# Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates[1]

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Received November 1, 1988

Accepted September 18, 1989

GRIFFITHS, R. P., CALDWELL, B. A., CROMACK, K., JR., and MORITA, R. Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. Can. J. For. Res. **20**: 211–218.

Monthly samples of ectomycorrhizal mat soils from a maturing Douglas-fir forest and adjacent nonmat soils were collected and analyzed for respiration, acetylene reduction activity, denitrification rates, extractable ammonium, nitrogen mineralization, microbial biomass, temperature, pH, percent moisture, total phosphate, nitrogen, and carbon. Seasonal patterns suggested complex interactions among the host tree, ectomycorrhizal fungus, and the mat microbial community as influenced by seasonal changes in moisture, temperature, and light availability. The most dramatic changes in rates were found during moisture–temperature transition periods in the spring and fall. Respiration within the mat community was highest during the period when tree growth is normally the greatest (in the spring and fall). In addition, there was a major respiration peak observed in the winter that we hypothesize was caused by the utilization of labile carbon by microheterotrophs. Differences were also observed between mat and nonmat soils in respiration rates, microbial biomass carbon, acetylene reduction activity, and levels of mineralizable nitrogen, which were all generally higher in the mat soils, and pH and denitrification rates, which were generally lower in nonmat soils. There is also evidence that suggests that nitrogen is very tightly coupled within the mat communities.

GRIFFITHS, R. P., CALDWELL, B. A., CROMACK, K., JR., et MORITA, R. Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. Can. J. For. Res. **20** : 211–218.

Des échantillons de sols à mors granulaires avec ectomycorhizes ont été prélevés mensuellement dans une forêt en maturation de Sapin de Douglas et dans une forêt adjacente sans mors granulaires et analysés pour la respiration, l'activité de réduction de l'acétylène, les taux de dénitrification, l'ammonium extractible, la minéralisation de l'azote, la biomasse microbienne, la température, le pH, le pourcentage d'humidité, le phosphore total, l'azote total et le carbone total. Les patrons saisonniers suggèrent des interactions complexes entre l'arbre hôte, le champignon ectomycorhizien et la communauté microbiologique dans le mor granulaire qui est influencée par les changements saisonniers en humidité, en température et en lumière disponible. Les changements les plus marqués ont été observés durant les périodes de transition dans l'humidité–température au printemps et à l'automne. La respiration à l'intérieur de la communauté à mors granulaires était la plus élevée durant la période où la croissance de l'arbre est normalement la plus forte (au printemps et à l'automne). De plus, il y avait un pic majeur de respiration observé durant l'hiver que nous croyons être causé par l'utilisation du carbone labile par les microhétérotrophes. Des différences ont aussi été observées entre les mors granulaires et les sols sans mors granulaires dans les taux de respiration : la biomasse microbienne, l'activité de réduction de l'acétylène et les niveaux d'azote minéralisable étaient généralement tous plus élevés dans les sols à mors granulaires et le pH et les taux de dénitrification étaient généralement plus faibles das les sols sans mors granulaires. Il y a aussi évidence suggérant que l'azote est très étroitement couplé dans les communautés avec mors granulaires.

[Traduit par la revue]

## Introduction

Ectomycorrhizal fungi are known to perform a number of important functions in their symbiotic relationships with trees, including the transport of nutrients to the plant and protection of the plant from certain pathogens and drought stress. In many cases, colonization of trees by ectomycorrhizal fungi may indeed be required for plant survival (Harley and Smith 1983). In this symbiosis, the tree provides energy in the form of carbohydrates, and the fungal component accumulates nutrients from a much greater volume of soil than could otherwise be exploited by the tree alone. The nutrients accumulated and transported include mono- and di-valent cations, P, and N. Although several studies based on measurements of litter, hyphal, and soil pools have suggested the importance of ectomycorrhizal nutrient cycling in United States Pacific Northwest coniferous forests (Fogel and Hunt 1983; Vogt et al. 1982), the microbial processes responsible have not been investigated.

Field observations in maturing coniferous forests have shown that new fine root formation and ectomycorrhizae predominate in the litter and humus layers rather than in the mineral soil (Mikola 1973; Harvey et al. 1978; Vogt et al. 1980). One plausible explanation for this is that although the mineral soil may contain a larger total organic matter

[1]Published as technical paper No. 9008, Oregon Agricultural Experiment Station.

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pool than the forest floor litter (Fogel and Hunt 1983), the litter is more slowly degraded than dead root material, which contains a greater proportion of polysaccharides and proteins. The more rapid mineralization of litter would lead to more N and P being available to the growing tree via ectomycorrhizae.

