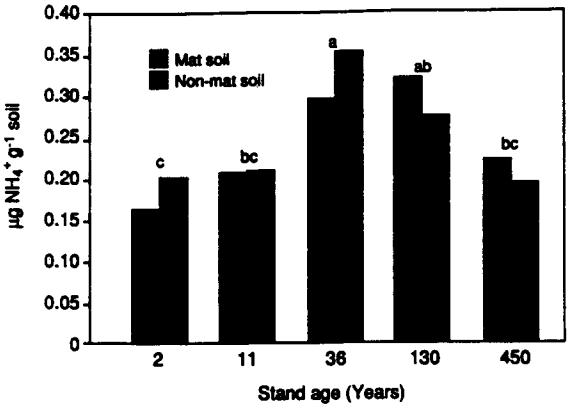

Fig. 4. Extractable NH_4^+ as μg N g^{-1} soil.

this pattern suggests that the mat communities were preferentially removing labile N from the soil.

Using ^{15}N tracer, Drury *et al.* (1991) have shown that organic matter mineralization processes release labile N as either NH_4^+ or NO_3^- and that these compounds remain in the soil until the N is either immobilized into microbial biomass or taken up directly by plants or their associated mycorrhizal fungi. Assuming that the input of labile N was the same in the mat and non-mat soils we studied, the lower amount of labile N in the mat soils compared to the non-mat soils indicates a higher rate of N mineralization in the mat soils. Thus, according to the tracer study results, the mat soils should have had higher concentrations of NH_4^+ or NO_3^- than the non-mat soils. However, this was not consistently the case (Table 1, Fig. 4). Nor did the mat soils contain consistently higher concentrations of microbial biomass than non-mat soils [Fig. 2(a) and (b)]. Additionally, denitrification rates are extremely low in mat soils (Griffiths *et al.*, 1990), so it is unlikely that the N was being lost from these soils in this manner. We therefore deduce that the N being released by labile N mineralization in the mat soils was being removed by the mycorrhizal fungi or tree roots and incorporated into the host trees.

The mean value for labile C was always greater in mat soils than in the corresponding non-mat soils (Fig. 5), although, as with labile N, the difference in values was significant only for the old-growth soils. The apparent buildup of labile C in mat soils of older stands is probably due to something other than increases in microbial biomass because numbers of bacteria and amount of fungal hyphae were not higher in these soils [Fig. 2(a) and (b)]. This leaves two possibilities: first, that the selective removal of labile N by the mats resulted in an enrichment of labile C; and second, that the mat fungi were excreting high C:N-ratio compounds such as organic acids. The high concentrations of oxalate (Graustein *et al.*, 1977) and lower pH values (Table 1; Griffiths *et al.*,

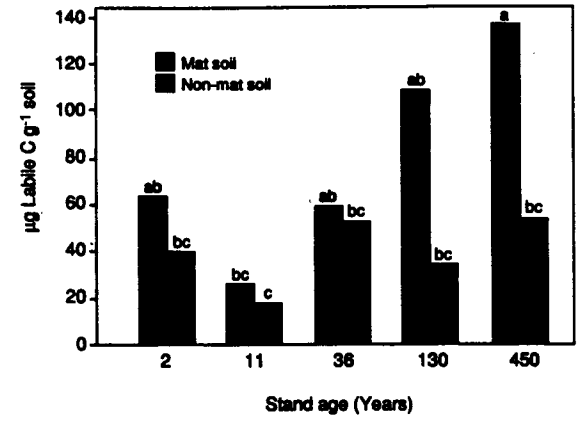

Fig. 5. Labile C as μg CO_2 carbon produced g^{-1} dry wt soil.

1991) found in ectomycorrhizal mat soils compared to non-mat soils supports the latter possibility. The patterns observed in our study suggest that both phenomena may be occurring.

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