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Variation in NH_4^+ mineralization and microbial communities with stand age in lodgepole pine (*Pinus contorta*) forests, Yellowstone National Park (USA)

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Abstract

Soils and vegetation were analyzed in 20 lodgepole pine (*Pinus contorta*) forest stands, varying in age from 50 to 350 years, that had initiated following stand-replacing fire. Our goal was to determine how nitrogen availability (NH_4^+–N) and microbial community composition varied with stand age-class and to determine whether differences could be explained by canopy, soil, or understory characteristics. Gross NH_4^+ mineralization was measured using laboratory isotopic pool dilution, and microbial community composition was evaluated using microbial membrane lipids. The microbial community composition of stands in the 300–350 age class was distinct from stands in younger age classes. Microbial community composition among sites varied with pH, % organic matter, and phosphorus. Gross NH_4^+ mineralization rates averaged 1.45 ± 0.07 mg NH_4^+ kg soil^{-1} d^{-1} while consumption averaged 1.37 ± 0.20 mg NH_4^+ kg soil^{-1} d^{-1}, resulting in low net NH_4^+ mineralization rates (0.08 ± 0.18 mg NH_4^+ kg soil^{-1} d^{-1}), but rates were not significantly different with stand age-class at $p < 0.05$. At $p < 0.10$, net NH_4^+ mineralization was significantly higher in the 300–350 age class compared to the 125–175 age class. None of the measured variables significantly explained NH_4^+ consumption and net mineralization patterns. However, gross NH_4^+ mineralization rates were best explained by information on microbial community structure (i.e. lipids). Variation among stands within a given age-classes was high, indicating that patterns of N cycling across landscapes reflect substantial heterogeneity among mature stands.

Keywords: Pool dilution; Microbial community composition; PLFA; Nitrogen; Chronosequence; Lodgepole pine; Gross mineralization

1. Introduction

Large, infrequent disturbances such as severe, stand-replacing wildfires may affect biogeochemical cycling over long time periods by altering stand structure and soil properties. In particular, nitrogen (N) is a limiting nutrient in many ecosystems (Vitousek and Howarth, 1991) and is particularly sensitive to volatilization by fire. Most previous research has focused on the immediate physical and chemical effects of fire on N cycling and little is known about how microbial communities mediate N cycling rates after disturbance, nor how N cycling changes with stand age in fire-dominated ecosystems. Evaluating N cycling and microbial communities among mature (>50 years old) fire-dominated ecosystems will help constrain estimates of N cycling and elucidate changes in N dynamics with stand development.

During and immediately after fire, N cycling is altered by the combustion of vegetation and forest floor organic matter, the transfer of elements to the ash layer, the pyrolysis of nutrients within existing soil organic matter, and the volatilization of elements to the atmosphere. Estimates of post-fire N availability vary but many studies show an immediate increase in inorganic N for up to a decade (Wan et al., 2001; Smithwick et al., in press). Longer term changes in N cycling are likely to be a function of changes in biotic (plant and microbial) and abiotic (soil exchange complex, climate) factors, as they control N mineralization, consumption, and loss. Severe wildfires in coniferous forests typically have return times of several centuries (Turner and Romme, 1994), and significantly alter

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canopy structure, understory composition, and forest floor organic matter (factors which may indirectly affect N cycling and microbial community dynamics over long time periods). Although N is the element that is most likely to be limiting in mature stands in temperate forests (Fahey and Knight, 1986; Vitousek and Howarth, 1991), it is not known when N becomes limiting in fire-dominated ecosystems, nor how N availability varies among mature stands. There have been limited studies in other ecosystems that have evaluated changes in N availability with stand age. For example, Marion and Black (1988) studied a chapparal fire chronosequence ranging in stand age from 4 to 85 years and concluded that N availability increased with increasing stand age up to 50–60 years, beyond which it declined, whereas MacLean and Wein (1977) studied jack pine and hardwood stands ranging in age from 1 to 57 years and concluded that N availability was inconsistent due to varying fire intensities and postfire conditions.

General hypotheses have been proposed to evaluate variation of soil N availability with forest stand development. As stands age, nitrate (NO_3^-) losses to streams are expected to increase as plant demand is saturated (Vitousek and Reiners, 1975). Similarly, NO_3^- losses to streams should increase in response to chronic N deposition exceeding plant demand (Aber et al., 1989), although plant uptake may persist for centuries (Goodale et al., 2000). Whether stands become more or less 'leaky' with increasing stand age is due to the complex interaction of abiotic and biotic processes, which vary spatially and temporally. Thus, even among relatively mature stands, N availability should vary with stand age and structural characteristics.

In contrast to soil N transformations, there are no comparable hypotheses for evaluating the variation of microbial communities with stand development after disturbance. It is well known that disturbance alters ecosystem function (Gorham et al., 1979; Bormann and Likens, 1979; Foster et al., 1997; Wirth et al., 2002), yet how microbial communities mediate these responses is not known. Often, ecosystem scientists measure and monitor soil processes in the absence of specific information on microorganisms, despite increasing microbiological information about how specific species control these processes. Ecosystem scientists often assume that microbes are passively reacting to abiotic and biotic stimuli rather than controlling soil processes explicitly (Balser et al., 2002). In a modeling context, this is especially apparent, as microbes are often treated as 'black boxes' (Balser et al., 2002). However, there is increasing evidence that the structure of microbial communities alters soil processes (Setälä et al., 1998; Breland and Eltun, 1999; Neher, 1999; Balser et al., 2002; Zak et al., 2003; Balser and Firestone, in press), and several authors have shown that microbial community structure and function change along primary successional sequences (Ohtonen et al., 1999; Merilä et al., 2002) and with management (Pennanen et al., 1999; Waldrop et al., 2000; Yao et al., 2000; Steenwerth et al., 2003). Litton et al. (2003) showed that soil-surface carbon dioxide efflux and microbial biomass carbon were higher in mature stands (~110 years old) compared to young stands (13 years after a stand-replacing fire). Yet, we lack a synthetic understanding of how microbial communities mediate ecosystem function in fire-dominated systems.

Forest canopy structure often differs among mature stands and may directly or indirectly affect N cycling and microbial community composition. Aboveground vegetation quality and quantity affect temperature and moisture conditions at the forest floor, the amount of nutrient and water throughfall, the productivity and species composition of the forest floor (Romme et al., 1986), and the quality and quantity of soil organic matter (Stottlemyer and Toczdlowski, 1999), which may affect nutrient turnover (Nohrstedt, 1985; Fahey and Knight, 1986; Epstein et al., 1998). Microbial composition may be affected by forest structure indirectly by changes in soil pH, temperature and moisture (Bardgett et al., 1997; Berg et al. 1998; Arao, 1999; Bardgett et al., 1999; Wilkinson et al., 2002; Bååth and Anderson, 2003) and changes in understory biomass and composition affecting substrate (rhizosphere or litter) quality and quantity (Frostegård and Bååth, 1996; Bardgett et al., 1997; Bossio et al., 1998; Bending and Turner, 1999; Ohtonen et al., 1999; Bending et al., 2002; Marschner et al., 2003). Microbial composition may be affected by forest structure directly through the distribution of individual trees (Pennanen et al., 1999; Wilkinson and Anderson, 2001).

