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Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi

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Abstract

Two complementary studies were performed to examine (1) the effect of 18 years of nitrogen (N) fertilization, and (2) the effects of N fertilization during one growing season on soil microbial community composition and soil resource availability in a grassland ecosystem. N was added at three different rates: 0, 5.44, and 27.2 g N m^{-2} y^{-1}. In both studies, *Schizachyrium scoparium* was the dominant plant species before N treatments were applied. Soil microbial communities from each experiment were characterized using fatty acid methyl ester (FAME) analysis. Discriminant analysis of the FAMEs separated the three N fertilizer treatments in both experiments, indicating shifts in the composition of the microbial communities. In general, plots that received N fertilizer at low or high application rates for 18 years showed increased proportions of bacterial FAMEs and decreased fungal FAMEs. In particular, control plots contained a significantly higher proportion of fungal FAMEs C18:1(*cis*9) and C18:2(*cis*9,12) and of the arbuscular mycorrhizal fungal (AMF) FAME, C16:1(*cis*11), than both of the N addition treatment plots. A significant negative effect of N fertilization on the AMF FAME, C16:1(*cis*11), was measured in the short-term experiment. Our results indicate that high rates of anthropogenic N deposition can lead to significant changes in the composition of soil microbial communities over short periods and can even disrupt the relationship between AMF and plants.

Keywords: Arbuscular mycorrhizal fungi; Fame; Microbial community composition; Nitrogen fertilization; Grasslands

1. Introduction

Anthropogenic nitrogen (N) deposition has the potential to affect soil microorganisms directly by altering competitive interactions with plants (Harte and Kinzig, 1993; Naeem et al., 2000). The effects of N on soil microorganisms have the potential to be manifested more indirectly, particularly in fungi involved in symbiotic relationships with plant roots (Egerton-Warburton and Allen, 2000; Corkidi et al., 2002; Johnson et al., 2003a; Gustafson and Casper, 2004). In either case, changes in soil microbial communities in response to N are likely to alter plant community dynamics.

Studies of N fertilization have demonstrated that the nature and quantity of fertilizer affects microbial enzyme activity, particularly lignolytic enzymes (Carreiro et al., 2000; Sinsabaugh et al., 2002; Frey et al., 2004) as well as the composition of soil microbial communities (Bardgett et al., 1999; Lundquist et al., 1999). Studies in both forests (Frey et al., 2004) and grasslands (Bardgett and Shine, 1999) have shown that N fertilization lowers fungal:bacterial ratios. More specifically, a growing body of evidence strongly suggests that N fertilization not only changes the species composition of both arbuscular mycorrhizal fungal (AMF) (Egerton-Warburton and Allen, 2000) and ectomycorrhizal communities (Avis et al., 2003), but it also

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selects for AMF species which are less beneficial to plants at lower nutrient concentrations (Johnson, 1993).

The objective of this study was to explore the response of soil microbial communities in a long-term N fertilization experiment at the Cedar Creek Long Term Ecological Research Site (part of the US LTER network) whose aboveground responses have been intensively studied (Tilman, 1987). The experiment began in 1982 (Fig. 1a) in a series of abandoned fields dominated by native, C_4 grasses. Twelve years of N fertilization increased the abundance of C_3 grasses, increased litter production, and lowered plant diversity (Figs. 1b and c) (Wedin and Tilman, 1996). Johnson (1993) has shown that fertilization changed the composition of the AMF spore communities. The plant species shifts were associated with decreased C:N ratios of plant tissues, increased net N mineralization, increased soil nitrate, and high N losses (Wedin and Tilman, 1996). The authors argued that changes in the composition of the plant communities appeared to be driving the nonlinear response. They also considered that the change from net immobilization to net mineralization was determined by a microbial threshold and the C:N ratio of detritus (Wedin and Tilman, 1996), but the response of the belowground microbial community in the long-term fertilization experiment has not been examined with the exception of the AMF studies (Johnson, 1993; Johnson et al., 2003a).

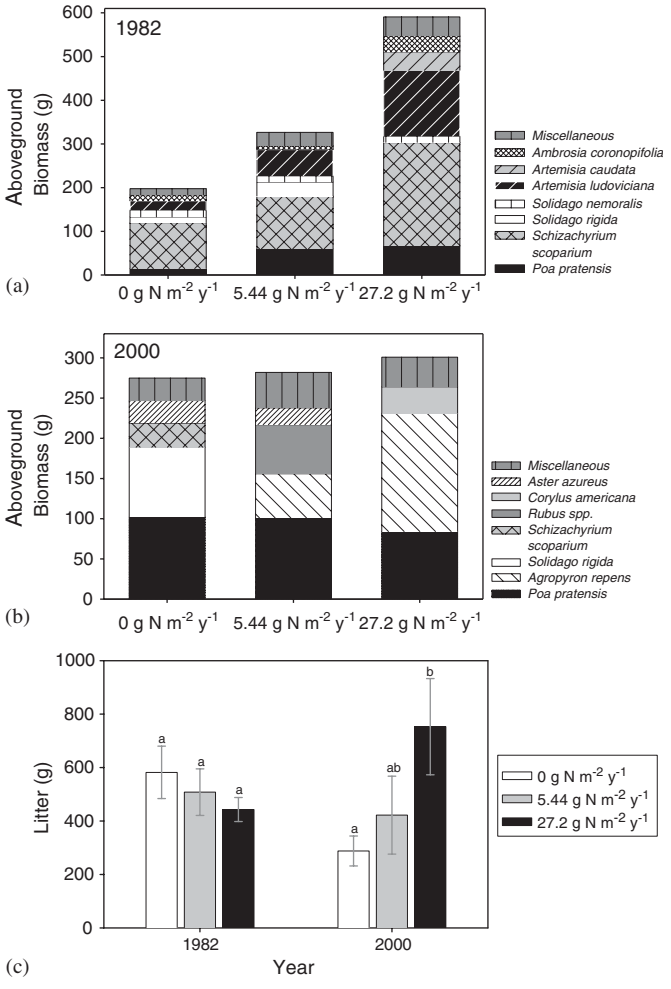

Fig. 1. Plant species abundances and litter accumulation from the plots whose soils were sampled in 2000 from the long-term N fertilization experiment. (a) Abundances of the most predominant plant species in 1982. (b) Abundances of the most predominant plant species in 2000. (c) Litter accumulation in 1982 and 2000. Treatment means followed by the same letter do not differ significantly at $P > 0.05$ by Tukey's HSD test.

It is well known that different plant species have different microbial communities associated with them. However, along the N fertilization gradient at Cedar Creek (eight fertilization treatments from 0 to 27.2 g N m^{-2} y^{-1}; see Tilman 1987 for details), changes in the plant community are confounded with differences in N availability. This makes it difficult to determine whether or not soil microbial community composition is altered in response to increased N availability and if these changes can affect plant community dynamics.

To begin to separate these factors, two complementary studies were performed to measure and compare changes in soil microbial community composition in response to: (1) 18 years of N fertilization and (2) one growing season of N fertilization. A one time pulse of N to plots dominated by *Schizachyrium scoparium*, a native C_4 grass, allowed a more direct assessment of its influence on the microbial community without changing the composition of the plant community during the growing season.