Another means by which mycorrhizal fungi might make N available to trees is by forming associations with N-fixing bacteria. In this manner, the relatively abundant energy source in the form of photosynthate from the tree could be converted to fixed N, which is often the limiting nutrient in coniferous forest systems. Indeed, a recent study has shown high N-fixation activity by *Azospirillum* sp. associated with ectomycorrhizal fungi taken from coniferous forest soils (Li and Castellano 1987).

Ectomycorrhizal fungi are known to form extensive mats within forest soils and litter layers that are characterized by a dense profusion of rhizomorphs. Similar mats have been reported in coniferous forest soils of Finland (Hintikka and Naykki 1967), Canada (Fisher 1972), and the United States Pacific Northwest (Cromack et al. 1979). The *Hysterangium setchellii* mats that we have chosen for this study form distinct morphological entities that are easily differentiated from adjacent noncolonized soil. Some of the earliest studies of these mycorrhizal fungi revealed that different physiological types of bacteria were associated with fungal rhizomorphs when compared with the surrounding soil (Neal et al. 1964). Both ammonia oxidizers and nitrate reducers were suppressed in rhizosphere soil relative to nonrhizosphere soils. More recent studies have shown that mat soils have different biological and chemical characteristics from adjacent noncolonized soils (Cromack et al. 1979; Cromack et al. 1988).

The objective of the present study was to investigate aspects of N cycling in these mats by comparing microbial activities and chemical characteristics in the mat and adjacent noncolonized soils.

## Materials and methods

*Site description and sample preparation*

Each month, five different soils colonized by the ectomycorrhizal mat forming fungus *H. setchellii* were collected along with five adjacent uncolonized soils from the Woods Creek watershed located 30 km southwest of Corvallis, Oregon. The site is a 60- to 75-year-old second-growth Douglas-fir stand at an elevation of approximately 460 m, with a gravelly loam derived from a colluvium of weathered basalt and sandstone. This location has been studied extensively in the past and has been previously described in detail by Fogel (1976) and Cromack et al. (1979).

Samples were collected to a depth of 10 cm, using a trowel, and placed in sealable plastic bags, which were placed in ice chests to keep them at approximately the soil temperature while being transported to the laboratory (20 min from location). These samples did not include the litter layer. All soil samples (both mat and nonmat) were mineral soils, described in detail by Hunt and Trappe (1987). Once in the laboratory, samples were incubated within 0.5° C of the original soil temperature. Rocks and root material were removed by hand prior to all treatments. Subsamples used to measure respiration were removed without being sieved. Field-moist soils used in all other procedures were sieved (2.0-mm mesh).

*Assay procedures*

The moisture content was determined by drying 10 g of field-moist sieved soil at 70°C for 24 h and was used to normalize the results of all assays, except where noted. Subsamples (5 g) were added to 50 mL distilled water and held overnight and until pH measurements were made, using a model 901 ion analyzer (Orion Instruments).

Respiration measurements were made as soon as the samples were received in the laboratory. Duplicate 100-g wet weight, non-sieved samples were placed in 400-mL sealable glass containers fitted with rubber serum bottle caps. The initial $CO_2$ concentration in the headspace was assayed at the beginning of the experiment and again 2 h later by gas chromatography (Hewlett Packard 5730A with a thermal conductivity detector; 2 m × 3 mm stainless steel Porapak R column and He carrier gas). The respiration rate was constant for 4 h after incubation was initiated, therefore, the initial 2-h incubation period was used routinely to measure respiration rates to insure a linear response. After analysis, soil samples were air dried and sieved through a 2 mm screen for use in normalizing respiration data to grams of dry weight and for total C, N, and P analyses.

Total N and P were determined from samples digested by microKjeldahl procedure (Nelson and Sommers 1980) and assayed colorimetrically using a Technicon autoanalyzer. Total C was determined using a LECO 12 induction furnace. Biomass C determinations were made on unsieved field-moist soils using the chloroform fumigation method of Jenkinson and Powlson (1976). Incubation at room temperature was initiated within 24 h of collection and was conducted for 10 days after the chloroform treatment was completed. A scintillation vial with water was included in each reaction chamber to prevent soil desiccation. The nonchloroformed $CO_2$ controls were subtracted from the $CO_2$ collected from chloroformed soils. After incubation, all soils were dried for dry weight determinations. Microbial biomass C ($B$) was calculated using Jenkinson and Powlson's (1976) formula: $B = (X - x)/K$, where $X$ is the $CO_2$ produced from the fumigated samples, $x$ is the $CO_2$ produced from unfumigated samples, and $K$ is the fraction of microbial C that is evolved as $CO_2$ during incubation (Vance et al. 1987).