Yellowstone National Park (YNP, Wyoming USA) is characterized by large and severe stand-replacing fires that occur approximately every 100–300 years (Romme, 1982). Fires are driven primarily by years of severe drought; thus, years of extreme climate events correlate strongly with years of large areas burned (Johnson, 1992; Renkin and Despain, 1992; Johnson and Wowchuck, 1993; Bessie and Johnson, 1995; Flannigan and Wotton, 2001). Fuels and fuel structure appear to have little bearing on the timing of large fire years (Turner et al., 2003) but forest structure resulting from severe fire results in a complex landscape mosaic. Specifically, 12 years after extensive fires in YNP in 1988, post-fire lodgepole pine density ranged 5 orders of magnitude and the coefficient of variation among individual stands was 231% (Kashian et al., in press). The coefficient of variation among stands unburned by the 1988 fires appears to remain high for centuries (91% for stands between 50 and 100 years old, reduced to 37% for stands 200–250 years old (Kashian et al., in press). Similarly, spatial variability of stand production (leaf area index and basal area index) was evident in stands at least one century following fire (Kashian et al., 2005). Thus, sub-alpine forests in YNP provide an excellent opportunity to evaluate N cycling and microbial communities across a wide range of stand ages and forest structures.

In this study, we assess the variation in gross rates of NH_4^+ mineralization and microbial community composition in mature lodgepole pine forest stands that burned between

50 and 350 years ago in YNP by asking three questions. (1) How do N (NH_4^+) cycling rates, microbial community composition, and associated stand characteristics (canopy structure, understory/forest floor cover, and soil properties) vary with stand age among mature lodgepole pine forest stands? Variation in N cycling and microbial community composition among mature stands may be minimal after the fire-initiated effects on forest structure (e.g. tree density) converge, indicating a coupling of aboveground ecosystem structure and belowground function. (2) What explains variation in N (NH_4^+) cycling rates and microbial community composition among mature stands? We expected that information on microbial community composition would improve explanation of NH_4^+ cycling rates beyond information on canopy structure, understory/forest floor characteristics, and soil properties. (3) Do stands exhibit similar patterns of variance in NH_4^+ cycling, microbial community composition, and stand characteristics with stand age, as observed by Kashian et al. (in press)? In the third question, we expected variation would be higher in the youngest stands and then decrease, following patterns of tree density observed by Kashian et al. (in press). We also expected that patterns of variance would be similar for the general stand characteristics, NH_4^+ cycling, and microbial community variables, suggesting they are closely linked.

2. Materials and methods

2.1. Site description

Yellowstone National Park encompasses 9000 km^2 across several high-elevation forested plateaus in northwest Wyoming. Approximately 80% of the park is dominated by lodgepole pine (*Pinus contorta*) forest, but subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), Engelmann spruce (*Picea engelmannii* Parry), and whitebark pine (*Pinus albicaulis* Engelm.) may be locally abundant (Despain, 1990). The climate in YNP is characterized by cool winter temperatures and dry summers. Average July air temperature is 10.8 °C and −11.4 °C in January. Mean annual precipitation is 565 mm (Dirks and Martner, 1982) mostly falling as snow. Elevation of the subalpine plateaus in the park, where our stands were located, ranges from 2100 to 2600 m.

Kashian et al. (in press, 2005) sampled stand density, basal area increment (BAI) and leaf area index (LAI) in 45 mature, 0.25 ha lodgepole pine stands in YNP. We selected a subset ($n=20$) of these stands, 5 in each of 4 age classes (50–100, 125–175, 200–250, 300–350), which varied in lodgepole pine tree density from 640 to 11,320 stems ha^{-1}. Most ($n=18$) of the stands were underlain by Quaternary rhyolite, a common volcanic substrate in YNP, which weathers into relatively dry, infertile soils. The remaining two stands were located above a mixed lake-bed sediment, which weathers into soil that is somewhat less infertile (Despain, 1990). A common problem of choosing spatially-distributed stands to represent an age gradient (the chronosequence approach) is that the stands vary in their unique site history, particularly with regard to disturbance and climatic variation. Local disturbance histories are difficult to determine, and these stands likely experienced different levels of insect attacks, canopy gaps, and low-intensity surface fires that occur within the long intervals between stand-replacing fire (Despain, 1990). However, site selection was rigorous; stand ages were determined from a stand age map and were corroborated using tree increment cores and climatic variation was calculated to be minimal based on historical records (see Kashian et al. in press, *Methods*).

2.2. Sampling of soil, understory and forest floor characteristics

At each site, a 20-m × 5-m belt transect was established to collect soil samples and estimate the percent cover of understory vegetation and forest floor cover. Every 2 m along the transect, a sampling location was positioned 2.5 m on alternating sides of the center transect ($n=10$ per transect). At each location, species-specific percent (%) cover of all graminoids, forbs, and shrubs within a 0.5 m^2 sampling frame was recorded. In addition, the % cover of litter, fine woody debris (FWD, <7.5 cm diameter), coarse woody debris (CWD, ≥7.5 cm diameter) touching the ground, rocks, and exposed mineral soil in the sampling frame were estimated visually. All cover values summed to 100%. Biomass of understory vegetation was calculated using species-specific allometric equations (Turner et al., in press) from estimates of % cover by species. At the center of each sampling frame, one soil sample was taken with a 5-cm diameter PVC core to a depth of 15 cm. Fresh forest floor litter was removed prior to sampling. Because no further disruptions of soil were made, soil samples from each site likely reflected different proportions of forest floor and mineral soil. Each soil sample was placed in a plastic bag and kept cool for transport to the laboratory.

Each soil sample was subsampled to create one composite core per stand for determination of general soils characteristics. These composite samples were homogenized, air dried, and sent to the Soils and Plant Analysis Lab at the University of Wisconsin, Madison. Soil pH was measured in water with a 1:1 mass:volume ratio. A micro-Kjeldahl procedure was used for total N determination (Jackson, 1958). Acid extractable phosphorus (P) was analyzed colorimetrically using the Truog method (Schulte et al., 1987) and potassium (K), calcium (Ca), and Magnesium (Mg) were measured by atomic absorption after extraction with H_2SO_4 (Schulte et al., 1987). Percent organic matter was determined by dry combustion using the Tekmar–Dohrman 183 TOC Boat Sampler DC-190 (Tekmar-Dohrman, Mason OH).

2.3. Isotopic $^{15}NH_4^+$ pool dilution

We calculated gross rates of NH_4^+ mineralization using ^{15}N isotope dilution (Kirkham and Bartholomew, 1954; Davidson et al., 1991; Hart et al., 1994) on a subset of the samples (randomly chosen, $n=4$ per site). We added 1 ml of ^{15}N ammonium sulphate ($(^{15}NH_4)_2SO_4$ ($15N_2$, 98%+) at a concentration of 17.81 μg ml^{-1}) into 30 g of soil. After mixing, initial ($t=0$) samples were prepared by extracting 15 g of soil with 75 ml of 2 M KCl. Samples were shaken for 30 min on a mechanical shaker, and filtered using KCl-rinsed, Whatman No. 2 filters. The remaining soil (approximately 15 g) was incubated at constant temperature and moisture for 24 h, long enough to improve the uniformity of the ^{15}N isotope within the soil and to obtain equilibrium of isotopic and indigenous N, but short enough that remineralization is unlikely (Murphy et al., 2003). The total amount of N added was approximately 1 μg^{15}N g-soil^{-1}. After the incubation period, the soil was extracted and filtered as described above. Extractants were frozen (−18 °C) until further analysis.

Samples were diffused using a ^{15}N diffusion procedure that volatilizes extracted N as NH_3, which is then collected on an acidified filter disk (Brooks et al., 1989; Herman et al., 1995). Diffusion efficiency was evaluated using standards until 90–95% recovery of sample was obtained. Samples were stored in 5×8 mm aluminum capsules (Elemental Microanalysis Ltd, Mason, Ohio) and analyzed by mass spectrometry (Europa Integra, UC-Davis Stable Isotope Facility, California).