2. Materials and methods

2.1. Study area

This study was conducted at the Cedar Creek LTER in Minnesota, USA. It is located 45 km north of Minneapolis on a glacial outwash plain, with sandy soils that are low in organic matter, N, and water holding capacity (Grigal et al., 1974). In 2000, the two complementary studies were performed to examine the long- and short-term responses of soil microbial community composition to N fertilization. *Schizachyrium scoparium* was chosen for the second experiment because it is typically a dominant C_4 grass in the prairie fields around Cedar Creek and was a dominant species in 1982 when the long-term fertilization experiment was started (Fig. 1a). It is replaced by C_3 grasses, including *Poa pratensis* and *Agropyron repens*, as N availability increases (Tilman, 1987) (Fig. 1b). Soil collection was identical in both experiments.

2.2. Collection of soils

Eight soil cores (15 cm deep by 2 cm wide) were randomly sampled from the entire area of the plot unless otherwise specified. To prevent contamination, gloves were worn at all times when handling the soil. Between each plot, the corer was wiped with a 50% ethanol solution to prevent any cross contamination between treatments or species. Cores were combined in a plastic bag. Soil was

transported in a cooler to the lab, then mixed in the plastic bag and immediately subsampled (~30 g soil oven dried.). Half of the subsample was immediately extracted with 1 M KCl to determine ammonium and nitrate concentrations. The other half was oven dried to determine field moisture. The remaining soil was stored at 4 °C for less than 72 h and then sieved (2 mm). The sieved soil was well mixed and then divided into thirds for air-drying, refrigeration, or freezing (−20 °C). Air-dried soil was used to assess pH using a 1:1 paste (10 g dry soil to 10 ml distilled water). On the same dates as soil was collected additional measurements were made. Soil CO_2 flux, soil temperature, and air temperature were quantified with a Li-Cor 6200 (Lincoln, NE). Soil moisture was measured using time domain reflectometry (TDR). Finally, air-dried soil from each plot was ground using a Wiley Mill and analyzed for total carbon (C) and N using a Costech Elemental Combustion Analyzer.

2.3. Long-term N fertilization experiment

Soil was collected from a subset of plots (4.0×4.0 m) located in a prairie field (Field C) that are part of an existing experiment at Cedar Creek. Briefly, these plots were established in 1982 on top of existing prairie vegetation that had reestablished itself in an abandoned upland field which had last been farmed in 1934 (Tilman, 1987). The experiment was established to assess the long-term impacts of N deposition on plant species diversity, productivity, and dynamics (Tilman, 1987). Soil at this site was a Zimmerman fine sand (mixed, frigid, Alfic Udipsamments, Entisols). Eight N fertilization treatments were established, and all plots received trace minerals and nutrients to ensure that N was the only limiting one. N was added as commercial pelletized NH_4NO_3 fertilizer and broadcast manually twice a year. Half was applied in mid-May, and the other half added in mid-June. In 1985, $CaCO_3$ was applied to adjust soil pH of several treatments. In 1989, limestone was used to make further adjustments. Plant biomass data for 1982 and for the year 2000 were obtained from the Cedar Creek LTER database. See Tilman (1987) for more details on the liming procedure and the collection of plant biomass.

In this study, soil was only collected from three fertilization treatments, $0\,g\,N\,m^{-2}\,yr^{-1}$ ($N = 6$), $5.44\,g\,N\,m^{-2}\,yr^{-1}$ ($N = 6$), and $27.2\,g\,N\,m^{-2}\,yr^{-1}$ ($N = 6$). Hereafter, these treatments will be referred to as control, low N, or high N. Soil collars were installed on 6 June in the center of a 0.5×4.0 m area along the north side of the plots. Cores were collected from this area as well on 9 June, 16 August, and 5 September.

2.4. Short-term N fertilization experiment

In order to assess the direct effects of N fertilization, a short-term N fertilization experiment was established. Eighteen 1.0×1.0 m plots were established around individual *S. scoparium* plants, which grow in clumps 30–60 cm in diameter. These plots were located along the east edge of a larger experiment known as Biodiversity I (Tilman et al., 1996). This entire experimental area encompassed 10 ha of what was once an abandoned agricultural field dominated by the C_3 grass *Bromus inermis*. The field was treated with herbicide and burned in August 1993, had 6–8 cm of soil removed to reduce the seed bank, was plowed and harrowed, divided into plots, and then seeded in May 1994 (Tilman et al., 1996). Soil was a Nymore sand with 0–6% slopes (mixed, frigid, Typic Udipsamments, Entisols).

The area where the *S. scoparium* plots were located had originally been seeded in 1994 to be a large monoculture (15×15 m) for the Biodiversity I experiment and was dominated by *S. scoparium* (90–95% of aboveground biomass, personal observation by K. Bradley). Soil collars were installed in the 1.0×1.0 m plots on 6 June, 15 cm from a randomly chosen edge. These plots were fertilized only once, on 11 June with either $0\,g\,N\,m^{-2}$ ($N = 6$), $5.44\,g\,N\,m^{-2}$ ($N = 6$), or $27.2\,g\,N\,m^{-2}$ ($N = 6$); these amounts are equal to the annual rate of N addition in the long-term fertilization experiment (see Section 2.3). Hereafter, these treatments will be referred to as control, low N, or high N. Composite soil cores were collected four times: 8 June (pre-treatment), 28 June (17 d after fertilization), 13 July, and 21 August.

2.5. Lipid extraction

Microbial community structure was determined on whole soil samples collected and frozen as described above by the use of fatty acid methyl ester (FAME) analysis (Johnson et al., 2004). This method compares favorably with phospholipid fatty acid (PLFA) analysis for discriminating among soils and management (Drijber et al., 2000) and has been used by several investigators as an alternative to the strong hydrolysis conditions in the 'MIDI-FAME' approach while maintaining a straight-forward extraction procedure applicable to processing large numbers of samples (Ritchie et al., 2000; Schutter and Dick, 2000; Nazih et al., 2001).

Briefly, a mild alkaline hydrolysis (0.2 M KOH in methanol) was used to extract whole cell fatty acids (FA) from the soil. This included FAs from phospholipids, glycolipids and neutral lipids. The FAME extraction residue was dissolved in hexane and frozen at −20 °C until run on the gas chromatograph (GC). Samples were prepared for the GC by evaporating the solvent and adding 250 μl of hexane containing methyl-nonadecanoate ($0.05\,\mu g\,\mu l^{-1}$) as an internal standard.

2.6. FAME quantification and identification

Released FAMEs were separated on a Hewlett Packard 5890 Series II GC using helium as the carrier gas. This instrument contained an Ultra 2 HP column (50 m, 0.2 mm I.D., 0.33 μm film thickness) and was run in split mode

(44:1) with a 0.75 min purge time. Injector and flame ionization detector was maintained at 280 and 300 °C, respectively, and oven temperature was ramped from 50 to 160 °C at 40 °C min^{-1} and held for 2 min, then ramped at 3 °C min to 300 °C and held for 30 min. Identification of FAMEs was by retention time and confirmed by gas chromatography mass spectrometry (GCMS). Unsaturated and substituted FAMEs were identified by GCMS as dimethyloxazoline derivatives (Yu et al., 1989).