Nitrogen fixation rates were assayed using the acetylene ($C_2H_2$) reduction activity (ARA) technique (Hardy et al. 1973). Duplicate 5-g subsamples of sieved field-moist soil were added to 25-mL Erlenmeyer flasks fitted with serum bottle stoppers. The headspace was purged with Ar, and the final atmosphere adjusted to 10% $C_2H_2$ and 1% $O_2$. The samples were incubated within 0.5°C of the *in situ* temperature for 7 days. The headspace was assayed at days 0 and 7 by injecting 0.3 mL into a Hewlett Packard model 5830A gas chromatograph fitted with a flame ionization detector and a column (2 m × 3 mm) of Porapak Q. The rate of ethylene production was calculated by subtracting the time 0 concentrations from the 7-day concentrations. Controls without $C_2H_2$ were run with approximately one-third of the samples to measure natural ethylene production, but none was observed. Spot checking of response linearity showed that the rates at 3 and 7 days were essentially the same.

Denitrification rates were determined by analysis of $N_2O$ production with a $C_2H_2$ block as described by Balderson et al. (1976). The samples were prepared in the same way as the ARA samples, except the headspace was purged of all oxygen. Initial and 7-day $N_2O$ concentrations were assayed using a Hewlett Packard model 5840A gas chromatograph fitted with an electron capture detector. Time series experiments conducted on winter samples showed that both ARA and denitrification rates after the first 24 h were linear up to 7 days. In some of the most active samples, denitrification rates were highest within the first 2–4 h, with reduced rates thereafter. Therefore, the denitrification rates we have reported are probably conservative. On a time scale of days, there did not appear to be an enhancement of ARA with time up to 1 week. The initial justification for using the 7-day incubation period for both ARA and denitrification was that we were using nonamended soils at *in situ* temperatures and the rates were very low. In most cases, the amount of $N_2O$ produced was too low in many samples to be detected until they had incubated for several days; this was partic-

TABLE 1. Seasonal range of environmental characteristics, soil chemistry, and microbial activities in mat and nonmat soils

| | Units | Mat soils | | Nonmat soils | |
|---|---|---|---|---|---|
| | | Avg. | Range | Avg. | Range |
| Temperature | °C | 9.8 | 2.0–19.0 | 9.8 | 2.0–19.0 |
| % moisture | | 47.0 | 21.4–76.0 | 46.6 | 18.9–68.6 |
| pH* | | 4.82 | 4.26–5.29 | 5.20 | 4.66–5.66 |
| $C_F$* | g biomass C/kg DW | 7.90 | 4.22–11.7 | 2.15 | 0.90–3.15 |
| $N_M$ | μg N/g DW | 247 | 118–363 | 193 | 82–440 |
| Respiration rate* | μg C/g DW | 6.63 | 2.93–11.2 | 1.4 | 0.49–2.57 |
| Extractable ammonia | μg N/g DW | 2.78 | 0.42–10.1 | 2.34 | 0.10–6.31 |
| Acetylene reduction | pmol ethylene/(g DW·day) | 66.8 | 10.5–142 | 25.0 | 7.69–45.8 |
| Denitrification | pmol $N_2O$/(g DW·day) | 8.32 | 0.5–32.9 | 23.9 | 2.85–22.7 |
| Respired C:$C_F$ | % biomass C respired per day | 2.37 | 0.76–5.86 | 1.76 | 0.85–3.38 |
| $C_F$:$N_M$ | | 36.6 | 22.1–61.5 | 13.0 | 6.68–24.0 |
| % total organic C* | | 10.8 | 8.94–12.5 | 8.60 | 5.45–9.74 |
| % total organic N | | 0.358 | 0.298–0.412 | 0.288 | 0.214–0.336 |
| Total P | ppm | 886 | 651–1047 | 761 | 732–831 |

NOTE: $C_F$, biomass C as calculated using the chloroform fumigation method; $N_M$, N mineralization; DW, dry weight.
*Those variables with a significant difference between mat and nonmat soils for all months.

ularly true in the winter samples. Ideally in a study such as this in which a wide range of environmental conditions are encountered during the course of the study, a time series should be run on each sample to bracket the ideal incubation time. However, this is not practical because of the time required for analyses.

Ammonium concentrations were measured by a ammonium electrode (HNU Systems Inc. Newton, MA) after extracting 10 g field-moist soil with 50 mL 2 M KCl for 1 h on a rotary shaker. Since the final concentrations were generally between $1 \times 10^{-4}$ and $1 \times 10^{-5}$ M, standard solutions at these concentrations were used to recalibrate every fourth sample.

Mineralizable N was determined using the anaerobic incubation technique as modified with a shorter incubation time and higher incubation temperature by McNabb et al. (1986). In the present study, 10 g of field-moist samples were placed in 20 × 125 mm screw cap test tubes to which distilled water was added so that no bubbles were present in the tube. Tubes were incubated at 40°C for 7 days and then assayed for ammonium concentration using the assay procedure described above. The standards used were $1 \times 10^{-3}$ and $1 \times 10^{-4}$ M ammonium chloride since ammonium concentrations in these samples were normally 10-fold higher than the ammonium extracted from the field soils.