Gross rates were determined from changes in atom percentage of ^{15}N excesses (APE) above background values and from N pool sizes of pre-and post-incubated soils. Pool sizes of all samples (NH_4–N) were determined on a Lachat QuikChem (Lachat Instruments, Milwaukee, Wisconsin, USA) autoanalyzer. Gross NH_4^+ mineralization was calculated using the equations from Kirkham and Bartholomew (1954):

$$m = (M_0 - M_t)/t * [\log(\mathrm{APE}_0/\mathrm{APE}_t)/\log(M_0/M_t)] \text{ and,}$$

$$c = m - [(M_0 - M_t)/t],$$

where m, gross NH_4^+–N mineralization rate of the soil (mg kg^{-1} d^{-1}); c, NH_4^+–N consumption rate of soil (mg kg^{-1} d^{-1}); t, time (1 day); APE_0, atom percent ^{15}N excess of NH_4^+ pool at time 0; APE_t, atom percent ^{15}N excess of NH_4^+ pool at time t; M_0, total NH_4^+–N in the soil at time 0 (mg NH_4^+–N kg^{-1}); M_t, total NH_4^+–N in the soil at time t (mg NH_4^+–N kg^{-1}). Estimates of net NH_4^+ mineralization rates were calculated by subtracting consumption rates from gross mineralization and thus were not independently derived.

Application of labeled $^{15}NH_4^+$ enables the measurement of gross mineralization (input flow) and the sum of $^{15}NH_4^+$ consumption processes (output flow), which may include $^{15}NH_4^+$ immobilization, $^{15}NH_4^+$ oxidization, gaseous loss and, least likely in a laboratory setting, plant uptake. We assume $^{15}NH_4^+$ immobilization accounts for the majority of the $^{15}NH_4^+$ consumption, although we refer to the processes as $^{15}NH_4^+$ consumption because it is not possible to separate immobilization with this method. There are several assumptions underlying $^{15}NH_4^+$ isotope pool dilution including minimal isotopic discrimination, uniform distribution of the applied isotope, and equilibrium of N pools (Murphy et al., 2003). However, pool dilution has the advantage of being able to discriminate consumption and production processes and therefore is the analysis that we expect to be most closely associated with changes in microbial community composition. Moreover, by using the pool dilution method, microbial community analysis and N cycling processes were measured on the same time step (versus other N measures, e.g. in situ resin cores).

2.4. Microbial community analysis

A hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) was used to analyze the microbial community composition on a subset of soil samples per site (randomly chosen, $n=4$). The procedure is based on the extraction of signature lipid biomarkers from the cell membrane and wall of microorganisms (White and Ringelberg, 1998). Immediately after sampling, soil samples were put in plastic bags and placed in coolers in the field and kept cool for transport back to the laboratory. Samples were shipped overnight to the University of Wisconsin (Madison, WI) where they were homogenized, frozen, and then freeze-dried before analysis. All glassware was baked at 475 °C for 4 h. We extracted, purified and identified PLFAs from microbial cell membranes in lyophilized whole-soil using a hybrid lipid extraction based on a modified Bligh and Dyer (1959) technique, combined with fatty acid methyl ester analysis (FAME) as described by Microbial ID Inc. (Hayward, CA). Briefly, lipids were extracted from 4 g of freeze-dried soil using a chloroform-methanol extraction with a phosphate buffer (potassium phosphate (3.6 ml), methanol (8 ml), and $CHCl_3$ (4 ml)) in 25-ml glass tubes, shaken for 1 hr and centrifuged. Supernatant was then decanted to 30-ml tubes and potassium phosphate buffer and chloroform were re-added and the tubes were vortexed for 30 s. The phases were allowed to separate overnight at room temperature. The top layer was aspirated off, saving the chloroform phase, and the volume was reduced in a RapidVap. We then follow the procedure for FAME as given by Microbial ID Inc; sodium hydroxide was added for saponification and the solution was heated in a water bath for 30 min, followed by mild alkaline methanolysis.

A 2 μl injection of the methyl-ester derivatives of the extracted phospholipid were analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a flame ionization detector and split/splitless inlet and a 25 m× 0.2 mm inside diameter×0.33 μm film thickness Ultra 2

(5%-phenyl, 95%-methyl) capillary column (Agilent) using hydrogen as the carrier gas, N as the make up gas, and air to support the flame. Gas chromatograph conditions are set by the MIDI Sherlock program (MIDI, Inc. Newark, DE). Peaks were identified with bacterial fatty acid standards and Sherlock peak identification software (MIDI, Inc. Newark, DE). Fatty acids were quantified by comparisons of peak areas from the sample compared to peak areas of two internal standards, 9:0 (nonanoic methyl ester) and 19:0 (nonadeconoic methyl ester), of known concentration. In all subsequent analyses we used only fatty acids that were identifiable and present at >0.5 mole percent. We included several 'summed' fatty acids that could not be uniquely identified by the GC software, due to their high relative abundance; we refer to these markers as 'unknown', although they represent two overlapping response peaks.

Lipids cannot confidently be used to represent specific species but are more commonly assigned functional guilds. Terminology to describe fatty acids is described by 'A:B ωC' where 'A' indicates the total number of carbon atoms, 'B' the number of double bonds (unsaturations), and 'ω' indicates the position of the double bond from the methyl end of the molecule. The prefixes '*i*' and '*a*' refer to iso and anti-iso methyl branching. Hydroxy groups are indicated by 'OH'. Cyclopropyl groups are denoted by '*cy*'. 10ME refers to a methyl group on the tenth carbon from the carboxylic end of the fatty acid (Arao, 1999; Bååth and Anderson, 2003; Steenwerth et al., 2003).

Several ratios of lipids were calculated, including: fungi/bacteria, Gram positive/Gram negative (Gm^+/Gm^-), cyclopropyl/monounsaturated (a measure of nutritional stress; Petersen and Klug, 1994), and iso-/anteiso-branching (a measure of environmental stress; Kieft et al., 1994). Bacterial biomass was estimated from the summed abundance of the following PLFAs: *i*15:0, *a*15:0, 15:0, 15:1ω8, 16:0 10Me, *i*16:0, 16:1ω7, *a*17:0, *cy*17:0, 17:1ω7, 18:1ω7 and *cy*19:0ω8 (Frostegård and Bååth, 1996; Wilkinson et al., 2002) and fungal biomass was estimated from concentrations of the marker 18:2ω6 (Federle, 1986; Frostegård and Bååth, 1996), 18:1ω9, and unknown 5 (identified as either 18:2ω6,9 or *a*18:0 by MIDI software) (Federle, 1986; Frostegård and Bååth, 1996; Bååth and Anderson, 2003). We also grouped lipids into functional 'guilds' as follows: Gram positive bacteria included *i*15:0, *a*15:0, *i*16:0, *a*17:0. Gram negative bacteria included 16:1ω7, *cy*17:0, 17:1ω7, 18:1ω7, and *cy*19:0ω8. Cyclopropyl lipids included *cy*17:0 and *cy*19:0, while monounsaturated lipids included 16:1ω7 and 18:1ω7 (Wilkinson et al., 2002). Anteiso included *a*11:0, *a*15:0, *a*17:0, and iso included *i*16:0, *i*18:0, *i*19:0, *i*17:1G, and *i*17:1ω10.

2.5. Statistics

General soil properties, isotopic pool dilution results, microbial lipid abundance, relative mole fraction of lipids within guilds, lipid ratios, above-ground stand biomass, and forest floor cover data, were compared using one-way Analysis of Variance (ANOVA) for the 20 sites, with age-class as a grouping variable. Stands were separated into four discrete age-classes (50–100 years, 125–175 years, 200–250 years, 300–350 years). Significant differences among means were tested using the Tukey–Kramer (95% confidence interval, family-wise comparisons, $n=5$) using R statistical software. All variables to be used in the ANOVAs were tested for normality using the Shapiro-Wilk goodness-of-fit method and were log transformed as appropriate.