For each sample, the abundance of individual FAMEs were reported in absolute amounts (nmol g^{-1} soil) and then converted to nmol percent. FAME ratios less than 0.02 and FAMEs occurring in fewer than five samples were omitted from the data set. FAMEs with retention times less than C14:0 and greater than C20:0 were also deleted from the dataset.

The following FAMEs were designated as bacterial: *i*C15:0, *a*C15:0, C15:0, *i*C16:0, *i*C17:0, 9,10*cy*C17:0, C17:0, and 11,12*cy*C19:0 (Frostegård and Bååth, 1996). C16:0, often the most abundant FAME, correlates well with total biomass (Zelles et al., 1992). C18:1(*cis*9) and C18:2(*cis*9,12) were used as indicators of saprophytic fungi (Frostegård and Bååth, 1996; Stahl and Klug, 1996). C16:1(*cis*11) was used to indicate AMF (Graham et al., 1995; Olsson et al., 1995); although it is also found in some bacteria (Nichols et al., 1985, 1986; Elvert et al., 2003). AMF also contain abundant amounts of C18:1 (*cis*11) (Olsson and Johansen, 2000) which is also common to gram-negative bacteria. The FAME 10MeC18:0 was used to indicate soil actinomycetes (Kroppenstedt, 1985), while C20:4(5,8,11,14) was designated as a marker for eukaryotic organisms.

2.7. Statistical analysis

SAS 8.0 was used for the statistical analyses. Seasonal and treatment differences between the absolute amounts of individual FAs and measured soil properties were assessed using repeated-measures mixed model ANOVA (Littell et al., 1996). The within-subject effects were sampling date and treatment by date. N treatment was designated the between-subject effect. The covariance structure was investigated graphically. As the correlation between observations did not follow a clear pattern over time, compound symmetry covariance structure was used in the repeated-measures mixed model ANOVA. Treatment differences in soil C and N were analyzed using one-way ANOVAs.

In the case of the short-term dataset, pre-treatment values for individual FAs were included as covariates in the analysis. Twenty-two of the 28 FAMEs were significantly influenced by their pre-treatment amounts, indicating there were initial differences between the plots. However, there were no significant interactions between treatment and covariate. There were no treatment differences in the amounts of individual FAs on the first sampling date ($P > 0.05$, one-way ANOVAs). The use of the pre-treatment amounts as a covariate was excluded in the six cases where it was not a significant factor.

Denominator df of freedom were estimated using the Kenward–Rogers adjustment to avoid type I error (Guerin and Stroup, 2000). For a majority of the FAs, date was a significant factor. We were more interested in the other factors, N treatment and the interaction terms, thus when one of these factors was significant, a protected Least Significant Difference (LSD) test was used (Littell et al., 2002). The overall type I error rate was addressed by only conducting pairwise comparisons when there was overall significance of an F test for either N treatment or the interaction term. We also controlled for comparisonwise type I error inflation by using the Tukey–Kramer adjustment (Littell et al., 2002). The simple effects of interaction terms were investigated to determine how treatment differences varied over time (Littell et al., 2002).

To assess seasonal (sampling date) and treatment differences in microbial community composition, stepwise and canonical discriminant analysis were used on the remaining 28 FAMEs (SAS, 1999). Stepwise discriminant analysis is used to select the most significant combination of FAMEs for maximum discrimination of the treatments. Canonical discriminant analysis is essentially a graphical version of MANOVA, and its purpose is to determine a linear combination of variables that maximize the separation between treatments (SAS, 1999). These linear combinations, known as canonical variables, summarize between-class variation; they can show large treatment differences even if none of the original variables do (SAS, 1999).

Though plots were randomly selected and treatments randomly assigned, there were some initial differences between treatments in the short-term experiment. These differences were enough to discriminate between the plots independent of any treatment effects. To correct for this, difference scores were calculated by subtracting the pre-treatment FA values, in nmol g^{-1}, from each set of corresponding post-treatment FA values. The difference scores were then used in the discriminant analysis. There were strong effects of the sampling date on the abundance of different FAs in both the long- and short-term datasets. In the case of the short-term dataset, treatment differences became stronger over time. In order to isolate which FAs were responding to the N treatment, discriminant analysis was used for each sampling date. In the case of the long-term dataset, pre-treatment FAME data did not exist. To standardize for any between plot variance in total FAME amounts, relative nmol percents were used in the discriminant analysis of the long-term dataset.

3. Results

3.1. Plant species composition in the long-term N fertilization experiment

The plots that were sampled in 2000 did not differ in aboveground plant productivity (Fig. 1b), but they did

differ in litter accumulation (Fig. 1c), which was significantly higher in the plots receiving 27.2 g N m^{-2} yr^{-1}. *S. scoparium*, dominant in 1982 when the plots were established (Fig. 1a), was only present in the control treatment. The control plots also had the highest number of forb species, which account for most of the species diversity in the prairie fields scattered around Cedar Creek (data not shown). Most of the forbs were lost from the high N plots, and two grass species, *P. pratensis* and *A. repens*, dominated. Overall, control (1.30 species ± 0.14) and low N (1.20 species ± 0.09) plots had significantly higher diversity than the high N plots (0.75 species ± 0.13) as determined using the Shannon–Wiener index ($P = 0.016$, $F = 5.5$, $df = 2$). An interesting difference in the species composition that occurred between 1982 and 2000 was the considerable increase of *P. pratensis*, particularly in the control plots (Figs. 1a and b). This species made up a third of the biomass in all three treatments, though the plant species composition of the plots differed between treatments (Fig. 1b).

3.2. Abiotic variables in the long-term N fertilization experiment

Overall, soil conditions were significantly affected by the sampling date (Table 1). However, there were no differences between the N treatments in soil moisture or CO_2 flux (Table 1). Percent soil C and N were significantly higher in the two fertilized treatments as compared to the control, but the soil C:N ratios did not significantly differ between the treatments (Table 2). Available soil nitrate but not ammonium concentrations were significantly higher in the high N plots (Table 1). The treatment differences in soil pH became larger over time. This can be explained by the timing of the fertilizer treatment, half of which is applied in mid-May and the other half which is applied in mid-June.

3.3. FAME analysis from the long-term N fertilization experiment

The most abundant FAME was C16:0 (see Section 2.6. for FAME designations). Other abundant FAs, which each represented 5% or more of the sample, included C16:1(*cis*11), C18:0, C18:1(*cis*9), C18:1(*cis*11), C18:2(*cis*9, 12), and 11,12*cy*C19:0. All 28 FAMEs exhibited significant differences across sampling dates (results not shown). The mean total amount of FAMEs extracted, with the standard error given in parentheses, was 112 nmol g^{-1} soil (±3.7) in June, 123 nmol g^{-1} soil (±3.6) in August, and 85 nmol g^{-1} soil (±3.7) in September. These mean totals did not differ significantly between treatments.