Statistical analyses were conducted using two statistical packages for the personal computer: Statgraphics and SAS. An analysis of variance (ANOVA) was performed on each of the original variables. Since separate samples were taken over time, it was not necessary to use a repeated measure design; therefore, a two-way factorial design was utilized. The degrees of freedom for date, soil, date × soil, and error were 15, 1, 15, and 118, respectively, for all variables except N fixation and denitrification that were measured for 12 months instead of 15 months. The residuals were then checked for normality and constant variance. Since only the dry weights did not violate these assumptions, the remaining variables were log transformed and residuals were again checked for normality and constant variance. Those variables that still violated the normality assumptions were analyzed following rank transformation (Conover and Iman 1981). Only denitrification and respiration (because of the great variability) violated these assumptions when rank transformation was used. Both of these variables were analyzed using weighted ANOVAs. Fisher's Protected LSD was used to determine significant differences ($p \leq 0.05$) in all variables except denitrification between mat and nonmat samples over time. Owing to the great variability in denitrification, the Bonferroni method, a more conservative separation of means test, was used.

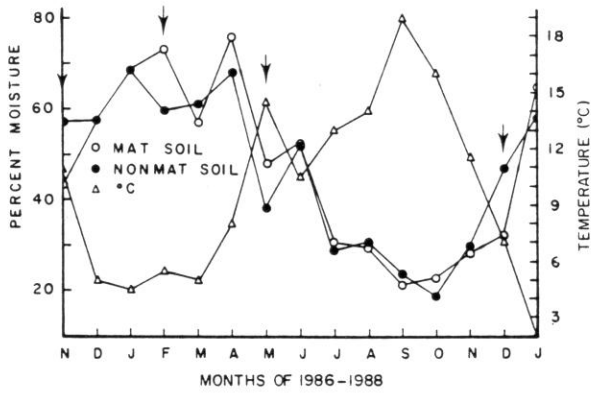


FIG. 1. Percent moisture and temperature (°C). Those months in which the differences between mat and nonmat soils were statistically different are indicated by arrows.

## Results and discussion

The means and ranges for microbial activities, chemistry, and environmental characteristics for monthly samples of ectomycorrhizal mat communities and adjacent uncolonized soils are listed in Table 1. Temperature and mat moisture were negatively correlated in mat ($r^2 = -0.540$) and nonmat soils ($r^2 = -0.642$). The correlation between temperature and moisture shows the inverse relationship typical of a Mediterranean climate with arm, dry summers and cool, moist winters (Fig. 1). Although there were differences in percent moisture between mat and nonmat soils, these differences were only significant for November, February, May, and December.