To gain the maximum 'information' from the multivariate data sets generated by the PLFA, we used ordination axes derived from non-metric multidimensional scaling (NMS) (PC-ORD (McCune and Mefford, 1999)) as summary variables describing the microbial community. We ordinated stands by their microbial community composition based on the relative mole fraction of individual lipids. We chose to use NMS because it avoids the assumption of linear relationships among variables and it uses rank distances, minimizing error produced by the 'zero-truncation' problem common to community data (McCune and Grace, 2002). All mole fraction data were arcsine squareroot transformed (McCune and Grace, 2002). After transformation, skew was <1 and coefficient of variation was 33.2% among sites and 46.2% among lipids. Outlier analysis on all variables was performed using Sorensen distance. Lipid 12:1ω7 was 4.50 standard deviations above the mean and was deleted from future analyses. One site was a mild outlier (2.94 standard deviations above the mean) but was included in the dataset because it is the youngest stand in the chronosequence (50 years old). NMS was run using the 'slow and thorough' autopilot option, with 40 runs with real data and 50 random runs. The NMS ordination resulted in 2 axes (confirmed by examination of the scree plots), which explained 95% of the variance. Most of the variance was explained by axis 1 (55%), while axis 2 explained 40%. Ordination scores of sites were averaged for each age-class and differences between age-classes were compared using Fisher LSD. Pearson correlation coefficients were used to explore the relationships between ordination scores or functional guilds and canopy, soil, understory/forest floor cover, and mineralization and consumption rates.

Backward-selection stepwise multiple linear regression models were run with stand characteristics and NMS axis scores as independent variables and NH_4^+ cycling rates as response variables. The goal was to determine which stand characteristics (canopy, understory/forest floor, soil, and microbial) provided the best statistical model of NH_4^+ cycling rates. Then, the variables selected within each category were combined into a second stepwise model to determine their relative importance. The variables that best explained NH_4^+ cycling rates were then combined in a linear model, and only those variables that significantly ($p<0.05$) contributed to the model were included in the final model. This 'nested' stepwise approach elucidated the relative strength of different ecosystem variables. Particularly, it

allowed us to determine whether microbial information is as useful as other stand characteristics to explain NH_4^+ cycling.

To determine whether stands exhibit similar patterns of convergence as observed by Kashian et al. (in press) for canopy structure, we calculated between-stand coefficients of variation for each variable.

3. Results

3.1. Variation with stand age (Question 1)

In general, canopy, understory/forest floor, and soil variables did not differ significantly among the 4 age classes, primarily due to wide within age-class variation (i.e. variation among stands) (Table 1). Among soil nutrients, only calcium varied significantly among age classes. Across all sites, soil organic matter averaged 4.3%, total N averaged 0.08%, and pH averaged 5.1. Among cover values, only downed wood (FWD, CWD) and exposed mineral soil were significantly different among age classes. Total biotic cover ranged from 14 to 30% across the 20 sites but was not significantly different among age classes. Since our stands were a subset of stands measured by Kashian et al. (in press, 2005), we used data reported therein to calculate changes in canopy variables with increasing age class for our stands. Similar trends were reflected in our stands subset compared to those determined by Kashian et al. (in press). BAI and tree density were significantly higher in the youngest age class than the older age classes. Using our 20-stand subset, stand tree density decreased from 5092 ($\pm$2047) trees ha^{-1} in the 50–100 age class to 1228 ($\pm$216) in the 300–350 age class, while BAI decreased from 6.1 ($\pm$1.3) $m^2\ ha^{-1}$ in the 50–100 age class to 2.5 ($\pm$0.3) in the 300–350 age class (Kashian et al., in press, 2005).

The average scores along the axes of the microbial NMS ordination differed between the 300–350 age-class and the 50–100 and 200–250 age classes (Fig. 1). Lipids that were most highly correlated ($r > |0.8|$) with axis 1 included fungal indicators (18:2ω6, 18:1ω9), unidentified lipids (unknown 7, unknown 3), 16:0N alcohol, and 16:1ω7 (Fig. 2). Lipids that were most highly correlated with axis 2 included an actinomycetes marker (10Me16:0), unidentified lipids (unknown 3, unknown 5), 18:1ω5, and *i*19:0.

Total PLFA lipid abundance ranged from 54.8 $\pm$ 15 nmol g^{-1} in the 125–175 age class to 110.0 $\pm$ 26 nmol g^{-1} in the 200–250 age class, but was not significantly different among age classes. PLFA abundance among functional guilds also did not differ significantly among age classes, although lower lipid abundance in the 125–175 age class was consistently observed (data not shown). Stress ratios of dominant functional guilds were not significantly different among age-classes. Three lipids (16:0, 15:1ω8, 18:1ω7) together accounted for almost 35% of the lipid biomass while another 5 (18:1ω9, unknown 5, *cy*17:0, 12:0, and 14:0) comprised an additional 25% (Table 2). Thus, over half the variation in PLFA abundance

Table 1
Average ($\pm$1 SE) stand characteristics by age-class (n=5)

Variable	50–100	125–175	200–250	300–350	p
Organic matter (%)	4.4 (0.8)	4.2 (1.2)	4.3 (0.6)	4.3 (0.3)	ns
Calcium (kg ha^{-1})	538 (96.0)ab	493 (39.8)ab	697 (53.7)a	489 (21.7)b	.
Magnesium (kg ha^{-1})	79 (17.0)	72 (7.6)	90 (12.3)	65 (9.0)	ns
Potassium (kg ha^{-1})	334 (38.3)	334 (53.1)	293 (22.0)	260 (21.2)	ns
Phosphorus (kg ha^{-1})	18 (2.9)	12 (2.0)	16 (3.6)	20 (5.3)	ns
Total Nitrogen (%)	0.07 (0.01)	0.07 (0.01)	0.08 (0.01)	0.08 (0.01)	ns
PH	5.14 (0.13)	5.12 (0.07)	5.10 (0.04)	4.98 (0.04)	ns
Total biotic cover (%)	13.9 (5.9)	19.0 (6.9)	29.8 (12.9)	24.8 (3.4)	ns
Rock cover (%)	0.3 (0.1)	0.1 (0.1)	0.3 (0.3)	0.8 (0.6)	ns
Fine woody debris cover (%)	6.5 (2.0)a	10.2 (0.6)b	10.5 (1.2)b	8.1 (0.7)ab	*
Coarse woody debris cover (%)	7.9 (4.8)a	3.0 (0.8)b	11.1 (3.4)ab	3.2 (1.4)ab	*
Mineral soil cover (%)	8.2 (0.6)a	5.4 (0.6)ab	2.0 (0.9)b	3.0 (1.0)ab	*
Litter cover (%)	69.5 (7.6)	54.6 (6.7)	53.8 (11.7)	55.2 (6.2)	ns
Forb biomass (kg ha^{-1})	3.4 (1.0)	7.5 (4.8)	20.3 (11.4)	9.6 (4.2)	ns
Graminoid biomass (kg ha^{-1})	52.6 (22.2)	34.7 (58.9)	58.9 (30.4)	57.1 (16.1)	ns
Shrub biomass (kg ha^{-1})	10.3 (9.2)	14.0 (14.0)	22.8 (16.3)	27.0 (15.7)	ns
Herb biomass (kg ha^{-1})	66.3 (31.4)	56.2 (28.7)	101.9 (52.3)	93.7 (11.6)	ns
Basal area increment ($m^2\ ha^{-1}$)[a]	6.1 (1.3)a	4.1 (0.5)ab	3.0 (0.6)ab	2.5 (0.3)b	*
Leaf area index ($m^2\ m^{-2}$)[1]	2.0 (0.3)	1.8 (0.1)	2.0 (0.2)	1.7 (0.1)	ns
Density (stems ha^{-1})[1]	5092 (2047)a	2916 (691)ab	1172 (195)b	1228 (216)b	*

P-value represents statistical differences among age-class means using ANOVA ('*' 0.05 '.' 0.1, ns=non-significant). Letters denote differences among age-classes using Tukey–Kramer HSD (90% confidence interval). Calcium, phosphorus, total biotic cover, rock cover, coarse woody debris cover, understory biomass, basal area increment, and density were log transformed before analysis.