A majority (21 of 28) of the FAMEs had significant treatment differences (results not shown), and the means of the 28 FAMEs, averaged by treatment, are represented in Fig. 2. FAMEs with significant treatment effects were further examined using an LSD test. Plots that received N fertilizer at low or high rates had increased proportions

Table 1
Mean and standard errors (in brackets) of the soil conditions and temperatures in the long-term fertilization plots

Soil conditions	9 June			16 August			5 September		
	Control	Low N	High N	Control	Low N	High N	Control	Low N	High N
NO_3^- (mg kg^{-1} soil)	1.24 ^{a} (0.68)	1.31 ^{a} (0.37)	16.1 ^{b} (2.02)	0.95 ^{a} (0.42)	1.98 ^{a} (0.72)	22.2 ^{b} (3.29)	0.52 ^{a} (0.23)	1.08 ^{a} (0.27)	15.3 ^{b} (1.3)
NH_4^+ (mg kg^{-1} soil)	5.45 ^{a} (0.91)	5.42 ^{a} (1.19)	10.2 ^{a} (1.96)	1.89 ^{a} (0.46)	3.66 ^{a} (0.52)	6.01 ^{a} (1.34)	1.18 ^{a} (0.31)	1.83 ^{a} (0.33)	4.29 ^{a} (1.82)
pH (water)	6.55 ^{a} (0.052)	6.15 ^{a} (0.052)	5.27 ^{b} (0.11)	6.25 ^{a} (0.082)	5.82 ^{b} (0.072)	5.27 ^{c} (0.12)	6.37 ^{a} (0.052)	5.92 ^{b} (0.056)	5.17 ^{c} (0.17)
Soil moisture (%)	7.4 ^{a} (0.36)	7.5 ^{a} (0.40)	6.8 ^{a} (0.43)	2.4 ^{a} (0.11)	2.9 ^{a} (0. 25)	3.1 ^{a} (0.12)	2.8 ^{a} (0.20)	2.7 ^{a} (0.19)	3.3 ^{a} (0.23)
CO_2 flux (μmol $CO_2/m^2/s$)	−3.17 ^{a} (0.35)	−3.67 ^{a} (0.21)	−3.89 ^{a} (0.69)	−1.57 ^{a} (0.42)	−1.79 ^{a} (0.14)	−1.97 ^{a} (0.15)	−1.36 ^{a} (0.11)	−1.61 ^{a} (0.09)	−1.98 ^{a} (0.22)

Letters indicate significant differences between treatments on a given sampling date. Data were analyzed using repeated-measures mixed model ANOVA. Overall, there was a significant effect of the sampling date for all five variables. Treatment means at a given date followed by the same letter do not differ significantly at $P > 0.05$ by Tukey's HSD test.

of six bacterial FAMEs and decreased proportions of two fungal FAMEs and two AMF FAMEs (Fig. 2). The high N plots had the largest proportion of tuberculostearic acid, 10MeC18:0.

FAMEs *i*C15:0, *a*C15:0, *i*C16:0, C16:1(*cis*9), C16:1 (*cis*11), 10MeC18:0 and C18:1(*cis*13) had significant treatment by date interactions. For these seven FAMEs, Fig. 2 reflects which treatment had the highest, middle, and lowest relative proportion of an individual FAME, though the magnitude of difference between the treatments varied at each sampling date. For *i*C15:0, *a* C15:0, C16:1(*cis*9), and C18:1(*cis*13), the maximal treatment differences occurred in June and decreased at the two subsequent dates. Maximal treatment differences occurred in August for *i*C16:0 and 10Me18:0, while the maximum treatment differences for C16:1(*cis*11) were in September.

Table 2
Mean and standard errors (in brackets) of the percent soil C, percent soil N, and the soil C:N ratio collected 9 June 2000 in the long-term fertilization experiment

	Control	Low N	High N
Soil %C	0.75 [a] (0.04)	0.90 [b] (0.06)	1.07 [c] (0.03)
Soil %N	0.06 [a] (0.003)	0.07 [b] (0.005)	0.09 [c] (0.002)
C:N ratio	12.1 (0.19)	12.1 (0.13)	11.8 (0.10)

Data were analyzed using one-way ANOVA. Treatment means followed by the same letter do not differ significantly at $P<0.05$ by Tukey's HSD test.

A total of 14 FAMEs were used to discriminate between treatments. The canonical discriminant analysis identified five significant canonical functions (CF) ($P<0.005$). The first canonical function (CF1) accounted for 64% of the total variance and the second function 19%. CF1 is the linear combination of variables that maximized the separation N treatments from each other while the second function separated the sampling dates (Fig. 3). This analysis indicates that the soil microbial communities associated with each treatment differed significantly from each other.

The control and low N treatments were negatively associated with CF1, while high N treatment was positively associated with it. Two of the FAMEs responsible for this separation were C18:2(*cis*9,12) and C18:1(*cis*11) were also strongly negatively correlated with CF1, but positively associated with the control and low N treatments. This