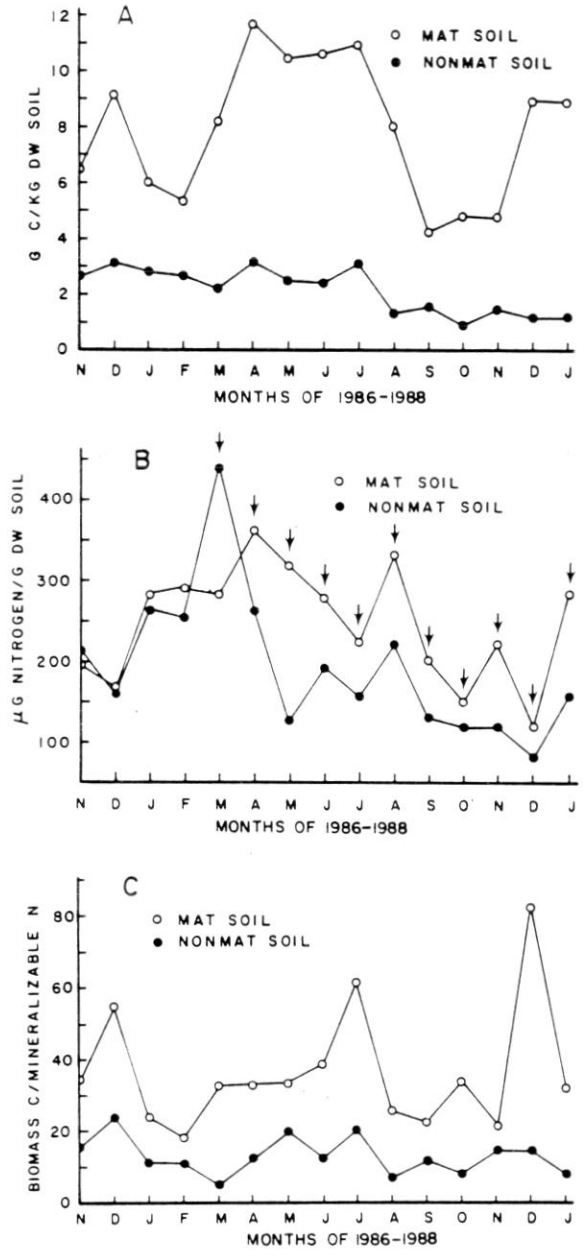


FIG. 2. (A) Biomass C data (chloroform fumigation). All differences between mat and nonmat soils were statistically significant. DW, dry weight. (B) Nitrogen mineralization (ammonia N) measured using anaerobic incubation. Those months in which the differences between mat and nonmat soils were statistically different are indicated by arrows. (C) Grams biomass C per gram N mineralized. All differences between mat and nonmat soils were statistically significant.

In a recent study of the same mat system using samples collected from the same location, Cromack et al. (1988) showed significantly higher concentrations of total N and C in mat soils than in adjacent nonmat soils. In the present study, a significant difference between total organic C in mat and nonmat soils was also found, but there was not a significant difference in total N or P (Table 1).

## *Biomass*

### *Chloroform fumigation as a measure of biomass C*

During each set of observations, the microbial biomass C in the mats as measured by chloroform fumigation ($C_F$) was significantly greater than that in nonmat soils (Fig. 2A). In all samples studied from March 1987 until January 1988, N mineralization ($N_M$) was highest in the mats (Fig. 2*B*), but the correlation between $C_F$ and $N_M$ was not significant.

The predominant ectomycorrhizal rhizomorphs (Cromack et al. 1979) and elevated microbial $C_F$ have been previously observed in these mats (Cromack et al. 1988). In addition to total organic C, Cromack et al. (1988) reported that populations of Collembola, mites, and nematodes were all greater in the mats. Seasonal shifts in protozoan and microarthropod populations were also observed.

Although these soils were acidic and contained relatively high concentrations of organic C, recent studies have shown that within the ranges we encountered in these soils, the technique that we used can be used to estimate microbial biomass C (Vance et al. 1987; Diaz-Ravina et al. 1988). If there were a reduction in $CO_2$ released during fumigation due to lower pH and higher organic C concentrations in mat soils, this would tend to reduce the estimated biomass C in mat soils relative to the nonmat soils, thereby underestimating the actual biomass C. Thus, the differential in $C_F$ we report between mat and nonmat soils should be considered conservative.

There is little agreement on exactly what the chloroform fumigation method measures (Ingham and Horton 1987). With what is known about potential sources of labile C in acid forest soils, it is unlikely that this method is an accurate measure of total microbial biomass C because lower percentages of fungal hyphae are lysed than bacteria or protozoa (Ingham and Horton 1987). Therefore, it is possible that most of the $CO_2$ released after fumigation was from the bacterial and protozoan biomass killed by fumigation rather than from the fungal biomass.

The low seasonal variation in nonmat soil $C_F$ suggests that even if there were significant seasonal shifts in labile C coming into the system, it was not reflected in shifts in $C_F$ (Fig. 2A). However, there were relatively dramatic seasonal shifts seen in mat soils, with peaks in December and March through August. The peak during the growing season would be expected if rhizosphere biomass were tied to primary productivity and to the release of labile compounds by the ectomycorrhizal hyphae to drive rhizosphere productivity (Waring and Schlesinger 1985).

### *Mineralizable N as a measure of biomass*

There was no significant difference in mineralizable N between the mat and nonmat soils from November 1986 to February 1987 (Fig. 2B). In March, however, there was a statistically significant increase in the nonmat soils. The nonmat soil $N_M$ levels dropped below those observed in mat soils in April and remained significantly lower throughout the balance of the study.

Both $C_F$ and anaerobic $N_M$ have been used as measurements of microbial biomass (Myrold 1987), but it is still unclear exactly what these measurements mean. It has been suggested that the $NH_4^+$ released during anaerobic incubation is a reflection of the N coming from aerobic microorganisms killed under anaerobic conditions (Adams and Attiwill 1986). During a comparative study of $N_M$ with C and N released during chloroform fumigation in a variety of Oregon coniferous forest soils, Myrold (1987) concluded that these techniques were measuring the same pool of organic N. He found a significant correlation ($r^2 = 0.66$)

between $N_M$ and $C_F$ using a fumigation technique in which no controls were subtracted. In our study, $r^2$ for the same variables were 0.04 in mat soils and 0.08 in nonmat soils. The main difference in these two studies was that in Myrold's study, a broad range of soils at the same time of year were compared and in the present study, measurements were made on two soil communities under a broad range of seasonal conditions.