[a] From data reported in Kashian et al. (in press, a)

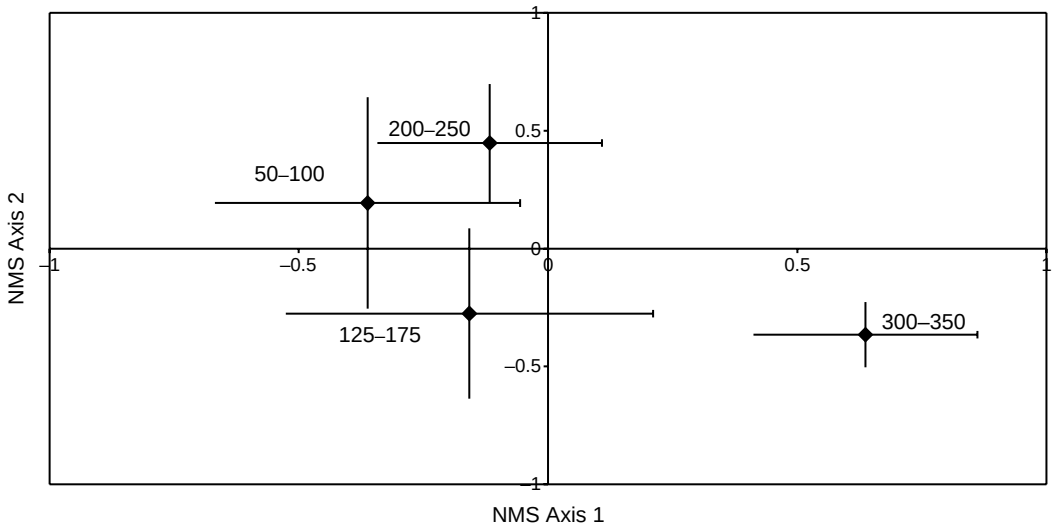

Fig. 1. Average (±1 SE) microbial community composition among age classes resulting from NMS ordination. ($n=5$ stands/age class).

could be explained by examining only 8 lipids. 'Unknown' lipids represented 12% of the total biomass in the 50–100 age class, but over 26% in the 300–350 age class, indicating that older forest microbial communities are less well characterized by the MIDI software. The relative abundance of only 1 lipid (*i*17:1ω10) was different among age classes, being significantly higher in the oldest age-class ($p<0.05$), although the biological significance of this result is probably limited due to this lipid's small relative abundance (~1%).

Gross NH_4^+ mineralization rates ranged from 0.49 to 3.82 mg kg-soil^{-1} d^{-1} (average = 1.45 ± 0.07 mg NH_4^+ kg-soil^{-1} d^{-1}). NH_4^+ consumption rates ranged from −0.03 to 4.67 mg NH_4^+ kg-soil^{-1} d^{-1} (average = 1.37 ± 0.20 mg NH_4^+ kg-soil^{-1} d^{-1}). Net NH_4^+ mineralization rates ranged from −1.07 to 1.05 mg NH_4^+ kg soil^{-1} d^{-1} (average = 0.08 ± 0.18 mg NH_4^+ kg-soil^{-1} d^{-1}) but were generally low (zero or negative) in the two youngest age-classes. The highest net NH_4^+ availability was observed in the oldest age-class, which also had the lowest NH_4^+ consumption. Gross NH_4^+ mineralization and NH_4^+ consumption, were not significantly different among age-classes ($p<0.10$), but net NH_4^+ mineralization was significantly higher in the 300–350 age class compared to the 125–175 age class (Fig. 3).

Because the % organic matter was low in these soils, rates of NH_4^+ mineralization and consumption expressed as per kilogram organic matter were much higher than rates expressed as total soil weight (e.g. gross NH_4^+ mineralization ranged from 12.31 to 70.83 mg NH_4^+ kg-om^{-1} d^{-1}). However, due to the narrow range of organic matter that we observed, rates did not differ among age-classes when expressed as per kilogram organic matter. NH_4^+ mineralization and consumption rates did not differ among age-classes if rates were divided by lipid abundance (e.g. specific rates of mineralization, Waldrop et al. (2000)).

3.2. Explaining patterns of microbial community composition and NH_4^+ cycling (Question 2)

Scores along axis 1 of the NMS ordination were positively correlated with gross NH_4^+ mineralization, % organic matter, % N, and the % of exposed rock, and negatively correlated with the % of exposed mineral soil (Table 3). Axis 1 was also weakly correlated with the cyclopropyl/ monounsaturated ratio ($r=0.45$, $p<0.1$, data not shown). Axis 2 scores were positively correlated with pH and the % of exposed mineral soil, and negatively correlated with %organic matter, %P, %N, graminoid and

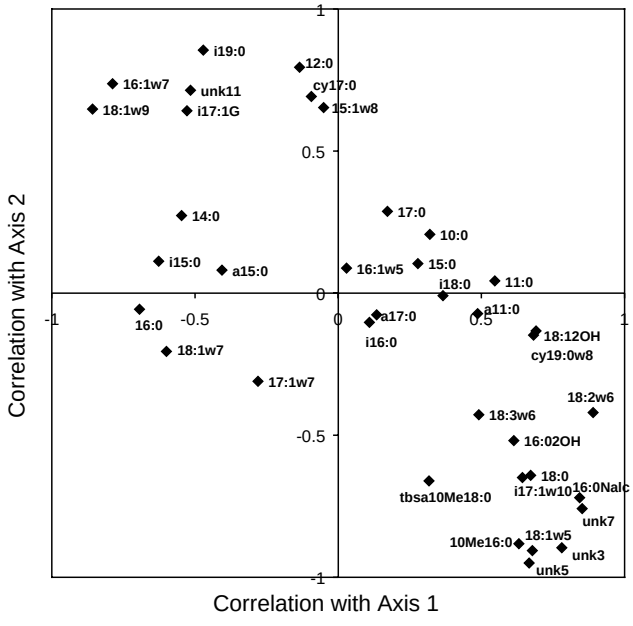

Fig. 2. Correlation of individual lipids with NMS ordination axes. Points closer to +1 or −1 are more strongly correlated with the ordination axes.