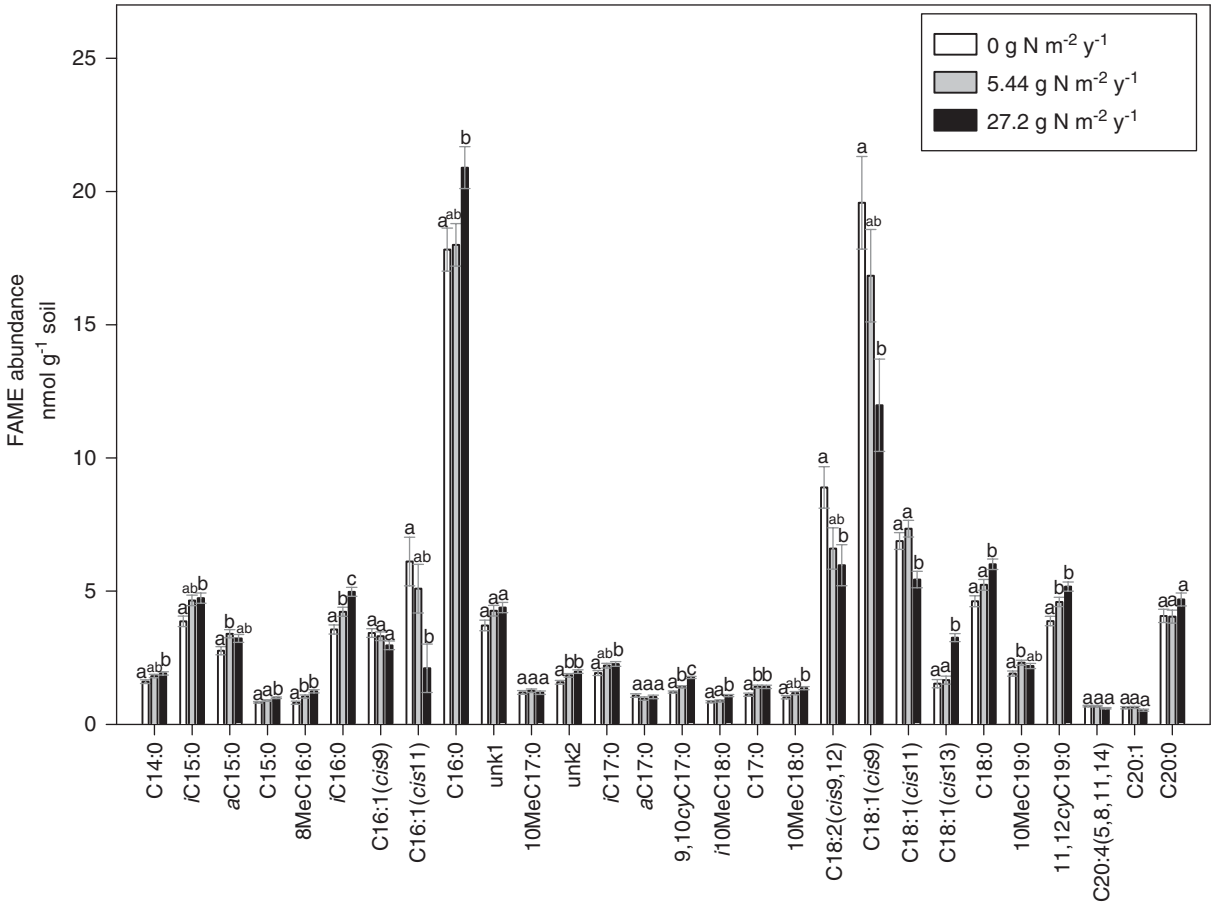

Fig. 2. The nmol amounts of FAMEs in plots at three different rates of N fertilization from the long-term N experiment. Values are treatment means averaged across sampling dates (±1 SE). For each FAME treatment means followed by the same letter do not differ significantly at $P>0.05$ by the Tukey–Kramer adjustment for multiple comparisons.

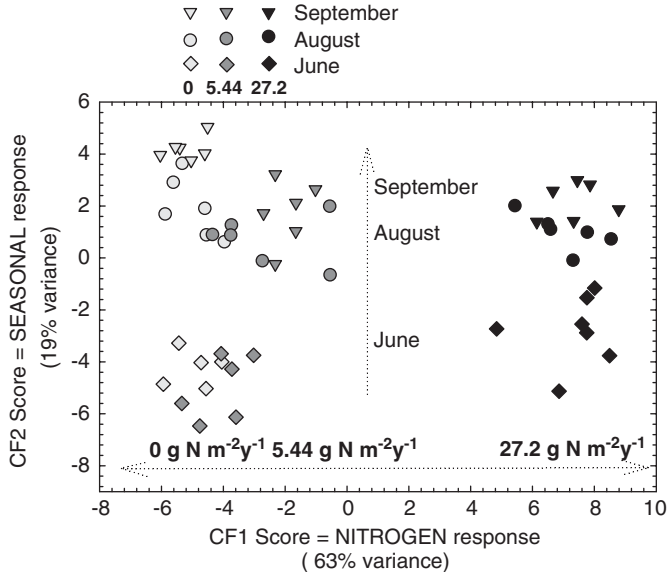

Fig. 3. Canonical discriminant analysis of the FAMEs from grassland plots fertilized with N since 1982. Discriminant scores of the N treatments are plotted for the first two significant CF. Microbial community composition was distinct among treatments, as shown by their separation along CF1.

result indicates that the fungal FAMEs as well as the AMF FAME negatively responded to high N fertilization. The second function mainly separated the June samples from the ones collected in August and September.

3.4. Abiotic variables in the short-term N fertilization experiment

All the soil conditions were significantly affected by the sampling date (Table 3). There were no significant treatment differences for soil moisture (Table 3), total soil C ($F = 0.92$, $P > 0.05$), total soil N ($F = 0.84$, $P > 0.05$), and soil C:N ratio ($F = 01.32$, $P > 0.05$). Both soil pH and soil available nitrate and ammonium, differed with sampling date and treatment (Table 3). The mean soil pH decreased at each sampling date within a given treatment, and pH was also significantly affected by the treatment (Table 3). The treatment by date interaction was also significant for pH; treatment differences became greater over time. The total soil available nitrate and ammonium did not differ between the treatments initially, but their concentrations were elevated in the high N plots following fertilization (Table 3).

3.5. FAME analysis from the short-term N fertilization experiment

Again, the most abundant FAME in the samples was C16:0 (see Section 2.6. for FAME designations). The other abundant FAs, each making up 5% or more of the sample area, included C16:1(*cis*9), C16:1(*cis*11), C18:1(*cis*9), C18:1 (*cis*11), and C18:2(*cis*9,12). Twenty-seven of the 28 FAMEs

Table 3
Mean and standard errors (in brackets) of the soil conditions and temperatures in the short-term fertilization plots

Soil conditions	8 June			28 June			13 July			21 August		
	Control	Low N	High N	Control	Low N	High N	Control	Low N	High N	Control	Low N	High N
NO_3^- (mg kg^{-1} soil)	0.12 ^{a} (0.05)	0.14 ^{a} (0.07)	0.11 ^{a} (0.04)	0.021 ^{a} (0.01)	1.63 ^{a} (1.17)	6.18 ^{b} (1.79)	0.035 ^{a} (0.01)	0.24 ^{a} (0.10)	4.17 ^{a} (1.31)	0.069 ^{a} (0.02)	0.14 ^{a} (0.05)	6.69 ^{b} (2.50)
NH_4^+ (mg kg^{-1} soil)	1.73 ^{a} (0.40)	1.59 ^{a} (0.27)	1.56 ^{a} (0.30)	0.92 ^{a} (0.21)	4.84 ^{a} (1.59)	7.57 ^{a} (3.13)	0.84 ^{a} (0.15)	1.70 ^{a} (0.37)	24.2 ^{b} (3.16)	1.10 ^{a} (0.24)	1.16 ^{a} (0.15)	10.8 ^{b} (2.05)
pH (water)	5.59 ^{a} (0.11)	5.69 ^{a} (0.08)	5.60 ^{a} (0.15)	5.46 ^{a} (0.03)	5.46 ^{a} (0.06)	5.49 ^{a} (0.08)	5.49 ^{a} (0.07)	5.40 ^{a} (0.08)	5.33 ^{a} (0.05)	5.44 ^{a} (0.07)	5.36 ^{a} (0.08)	5.12 ^{b} (0.04)
Soil moisture (%)	8.0 ^{a} (0.48)	8.3 ^{a} (0.55)	8.5 ^{a} (0.62)	7.6 ^{a} (0.50)	7.4 ^{a} (0.59)	7.7 ^{a} (0.62)	6.6 ^{a} (0.46)	6.1 ^{a} (0.62)	6.8 ^{a} (1.12)	4.0 ^{a} (0.13)	3.9 ^{a} (0.26)	3.4 ^{a} (0.25)
CO_2 flux (μmol CO_2 m^{-2} s^{-1})	−1.95 ^{a} (0.20)	−2.40 ^{a} (0.21)	−1.88 ^{a} (0.07)	−4.55 ^{a} (0.18)	−5.03 ^{a} (0.37)	−3.88 ^{a} (0.46)	−4.68 ^{a} (0.46)	−5.37 ^{a} (0.29)	−4.17 ^{a} (0.24)	−2.79 ^{a} (0.28)	−3.43 ^{a} (0.28)	−2.97 ^{a} (0.34)

Data were analyzed using repeated-measures mixed model ANOVA. Overall, there was a significant effect of the sampling date for all five variables. Treatment means at a given date followed by the same letter do not differ significantly at $P > 0.05$ by Tukey's HSD test.

were significantly different across sampling dates (data not shown). The LS means of total FAMEs with their standard errors in parentheses for 8 June, 28 June, 13 July and 21 August were 100 nmol g^{-1} soil ($\pm$3.4), 91 nmol g^{-1} soil ($\pm$3.4), 104 nmol g^{-1} soil ($\pm$3.4), and 116 nmol g^{-1} soil ($\pm$3.4). There were no treatment differences in the total amount of FAMEs.