If compositions of the microbial assemblages in both mat and nonmat soils are approximately the same and if both of these techniques accurately measured microbial biomass, we would expect the $C_F$:$N_M$ ratios in mat and nonmat soils to be approximately the same. This was not the case; these ratios were significantly greater in mat soils throughout the study (Fig. 2C). In addition, if one compares the seasonal patterns of $C_F$ (Fig. 2A) with $N_M$ (Fig. 2B), one must conclude that either there are wide seasonal shifts in the composition of the microbial assemblages or that these two techniques are measuring different N and C pools.

It seems likely that the higher $C_F$:$N_M$ ratios found in the mat soils may be due to the higher concentration of fungi in those soils, since the mats are defined by the presence of extensive fungal rhizomorph material. It is known that bacteria and fungi can have much different C:N ratios. Those of bacteria are often near 6, but fungi can have C:N ratios of over 30. If the nonmat soils were dominated by bacteria and the mat soils dominated by fungi, we would expect to find higher $C_F$:$N_M$ ratios in the mat soils than in the nonmat soils. In a recent seasonal study of this system where direct counts of bacteria and fungi were made seasonally (E.R. Ingham, unpublished data), the ratio of bacterial to fungal biomass was found to be the same in mat and nonmat soils, although the totals were greater in the mat soils. This strongly suggests that the different patterns we are observing using these two techniques cannot be explained in terms of relative shifts in the composition of the microbial assemblages.

When one compares the differences between the average $CO_2$ released from mat and nonmat soils during $C_F$ determinations (Table 1) with the ammonium produced during mineralization, it is apparent that different sources of organic matter are being measured. The mean $CO_2$ released after mat soil fumigation was higher than that released from nonmat soils by a factor of 3.67 (Table 1). The analogous ratio for ammonia released after anaerobic mineralization was 1.28. If one assumes that respiration rates in bulk soil is a rough approximation of microbial biomass (Nannipieri et al. 1978; West et al. 1986*a*, 1986*b*), then the ratio of respiration in mat vs. nonmat soils should be approximately the same as the biomass ratios in the same soils. The respiration ratio is 4.74, which is much closer to the fumigation biomass ratio of 3.67 than the mineralizable N ratio of 1.28. If our assumptions are correct, it would appear that $C_F$ estimations may indeed provide a more accurate estimation of microbial biomass than the $N_M$ technique in acid forest soils of the Pacific Northwest.

During most of the study, $N_M$ was significantly greater in the mat soils than in adjacent nonmat soils. This may be another reflection of the input of energy by the tree that supports an elevated population of soil organisms, thus immobilizing N. In Myrold's (1987) study, the mean $C_F$:$N_M$ ratio was 10.5 (range 6.7–14.3), which was close to the mean ratio we observed in nonmat soils (13.0; range 6.7–24). In contrast to this, the mean ratio in the mat soils was 36.6 (range 22–61.5, Table 1).

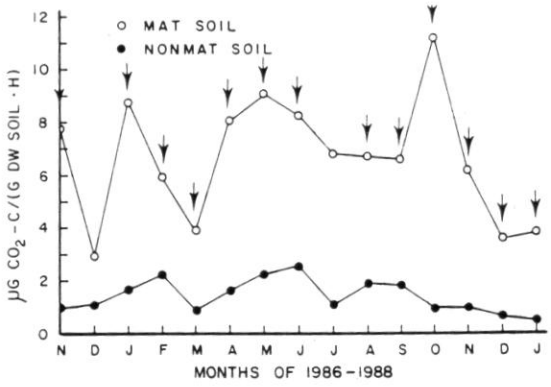


FIG. 3. Respiration rates. Those months in which the differences between mat and nonmat soils were statistically different are indicated by arrows. DW, dry weight.

*Respiration*

As one would expect from the $C_F$ data, respiration rates in mat soils were significantly greater than that in nonmat soils, except for December 1986 and June 1987 (Fig. 3). Respiration rates were elevated in November 1986, but reduced significantly by December 1987. In the 1st year, there was a peak in mat respiration rates from December to January, with a decline from January to March, after which there was an increase that peaked in May. This pattern is similar to one observed in forest soils in western Washington state (Vogt et al. 1979). Vogt et al. (1979) concluded from their study, and from those of others studying respiration of coniferous forest ecosystems of the Pacific Northwest, that temperature and moisture become limiting factors in different seasons. For this reason, we would predict that correlations between respiration and both temperature and moisture would be low if the respiration for all seasons were included in the statistical analysis. There was no significant correlation between respiration and moisture for either mat or nonmat soils. There were significant but low correlations between temperature and respiration in mat ($r^2 = 0.17$) and nonmat ($r^2 = 0.07$) soils. If both temperature and moisture are factored in, the $r^2$ values become 0.29 and 0.32, respectively, for mat and nonmat soils. We conclude from this that there must be other factors influencing the seasonal respiration patterns we have observed.