Table 2
Relative mole percent of lipids in each age-classes

PLFA Marker	50–100	125–175	200–250	300–350
Saturated				
10:0	0.50	0.20	0.40	0.36
11:0	0.80	0.38	0.86	1.09
12:0	5.77	4.88	6.00	5.61
14:0	6.50	6.31	7.60	5.36
15:0	0.47	0.09	0.23	0.16
16:0	13.59	12.59	11.80	9.75
17:0	1.75	1.31	1.26	1.80
18:0	0.39	0.64	0.35	0.78
Branched				
*a*11:0	1.12	0.32	1.34	1.89
*a*15:0	1.81	1.58	1.65	1.55
*i*15:0	3.19	2.80	2.98	2.32
*i*16:0	1.11	0.78	0.71	0.88
*a*17:0	1.57	1.23	1.15	1.45
*i*18:0	0.38	0.42	0.35	0.60
*i*19:0	2.42	1.16	2.15	0.00
*i*17:1G	1.70	1.73	1.42	0.34
*i*17:1ω10	0.37	0.55	0.32	1.73
Monounsaturated				
15:1ω8	11.60	10.94	15.25	12.39
16:1ω7	1.75	1.65	1.78	0.46
17:1ω7	2.58	2.43	1.84	1.79
18:1ω5	1.23	1.46	0.96	1.92
18:1ω7	11.18	11.03	8.02	6.94
Arbuscular mycorrhizal fungi (AMF)				
16:1ω5	0.71	0.46	1.11	0.69
Actinomycetes				
16:0 10Me	0.82	1.26	0.91	1.46
18:0 10Me	0.33	0.38	0.08	0.21
Cyclopropyl				
*cy*17:0	6.27	5.32	6.87	5.83
*cy*19:0ω8	0.24	0.39	0.37	0.66
Hydroxy				
16:0 2OH	0.61	0.58	0.32	0.78
18:1 2OH	0.19	0.23	0.59	0.64
Alcohol				
	16:0 N 1.17	1.59	0.95	1.62
Protozoa				
18:3ω6,9,12	0.93	0.72	0.46	1.00
Fungi				
18:1ω9	3.49	2.41	2.58	0.67
18:2ω6,9	0.53	0.63	0.65	1.02
Unk5 (18:2 ω 6,9/*a*18:0)	3.29	4.15	2.12	4.48
Summed lipids				
Unk11 (*i*15:02OH/16:1ω7)	1.17	2.24	3.79	0.73
Unk3 (*i*15:0 2OH/ 16:1ω7)	2.48	3.00	2.12	3.97
Unk7 (19:1ω6/.846/*cy*19)	6.04	12.16	8.67	17.09

Lipids are grouped by functional guild.

shrub biomass and gross NH_4^+ mineralization (Table 3). The strongest correlation ($p<0.001$) for both axes was with total PLFA abundance ($r=0.81$ and -0.77 for axis 1 and 2, respectively, data not shown). In general, microbial functional guilds were positively correlated with % organic matter, understory biomass (particularly shrubs), and % N, and were negatively correlated with litter and mineral soil (Table 3). Lipid biomarkers that most likely represent the actinomycetes guild (both 10Me18:0 and 10Me16:0) were significantly correlated with % exposed rock and gross NH_4^+ mineralization. Higher organic matter was also associated with more acidic soils, resulting in opposing relationships with organic matter and pH. For example, the fungi/bacteria ratio was positively correlated with pH ($r=0.46$, $p<0.05$) and negatively correlated with organic matter ($r=-0.59$, $p<0.01$).

Gross NH_4^+ mineralization was best explained by microbial community composition (NMS Axis1, iso/ante ratio) rather than general stand characteristics (Table 4). Gross NH_4^+ mineralization was positively correlated with % organic matter and total N but the relationships were not strong ($r=0.42$, $p<0.1$, and $r=0.43$, $p<0.1$, respectively, data not shown). Gross NH_4^+ mineralization was positively correlated with abundance of many different guilds (actinomycetes, hydroxy and protozoa guilds ($p<0.05$)) (Table 3) and numerous individual lipids (17:0, 18:0, 18:3ω6, unknown 3, and *cy*19:0ω8, data not shown).

In contrast to gross rates, the final stepwise models for NH_4^+ consumption and net NH_4^+ mineralization were not significant. The only significant relationships we observed for NH_4^+ consumption and net NH_4^+ mineralization were with individual functional guilds. NH_4^+ consumption was positively correlated with a lipid biomarker for actinomycetes (10Me18:0; $p<0.01$), while net NH_4^+ mineralization was negatively correlated with this same lipid ($p<0.05$) (Table 3) in addition to *i*18:0 ($p<0.05$). However, NH_4^+ consumption and net NH_4^+ mineralization were correlated with a narrower portion of the microbial community compared to gross rates. Interestingly, several microbial variables (total PLFA abundance, iso/ante, and NMSaxis1) were selected as important microbial variables for both NH_4^+ rates. Also, weak relationships between NH_4^+ consumption and characteristics of the forest floor may exist, reflected by the number of forest floor variables selected in the stepwise model.

3.3. Patterns of variance with stand age (Question 3)

For all stands, the coefficient of variation (CV) averaged 26% for soil, 37% for canopy, 67% for microbial community, 68% for gross mineralization and consumption, and 100% for understory cover variables. The CV of canopy variables for stands in the 50–100 age class averaged 60% but was half as large (26–33%) in the older age class (Fig. 4). However, only variance in stand tree density was significantly different among age-classes (Tukey's family-wise comparison, $p=0.05$), as Kashian et al. (in press) showed for the larger set of tree density data. The lack of significant differences in CV among age-classes for most variables indicates that between-stand variation was greater than variation among age-classes. The CV in NH_4^+ cycling rates had the widest range (26–96%) among age-classes but differences among age-classes were not significant. Contrary to trends in convergence for canopy and cover values, the average CV for gross NH_4^+ mineralization and

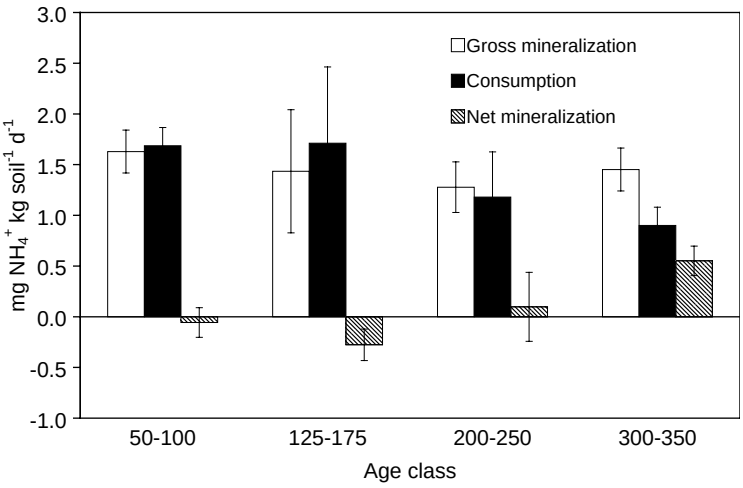

Fig. 3. Average (±1 SE) rates of gross NH_4^+ mineralization, NH_4^+ consumption, and net NH_4^+ mineralization among age classes. ($n=5$ stands/age class).

consumption was least in the 50–100 age-class compared to older age-classes indicating higher stand-to-stand variance among older stands.

4. Discussion

Observed rates of gross NH_4^+ mineralization among these mature lodgepole pine stands is comparable to rates of N cycling observed in other forested ecosystems, although at the low end. Rates for total N (NH_4^+ plus NO_3^-) gross mineralization range are typically <1 mg N kg^{-1} d^{-1} although they may range up to 50–200 mg N kg^{-1} d^{-1} (see citations in Mary et al., 1998). Similar to our mean rate of 1.45 mg kg^{-1} d^{-1}, gross N mineralization and immobilization were approximately 1 mg N kg^{-1} d^{-1} in a low productivity, 70-year old, coniferous forest stand in Washington (USA) (Hart et al., 1997, Fig. 1a and b) and approximately 1.5 mg kg^{-1} d^{-1} for a 13-year old pine forest in North Carolina (USA) (Finzi and Schlesinger, 2003, Fig. 2).

The range in gross NH_4^+ mineralization that we observed (0.49–3.82 mg kg^{-1} d^{-1}) in soil in mature lodgepole pine stands in YNP was twice as large as the range observed by Myrold and Tiedje (1986) when comparing agricultural and hardwood forest soils (0.6–1.5 mg kg^{-1} d^{-1}). The range we observed was also comparable to the range ($<$1–4.5 mg ($NO_3^- + NH_4^+$) kg^{-1} d^{-1}) observed by Hart et al. (1997, Fig. 1a) in low and high-productivity coniferous forest sites in the Pacific Northwest.