Treatment was a significant factor for C16:1(*cis*11), C16:0, and C20:1, and treatment was marginally significant for C20:4(5,8,11,14). These FAMEs were examined further using an LSD test for the treatment means at each sampling date. The control plots had significantly higher amounts of C16:0, C16:1(*cis*11), and C20:4(5,8,11,14) than the high N plots (Fig. 4). FAME C16:1(*cis*11) had a significant treatment by date interaction; the differences between treatments became larger at each sampling date.

To determine how the soil microbial communities diverged in response to N fertilization, discriminant analysis was used to determine which FAMEs maximally separated each treatment. Separate discriminations were performed for each sampling date. For 28 June, five FAMEs were needed to discriminate between treatments. Ninety-two percent of the variation in the data was captured by CF1. This analysis indicates that after only 2 weeks the three treatments were significantly different from each other along CF1 (Fig. 5a).

For the 13 July sampling date, only four FAMEs were needed to discriminate between treatments. Eighty-three percent of the variation in the data was captured by CF1. The three treatments were separable along CF1 indicating that significant treatment differences persisted a month after the treatment was applied (Fig. 5b).

For the final sampling date, 21 August, eight FAMEs were needed to discriminate between treatments. Ninety-seven percent of the variation in the data was captured by CF1. Ten weeks after the N treatment was applied, separation between treatments along CF1 was at its greatest (Fig. 5c), indicating that the soil microbial communities diverged over time in response to added N.

4. Discussion

4.1. Soil microbial community composition in the long-term N fertilization experiment

The first objective of this study was to assess the effects of chronic N fertilization on soil microbial communities in a sandy, prairie field. Fertilizing these plots with nitrate

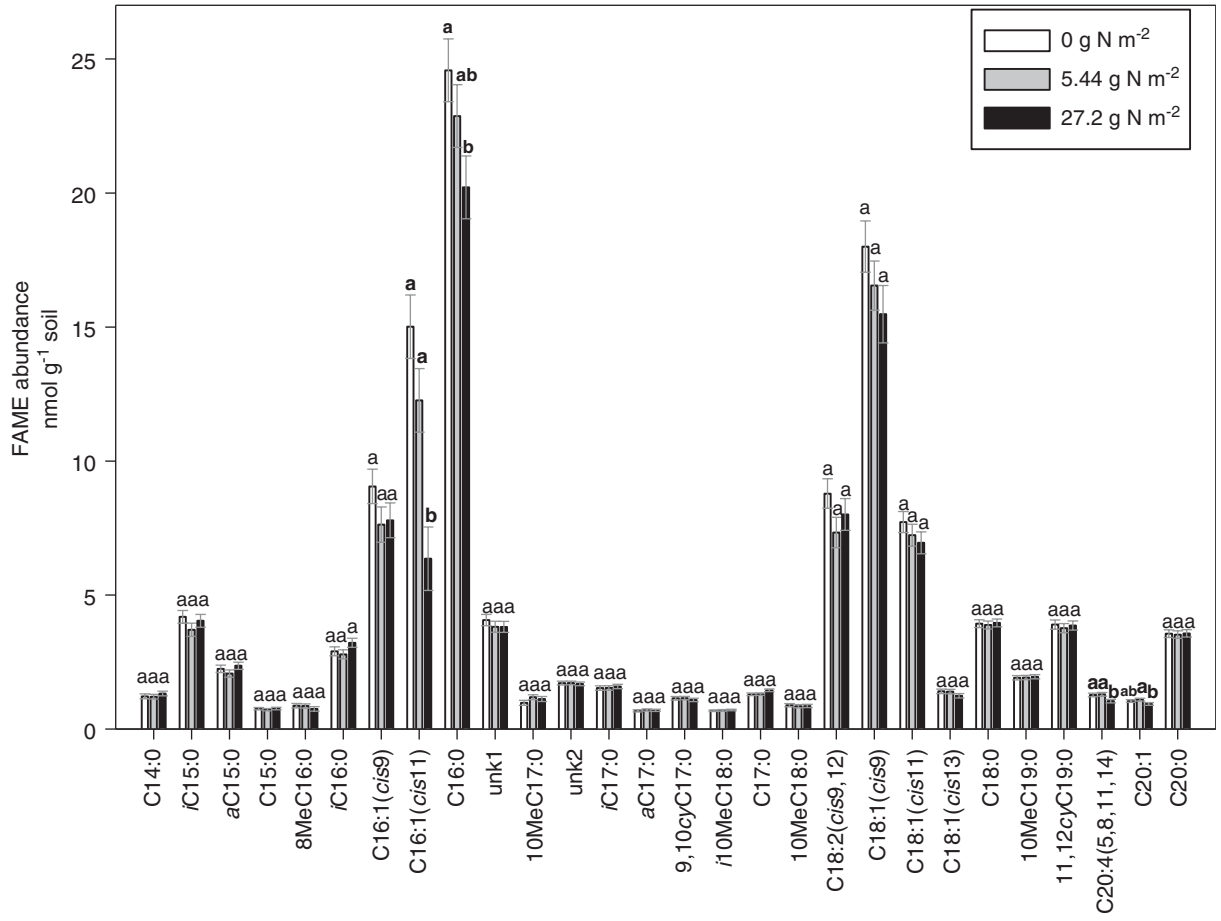

Fig. 4. The nmol amounts of FAMEs in plots at three different rates of N fertilization from the short-term N experiment. Values given are the LS means ($\pm$1 SE) for each treatment from 21 August, the last sampling date. For each FAME, the treatment means followed by the same letter do not differ significantly at $P>0.05$ by the Tukey–Kramer adjustment for multiple comparisons.