It is possible that the reduction in respiration rates from November to December 1986 and October to December 1987 reflect the amount of energy available to the system directly from photosynthate transported by the mycorrhizal fungi. If the heterotrophs in the mat communities were totally dependent on tree photosynthate for energy, we would expect the respiration rates to reach very low values in the winter (possibly approaching that observed in nonmat soils). In the 1st year, there was a large spike in January after an initial low point in December. This spike was not repeated in January of the 2nd year; possibly because of the lateness of the fall rains, which started approximately 6 weeks later in 1988 than in 1987. This spike in activity may be linked to metabolic activity of soil bacteria.

It is possible that the microheterotrophs are utilizing fungal and fine-root biomass that was killed as the result of decreased photosynthate coming into the system in the

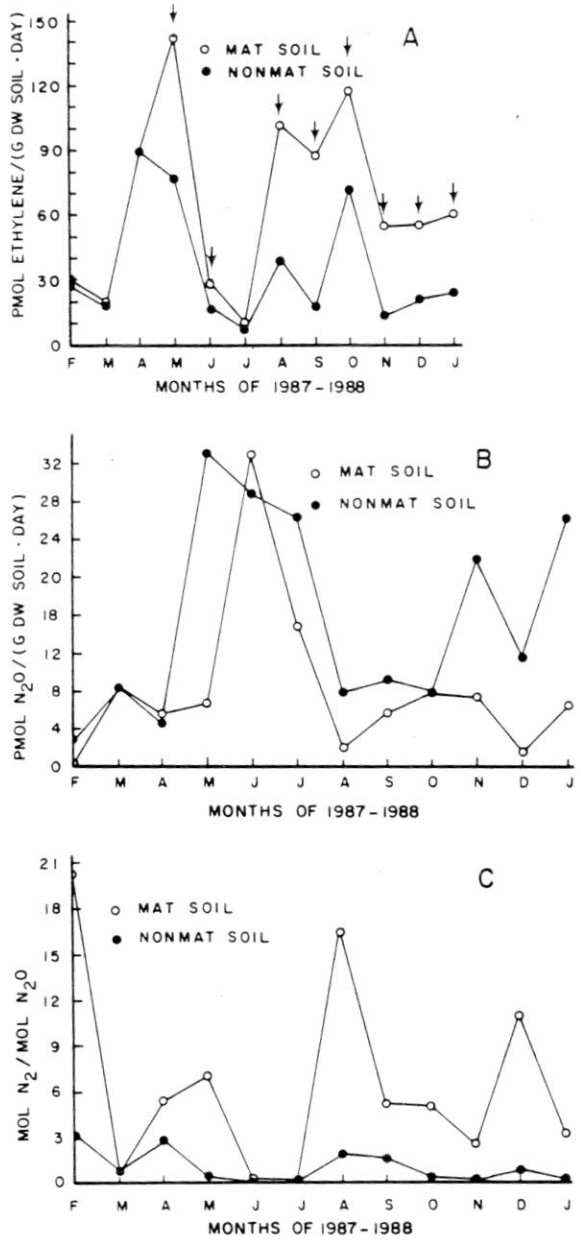


FIG. 4. (A) Acetylene reduction rates. Those months in which the differences between mat and nonmat soils were statistically different are indicated by arrows. DW, dry weight. (B) Denitrification rates. (C) Moles N fixed per mole N denitrified ($C_2H_4 \times 0.33 = N_2$).

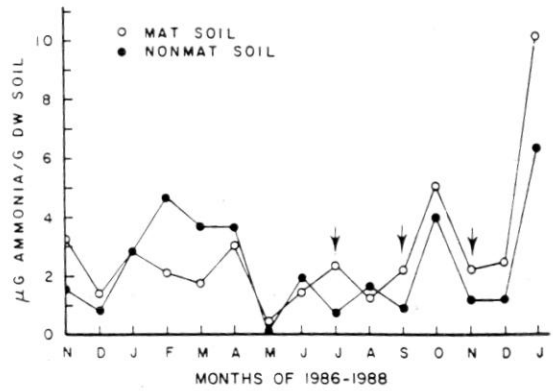


FIG. 5. Extractable ammonia. Those months in which the differences between mat and nonmat soils were statistically different are indicated by arrows. DW, dry weight.

fall. At the same time that there was an increase in respiration, there was a concomitant decrease in the $C_F$:$N_M$ ratio, suggesting that this rise in respiration may be tied to an increase in bacterial biomass C during the same period.

In the late summer, the increased respiration rates may be caused by another mechanism. When moisture becomes limiting and carbohydrate concentrations in the tree sap become elevated (Waring and Schlesinger 1985), it is likely that this would result in a high carbohydrate gradient between the tree and the mycorrhizal fungi, resulting in an increase in energy in the form of carbohydrate coming into the mats. This, in turn, may result in higher respiration rates by mat heterotrophs.