We did not observe significant patterns of convergence in NH_4^+ mineralization, as Kashian et al. (in press) reported for tree density. One reason may be the high variance we observed for all age-classes. Our study suggests that soil N cycling is heterogeneous among mature lodgepole pine stands. There is some precedence for this conclusion. Christensen and MacAller (1985) observed large between and within site variation in NO_3^- and NH_4^+ dynamics that exceeded variation among successional stage in a chronosequence in the Piedmont of North Carolina. Moreover, Turner et al. (in press) observed high between-stand variation in aboveground net primary productivity and LAI within a successional stage in lodgepole pine forests in YNP, which they suggested was similar in magnitude to temporal variation observed through succession.

4.1. Implications

Interestingly, the pool dilution results show the highest net NH_4^+ mineralization rates in the oldest age-class. Higher net NH_4^+ mineralization rates in older versus younger forests is not unprecedented and is consistent with fundamental nutrient cycling hypotheses (Vitousek and Reiners, 1975). In a primary successional sere near Lake Michigan, the highest rates of net NH_4^+ mineralization were observed in the oldest site (approximately 250 yrs) (Robertson and Vitousek, 1981). Yet, higher net NH_4^+ mineralization in the oldest age class suggests NH_4^+ is less limiting to microbes and plants than in younger stands, which seems to challenge theories of persistent N limitation in these lodgepole pine forests. Fahey and Knight (1986) proposed a positive feedback mechanism to explain persistent N limitation in lodgepole pine forests. They suggested that low N uptake by vegetation results in litter with high C:N ratio, which stimulates microbial immobilization of N during litter decay and furthers N limitation until N is returned via tree mortality or fire. However, increased net mineralization in older age classes is possible if C substrates are increasingly recalcitrant, reducing microbial immobilization (Hart et al., 1994; Merilä et al., 2002). Litton et al. (2003) showed that microbial biomass C and soil-surface carbon dioxide efflux varied with stand age in fire-dominated ecosystems, although their mature stands were $<$120 years. They attributed differences to the lack of

Table 3
Pearson correlations (*r*) between microbial functional guilds and NMS axis scores with mineralization rates and canopy, soil, or understory/forest floor variables

	NMS axis1	NMS axis2	Functional guild											
			1	2	3	4	5	6	7	8	9	10	11	12
	Mineralization rates													
Gross mineralization	$0.46^{(.)}$	$-0.40^{(.)}$	$0.46^{(*)}$	ns	ns	$0.39^{(.)}$	$0.38^{(.)}$	$0.38^{(.)}$	$0.50^{(*)}$	$0.58^{(**)}$	ns	$0.54^{(*)}$	ns	$0.39^{(.)}$
Consumption	ns	ns	$0.57^{(**)}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Net mineralization	ns	ns	$-0.46^{(*)}$	ns	$0.43^{(.)}$	ns	ns	ns	$0.38^{(.)}$	ns	ns	ns	ns	ns
	Canopy													
Density	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Basal area increment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Leaf area index	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	$0.42^{(.)}$	ns
	Soil													
pH	ns	$0.46^{(*)}$	ns	ns	ns	ns	$-0.44^{(.)}$	ns	ns	ns	ns	ns	ns	ns
%organic matter	$0.40^{(.)}$	$-0.45^{(*)}$	ns	ns	ns	$0.43^{(.)}$	$0.55^{(*)}$	ns	$0.42^{(.)}$	ns	$0.48^{(*)}$	ns	ns	$0.45^{(*)}$
Calcium	ns	ns	ns	ns	$0.38^{(.)}$	ns	ns	ns	ns	ns	ns	ns	ns	ns
Magnesium	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Phosphorus	ns	$-0.51^{(*)}$	$-0.39^{(.)}$	ns	$0.42^{(.)}$	$0.42^{(.)}$	ns	ns	ns	ns	ns	ns	ns	ns
Potassium	ns	ns	ns	$0.42^{(.)}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Nitrogen	$0.39^{(.)}$	$-0.38^{(.)}$	ns	ns	ns	ns	$0.40^{(.)}$	$0.39^{(.)}$	$0.52^{(*)}$	ns	ns	$0.41^{(.)}$	$0.50^{(*)}$	ns
	Understory/forest floor													
%Rock	$0.40^{(.)}$	ns	$0.73^{(***)}$	$0.40^{(.)}$	ns	ns	ns	$0.42^{(.)}$	ns	$0.45^{(*)}$	ns	$0.54^{(*)}$	ns	ns
%Litter	ns	ns	ns	ns	$-0.77^{(***)}$	$-0.51^{(*)}$	ns	$-0.49^{(*)}$	$-0.54^{(*)}$	ns	$-0.39^{(.)}$	$-0.39^{(.)}$	ns	ns
%Mineral soil	$-0.49^{(*)}$	$0.46^{(.)}$	ns	ns	ns	$-0.40^{(.)}$	$-0.62^{(**)}$	$-0.39^{(.)}$	$-0.57^{(**)}$	$-0.40^{(.)}$	$-0.57^{(**)}$	$-0.39^{(.)}$	$-0.44^{(.)}$	$-0.55^{(*)}$
%Coarse woody debris	ns	ns	ns	ns	ns	ns	ns	ns	ns	$0.41^{(.)}$	ns	$0.38^{(.)}$	ns	$0.42^{(.)}$
%Fine woody debris	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
%Total cover	ns	ns	$-0.38^{(.)}$	ns	$0.48^{(*)}$	ns	ns	ns	ns	ns	ns	ns	ns	ns
Forb biomass	ns	ns	ns	ns	$0.43^{(.)}$	ns	ns	ns	ns	ns	ns	ns	ns	ns
Graminoid biomass	ns	$-0.40^{(.)}$	ns	ns	ns	$0.39^{(.)}$	ns	ns	ns	ns	ns	ns	ns	ns
Shrub biomass	ns	$-0.40^{(.)}$	ns	ns	$0.43^{(.)}$	$0.46^{(.)}$	$0.45^{(*)}$	$0.43^{(.)}$	$0.43^{(*)}$	ns	$0.49^{(*)}$	$0.43^{(*)}$	ns	ns
Herb biomass	ns	ns	ns	ns	$0.43^{(.)}$	$0.42^{(.)}$	ns	ns	ns	ns	ns	ns	ns	ns

Independent variables (except pH) were log transformed. Significance levels are denoted in parentheses ('***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1). ns, not significant. Lipid biomarkers that represent the actinomycetes functional guild were analyzed separately. 1, Actinomycetes (10Me18:0); 2, Alcohols; 3, Arbuscular Mycorrhizal Fungi; 4, Branched; 5, Cyclopropyl; 6, Fungi; 7, Hydroxy; 8, Actinomycetes (10Me16:0); 9, Monounsaturated; 10, Protozoa; 11, Saturated; 12, Unknown ('summed').

Table 4
Variables selected in canopy, soil, understory/forest floor, and microbial categories in individual backward stepwise regression model on each category to explain mineralization rates

	Dependent Variable			
	Gross mineralization		Consumption	Net mineralization
Canopy	**Basal area increment**		–	–
	Density			
Soil	Organic matter		Magnesium	**pH**
	Magnesium			
Understory/Forest floor	Coarse woody debris		**Total % cover**	**Total % cover**
	Graminoid biomass		**Coarse woody debris* fine woody debris graminoid biomass* shrub biomass forb biomass** *	**Coarse woody debris forb biomass mineral soil**
Microbial	Total PLFA abundance **iso/ante* NMSaxis1 ****		Total PLFA abundance iso/ante NMSaxis1	**Total PLFA abundance** iso/ante NMSaxis1
Stepwise model	R^2	0.650	0.442	0.379
	AdjR^2	0.489	0.180	0.214
	F	4.027	1.704	2.290
	P	0.017	0.198	0.108
Final model	R^2	0.414	n/a	n/a
	AdjR^2	0.345		
	F	6.001		
	P	0.011		

The selected variables were run in a second backward stepwise model and the selected variables (in bold) were included in a linear model. Final model for gross mineralization includes only the variables that were significant (*$p<0.05$, **$p<0.01$) in the linear model. Linear models were not significant for consumption or net mineralization.

belowground plant substrates in young stands, but did not evaluate the variability of these substrates among mature stands due to a low sample size. Clearly, more data on soil and rhizosphere N and C substrates is needed to fully evaluate N dynamics in mature stands. It is difficult to substantiate N limitation theories with our pool dilution data because (a) mineralization and consumption from pool dilution are likely a better measure of cellular processes than soil organic matter processes (Fierer et al., 2001) and (b) plant N limitation is likely a function of the degree to which plants can access organic N sources via organic N depolymerization and mycorrhizal uptake processes (Schimel and Bennett, 2004), which cannot be measured using isotopic pool dilution. Moreover, we only evaluated NH_4^+ production, although we expect NO_3^- or organic N turnover may be significant.