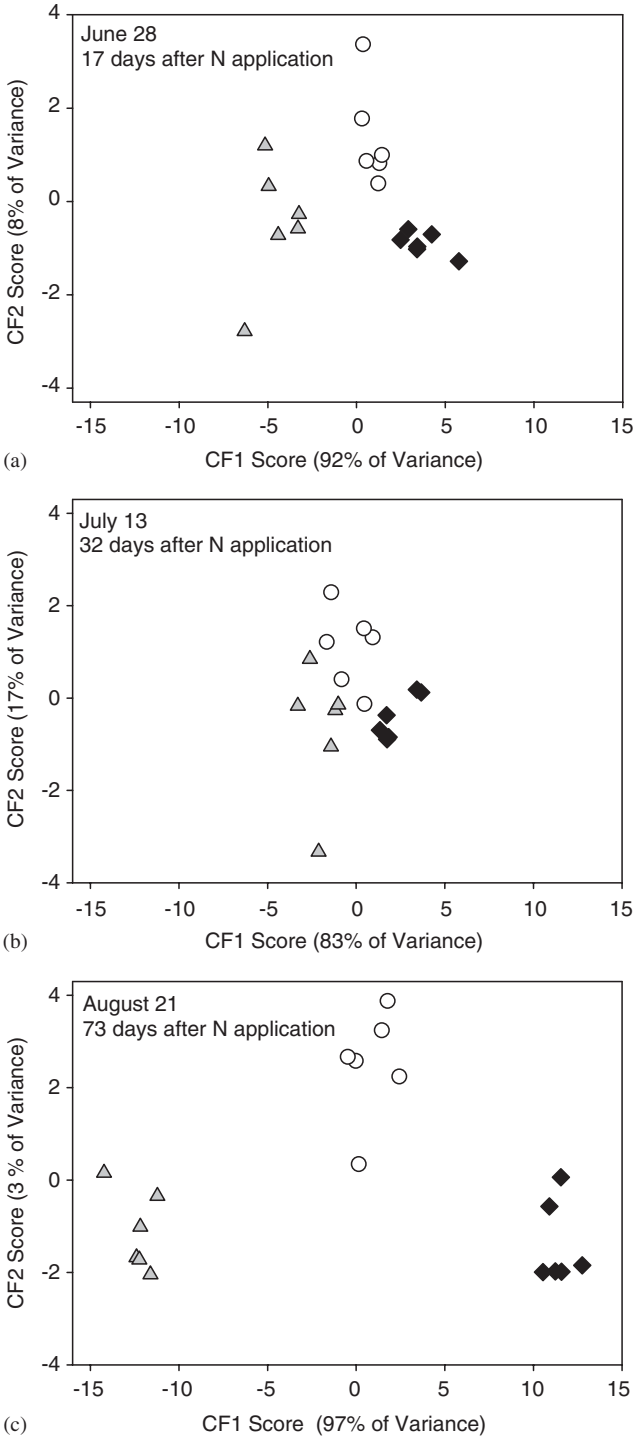

Fig. 5. Canonical discriminant analysis of the FAMEs from the short-term fertilization experiment. Discriminant scores of the N treatments (a, b, and c) are plotted for the first two significant CF. Scores are from (a) 28 June, (b) 13 July, and (c) 21 August. White circles represent the plots with $0\,g\,N\,m^{-2}\,yr^{-1}$, gray triangles represent the plots with $5.44\,g\,N\,m^{-2}\,yr^{-1}$, and the black squares represent the $27.2\,g\,N\,m^{-2}\,yr^{-1}$plots. Microbial community composition diverged among treatments along CF1, with the most separation between treatments occurring at the final sampling date.

and ammonium for 18 years has led to the divergence of not only the plant community (Figs. 1a, b) but also the soil microbial communities associated with different rates of N application (Fig. 3). Though *P. pratensis* was a substantial component of the plant communities in all three treatments, the identity of the other dominant plant species (Fig. 1b) were distinctly different in each treatment. In general, the control treatment plots were more diverse (see Section 3.1) and had a more substantial native prairie flora component than the most heavily fertilized plots.

The FAME analysis demonstrated that there were large differences in the composition of the soil communities between treatments (Figs. 2 and 3). The FAME analysis also discriminated between the sampling dates, though the treatment differences were evident at each date. However, the seasonal responses of individual FAMEs were similar between treatments. The total amount of FAMEs was highest in early August, likely coinciding with maximal rates of photosynthesis and a high influx of labile C compounds into the soil (Kuzyakov and Cheng, 2001). In September, the FAME abundance declined by a third, possibly in response to decreased input of labile C substrates by plants. However, treatment differences were much larger than the seasonal responses.

Treatment differences in the soil microbial communities in this study were characterized by increases in bacterial FAMEs and decreases in fungal and mycorrhizal FAMEs with N fertilization. Our results are consistent with another grassland study, which used PLFA to assess the soil community, and documented that unfertilized fields had a larger fungal component than fertilized fields (Bardgett and Shine, 1999). Similarly, gradient studies have shown that increased N availability (Högberg et al., 2003) and/or increased rates of N cycling (Myers et al., 2001) are associated with a bacterially dominated soil community accompanied by decreases in fungal (Myers et al., 2001) and mycorrhizal (Egerton-Warburton and Allen, 2000; Högberg et al., 2003) populations.

In particular, plots with the highest rates of N fertilization showed substantial depression of FAMEs C18:2(*cis*9,12), C18:1(*cis*9), C16:1(*cis*11), and C18:1 (*cis*11). The first two FAMEs are recognized as fungal biomarkers, with C18:1(*cis*9) composing 3–50% of the total FA content in fungal tissues (Stahl and Klug, 1996). The large decrease in both C18:2(*cis*9,12) and C18:1(*cis*9) in the heavily fertilized plots (Fig. 2) is consistent with data from other studies which have shown that N enrichment can depress fungal growth, particularly the mycelium (Berg and Verhoef, 1998; Hogervorst et al., 2003).

Both C16:1(*cis*11) and C18:1(*cis*11) are important components of the neutral lipid fraction in AMF (Graham et al., 1995; Olsson and Johansen, 2000; van Aarle and Olsson, 2003). Neutral lipids are important for C transport and metabolism in AMF and are thought to be the primary substrate for respiration in extraradical mycelium (Bago et al., 2002). As a majority of AMF biomass is found outside the roots as extraradical mycelium and spores (Olsson et al., 1999), it seems reasonable to hypothesize that these two FAMEs, particularly C16:1(*cis*11),

originated from AMF in this study (van Aarle and Olsson, 2003). Attributing the decrease in both FAMEs to a decrease in AMF is supported by more definitive work on AMF at Cedar Creek (Johnson et al., 1992).

Johnson et al. (1992) demonstrated that the composition of AMF communities is influenced both by soil type and plant species. The dominant plant species in this study, including *P. pratensis*, *S. scoparium*, and *A. repens*, were also found in the study above (Johnson et al., 1992). More spores were recovered from soils associated with *S. scoparium* than from *P. pratensis* and *A. repens*. This evidence is consistent with data presented here. *A. repens* and *P. pratensis* were the dominant plant species at the highest rate of fertilization. The decrease in the biomarker C16:1(*cis*11) could have resulted because the plant species which dominate the highly fertilized plots are associated with lower quantities of spores. Though our method may not be very efficient at extracting lipids from spores (Olsson and Johansen, 2000; personal observation by R. Drijber), decreases in spore abundance have been shown to be correlated with declines in extraradical mycelium (Egerton-Warburton and Allen, 2000).

Thus it seems more likely that the declines in C16:1(*cis*11) and C18:1(*cis*11) resulted from a decrease in AMF extraradical mycelium (Johnson, 1993; Berg and Verhoef, 1998; Treseder and Allen, 2002). The decrease in AMF extraradical mycelium/hyphae may initially be triggered as plants respond to mineral nutrient availability in the soil by changing their C allocation patterns (Johnson et al., 2003a, b). However, it is important to note here that the N:P ratio of the soil is an important determinant of whether the plant response to N enrichment is to increase or decrease C allocation to AMF (Treseder and Allen, 2002; Johnson et al., 2003a). If the availability of mineral N increases, and N:P ratios are low, plant C allocation belowground to mutualistic organisms such as AMF is expected to decrease, thus leading to a decline in the amount of external AMF structures. This phenomenon has been previously documented at Cedar Creek (Johnson et al., 2003a).

The pH was also significantly different between treatments; control plots were much less acidic than the most heavily fertilized plots (Table 1). The control plots, which had pH values ranging from 6.25 to 6.55, contained proportionally higher quantities of fungal FAMEs and lower proportions of bacterial FAMEs compared to the most heavily fertilized plots (Fig. 2b) whose pH was 5.17–5.27. Though soil fungi are more tolerant of acidic soil conditions than many other microorganisms (Coyne, 1999), AMF may be more adapted to a higher soil pH (Högberg et al., 2003).