Since these measurements were made on soil samples within 4 h of sampling, the $CO_2$ released could be caused by both soil organisms and respiration by root fragments. Root respiration should have been minimal, however, since visible roots were removed from the subsamples prior to analysis. The concentration of fine-root biomass in mat and nonmat soils has been measured and not found to be significantly different in these soils (K. Cromack, Jr., unpublished data). For these reasons, we are assuming that the differences we have seen in respiration rates in mat and nonmat soils are not due to differences in root respiration.

*Nitrogen fixation (acetylene reduction)*

ARA was significantly greater in the mat than in nonmat soils in May and June, and August through January. (Fig. 4A). There were two peaks in ARA observed in both soils: one in April and May and a second peak in the fall.

Nitrogen is usually considered an important limiting factor in coniferous forests of the Pacific Northwest. Because of the energy that should be available to these fungal mat communities, it was thought that N fixation might represent a significant input. This supposition was further reinforced by a recent study by Li (1987), which shows high rates of ARA associated with sporocarps of other ectomycorrhizal fungi found associated with Douglas-fir. When we measured ARAs in mat and adjacent nonmat soils, the rates were generally higher in mat soils (Fig. 4A).

The ARAs observed in this study are much lower (by up to 3 orders of magnitude) than those reported in forest litter (Baker and Attiwill 1984; Lindberg and Granhall 1985), but are close to those reported by Lindberg and Granhall (1985) in mineral soils of a mature Scots pine stand. In a study of ARA in litter from a southwestern Australia *Eucalyptus* forest, Baker and Attiwill (1984) showed that seasonal variations in $CO_2$ production and ARA were related to both temperature and moisture, where the highest rates were observed in winter and the lowest in the summer. Moisture has also been shown to be an important factor affecting ARA in soil by other investigators (Granhall and Lindberg 1978). In the present study, the lowest ARAs were in the summer in both mat and nonmat soils; some of the highest rates observed in the mat soils occurred in September and October 1987 when the percent moisture was at the lowest level for the year (Fig. 1). There was also no significant correlation between $C_2H_2$ reduction and temperature.

Compared with other possible sources of N within this system, that from N fixation is probably insignificant in terms of nutrient supplied to the tree; however, this activ-

ity may still have a significant function to the mat community itself. It has been reported by Fogel (1976) that sporocarp production by hypogeous fungi in western Oregon was greatest during the months of May and October. It may be more than a coincidence that N fixation rates were also elevated in these same months. It is possible that organisms within the mat community are fixing N that is in some way utilized in sporocarp production, which requires relatively high amounts of N.

*Denitrification and extractable ammonium*

The rates of denitrification were often higher in nonmat soils than in mat soils, but this was not statistically significant because of the high sample variability (Fig. 4B). As has been reported by others (Robertson and Tiedje 1984; Parkin et al. 1987), there was a great deal of heterogeneity in the observed rates at any given time and even among identical subsamples, with denitrification variances ranging from 0.11 to 2584. In general, the denitrification rates in mat soils were elevated only in June and July and were consistently low for the balance of the year. In contrast to this, denitrification rates in nonmat soils were elevated from May to July and again in November to January.

In a study of denitrification potential in forest soils, Binstock (1984) concluded that moisture was a primary determinate for denitrification. Although the denitrification rates were lowest in both mat and nonmat soils during the driest season of the year, there was no significant correlation between denitrification rates and moisture.

If the ratios of N fixation to denitrification are compared in mat and nonmat samples, it is apparent that in terms of atmospheric sources and sinks of fixed N, the mat soils appear to be more effective than nonmat soils in accumulating fixed N (Fig. 4C).

As was true with the denitrification data, there was a great deal of intersample heterogeneity among soil extractable ammonium, and the mean values showed no consistent seasonal trend (Fig. 5). The only differences that were statistically significant were those in July, September, and November 1988.

## Conclusions

Increases in respiration observed in the spring and fall associated with periods of maximum tree growth suggest that tree physiology is a major factor influencing biological activity within ectomycorrhizal mat communities. Although temperature and moisture regimes may have influenced respiration rates, correlations between respiration and these variables were low.

It has been suggested that anaerobic N mineralization and biomass C as measured with the chloroform fumigation technique both measure microbial biomass C. The seasonal patterns that we observed in these two variables in mat and nonmat soils suggest that these two techniques may not be measuring the same sources of organic N and C.

## Acknowledgements

The authors thank James Entry for providing the biomass C data, Michael Castellano for fungal mat identification, Elaine Ingham and David Myrold for their critical review of the manuscript, and Thomas Sabin for the statistical analysis. This work was supported by United States Department of Agriculture competitive grant No. 86-FYT-9-0224 and National Science Foundation grant No. BSR 8717849.

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