The lack of response of gross mineralization to age-class suggests that patterns of gross mineralization may not be related to age. We only examined stands >50 yrs, whereas many studies include younger stands with an N-fixing vegetation phase where large N sources may cause increased net mineralization (Stottlemyer and Toczdlowski, 1999; Merilä et al., 2002) resulting in a perceived reduction in net N mineralization rates with stand age. Perceived

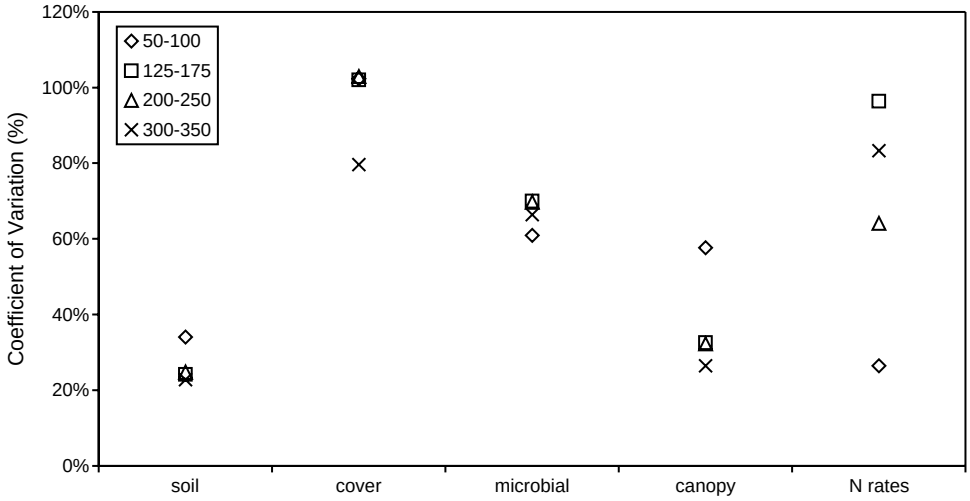

Fig. 4. Average coefficient of variation for soil, vegetative cover, microbial, canopy, and N cycling variables, by age class.

trends in N cycling rates may be a function of the age range of the sites that were sampled (Robertson and Vitousek, 1981) such that N cycling rates in mature stands may appear to be low if compared to high rates early in succession.

Factors other than stand age-class explaining gross NH_4^+ mineralization include the total amount of extracted microbial lipids, microbial community composition, and organic matter (or P, since they are correlated). Thus, microbial communities appear to be important in explaining gross N cycling rates among mature stands. Traditionally, N cycling models ignore microbial community composition when estimating N availability to plants, although recent research has challenged this phytocentric perspective (Neher, 1999; Lindahl et al., 2002). Linking microbial communities to N cycling has generally been attempted using information about microbial activities and chloroform-labile biomass (e.g. Davidson et al., 1992) since traditional methods of characterizing microbial composition using plate counts or DNA have been prohibitive for widespread use. Microbial lipid analysis represents an efficient method for characterizing microbial community composition, yet only a few studies have tried to relate changes in microbial community composition using lipid profiles to changes in N cycling (e.g. Balser and Firestone, in press). We conclude that lipid profiles were useful to explain variation in gross NH_4^+ mineralization. Moreover, our stepwise regression model selected microbial variables in preference to other measured environmental variables. Other evidence in support of the importance of the microbial community to explain N cycling is indirect. The oldest age class had a unique microbial community composition and was also characterized by the highest net NH_4^+ mineralization rates. Positive correlations between functional guilds and understory biomass and organic matter, and negative correlations between litter and mineral soil, suggests microbial guilds are responsive to changes in substrate, which are known to affect mineralization rates.

Ratios of functional guilds ('stress ratios') reflect the response of microbial communities to predators or stressful environmental situations (Vestal and White, 1989; Kieft et al., 1994; Petersen and Klug, 1994) and may, in turn, indicate altered nutrient cycling conditions, e.g. fungi and bacteria have different C:N ratios and may affect nutrient cycling differently (Bååth and Anderson, 2003). In our study, none of the stress ratios were correlated significantly with NH_4^+ cycling rates directly and there were no significant differences among age-classes due to large within-age-class variation. However, the iso-/ante-branching ratio, and Axis 1 of the NMS ordination (which was strongly correlated with the cyclopropyl/monounsaturated ratio), were included in the stepwise model, indicating the potential importance of evaluating stress ratios to explain N cycling.

Even small changes in environmental conditions may be perceived as 'stressful' to portions of the microbial community. As we observed, the ratio of PLFA-derived fungi to bacteria may increase among narrow ranges of pH (Bååth and Anderson, 2003) and may decrease with increasing organic matter (Marschner et al., 2003). Small changes in pH (3.32–4.84) have been shown to modify nitrification (Merilä et al., 2002), however, whether such modification in ecosystem function is a result of changes in microbial community composition or simple changes in the ratio of the NH_4^+/NO_3^- substrates is unknown. In general, how microbial communities respond to narrow changes in environmental conditions and, in turn, how subtle changes in the microbial community affect ecosystem function relative to possible simultaneous changes in substrate remains a challenge for soil and ecosystem scientists.

Our sampling was conducted during the growing season in Yellowstone (approximately mid-June to mid-August) and therefore the results reflect the active time of vegetative production and are appropriate for evaluating relative differences among age-classes. However, soils may exhibit different rates of NH_4^+ cycling and different microbial composition due to seasonal differences in temperature and moisture (Vestal and White, 1989; Binkley et al., 1994; Petersen and Klug, 1994; Stark and Firestone, 1995; Frostegård and Bååth, 1996; Berg et al. 1998; Bardgett et al., 1999; Ley and Schmidt, 2002; Wilkinson et al., 2002), so our results may not reflect microbial community composition or NH_4^+ cycling rates in other seasons or climates.

5. Conclusions

The simultaneous measurement of microbial community composition and NH_4^+ cycling across a heterogeneous landscape represents a new and exciting avenue of research, bridging the disciplines of landscape ecology, microbial ecology and terrestrial biogeochemical cycling. We suggest that microbial communities and N (NH_4^+) cycling are related in complex ways across mature lodgepole pine stands. While some trends with age were apparent, factors other than age largely explain N cycling and microbial community composition. Older age-classes are characterized by a unique microbial community composition and exhibit the highest net NH_4^+ mineralization, perhaps indicating C or other nutrient limitations are increasingly important in older stands. More research on soil organic matter quality, as well as independent verification of net N cycling rates, is needed in lodgepole pine ecosystems. In addition, this study supports the notion that microbial community information helps explain patterns of N (NH_4^+) cycling. The optimum way to quantify microbial changes, however, is still unclear since we found significant relationships at all levels of microbial community organization (e.g. community, guild, lipid). Finally, this study suggests that significant spatial and temporal variability in N cycling and microbial communities exists among relatively mature forests stands recovering from natural disturbance.

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