This trend is supported further by the correlations of discriminating FAMEs with the CF1. Five of the eight discriminating FAMEs (*i*C15:0, C15:0, *i*C16:0, 9,10*cy* C17:0, and C18:1(*cis*13)) were bacterial markers. They also strongly, positively correlated with CF1, the axis that separated the N treatments (Fig. 3) while a major fungal marker C18:2(*cis*9,12) was negatively correlated with the function. Högberg et al. (2003) documented a similar pattern in the lipid fungal marker C18:2(*cis*9,12), which decreased from 15% of the total PLFA in soils of low N availability to 1% in soils with higher N availability. Interestingly, their mycorrhizal marker, C16:1(*cis*11), showed the opposite pattern and doubled from 2% to over 4% of the total PLFA as N availability increased (Högberg et al., 2003). However, unlike our study, the soil became less acidic, increasing from 3.9 to 6.8, as soil N increased (Högberg et al., 2003), indicating that the microbial response to pH may also depend on the nutrient status of the soil. Given these results, the pH changes due to fertilization may have also contributed to the changes in the relative predominance of fungi, mycorrhizae and bacteria between the treatments.

4.2. Soil microbial community response in the short-term N fertilization experiment

The second objective of this study was to characterize the response of soil microbial communities associated with the prairie bunch grass, *S. scoparium*, to a one time N fertilizer application. Most FAMEs were more strongly affected by the sampling date and/or their initial abundance than by the fertilizer treatment. However, we did find significant treatment differences in the soil microbial communities at each sampling period following the fertilizer application (Figs. 4 and 5). The distances between treatments were largest at the final sampling date (Fig. 5c), which indicated that treatment effects grew larger with time.

There were only four FAMEs which exhibited significant treatment responses: C16:0, the most abundant marker, C20:4(5,8,11,14), a faunal marker, and C20:1 were significantly lower at the highest rate of fertilization compared to the controls (Fig. 4). In August, N availability remained elevated above ambient concentrations (Table 3), while the amount of C16:1(*cis*11) present in the highly fertilized plots was significantly less than the other treatments (Fig. 4). The decrease in the AMF marker, C16:1(*cis*11), suggests that after 73 d plants have responded to increased N availability by allocating less C to their AMF partners.

4.3. Soil microbial community composition: comparing experimental results

The third objective of this study was to compare the soil microbial community responses between the two N fertilization experiments. Both experiments had significant treatment differences in the composition of their microbial communities. Nine of the same FAMEs [C15:0, *i*C15:0, *i*C16:0, C16:1(*cis*9), 9,10*cy*C17:0, C18:1(*cis*11), C18:2(*cis*9,12), C20:1, and C20:4(5,8,11,14)] used to generate canonical functions and discriminate between the three treatments in the long-term experiment were important in at least one or more of the separate discriminant analyses done for the short-term

experiment. Unlike the long-term experiment, no significant effects of N fertilization on the bacterial or fungal FAMEs were detected in the short-term experiment (Fig. 4; see Section 2.6. for FAME designations). Both experiments had a significant decline in the amount C20:1 at the highest rate of fertilization. The most striking similarity between the two experiments was the response of the C16:1(*cis*11) marker.

In the high N plots of both experiments, N fertilization seemed to prevent significant increases of the C16:1(*cis*11) marker during the growing season, suggesting less hyphal proliferation. The inhibition of the AMF marker at high N in the short-term experiment demonstrates that soil communities can respond to anthropogenic N long before responses are evident in plant communities. Further, changes in the soil community are very likely to influence interspecific interactions of the existing plant community (Johnson, 1993; Corkidi et al., 2002; Johnson et al., 2003a; Gustafson and Casper, 2004), and may ultimately alter its composition, productivity and/or diversity.

4.4. Interactions between N & AMF and the implications for plant communities

The various plants at Cedar Creek including, *S. scoparium*, *P. pratensis, A. repens*, and *Solidago rigida,* respond differently to the interactive effects of AMF and N (Johnson et al., 2003b). The growth of *S. scoparium* and *S. gigantean* was largest in mixed plant communities with AMF and at low soil N (Johnson et al., 2003b). In contrast, *P. pratensis* grew largest without AMF and at high soil N, while *A. repens*, which is nonmycorrhizal, performed best at high soil N whether AMF were present or absent (Johnson et al., 2003b). Similarly, we found that extraradical AMF biomass was sensitive to species composition. There were higher absolute amounts of C16:1(*cis*11) in the short-term experiment (nmol FAME g^{-1} soil: control $= 10.3 \pm 0.97$, low N $= 8.7 \pm 0.72$, high N $= 5.4 \pm 0.20$) where the plots were dominated by *S. scoparium* as compared to the control plots in the long-term experiment (nmol FAME g^{-1} soil: control $= 6.4 \pm 0.88$, low N $= 5.1 \pm 0.40$, high N $= 2.1 \pm 0.12$) which had lower abundances of *S. scoparium* (Fig. 1b).

These results, along with the soil microbial community data, also offer a slightly different explanation for the observed changes in the plant community in the long-term N fertilization experiment (Figs. 1a and b). In 1982, when the experiment was started, all the plots were dominated by plant species, including *S. scoparium* and two *Solidago* species, which have been shown to be most competitive when associated with AMF fungi and at low soil N availability. By the year 2000, *Solidago* and *S. scoparium* were significant components of the flora only in the control plots, while *A. repens* and *P. pratensis* were major components in both the low and high N plots. These results are consistent with the hypothesis of Johnson et al. (2003b) which suggests that (1) mycotrophic plants do not compete well at high soil N and (2) mutualistic AMF interactions are negatively affected by N. Thus, the abundance of AMF, along with the mycorrhizal dependency of the plant species (Johnson et al., 2003b), may have helped to mediate the plant community responses to yearly N fertilization.

5. Conclusions

Together, these results suggest that N fertilization affects the composition of soil microbial communities. The major compositional changes included increased bacterial abundance and decreased saprophytic fungal abundance in the long-term experiment and decreased AMF abundance in both experiments. The effects of N on the microbial community were most likely indirect, mediated through the physiological responses of the plants to an increase in soil available N. These data support the hypotheses that high N supply (1) can lead to significant changes in the composition of soil microbial communities over relatively short periods and (2) negatively affect the mutualistic relationships between plants and AMF. Shifts in the makeup of soil communities in response to anthropogenic N could alter the dynamics of plant populations (Bever, 1994; Johnson et al., 2003b).

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References

Avis, P.G., McLaughlin, D.J., Dentinger, B.C., Reich, P.B., 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytologist 160, 239–253.

Bago, B., Zipfel, W., Williams, R.M., Jun, J., Arreola, R., Lammers, P.J., Pfeffer, P.E., Shachar-Hill, Y., 2002. Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. Plant Physiology 128, 108–124.

Bardgett, R.D., Shine, A., 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology & Biochemistry 31, 317–321.

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., Davies, W.J., 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology 13, 650–660.

Berg, M.P., Verhoef, H.A., 1998. Ecological characteristics of a nitrogen-saturated coniferous forest in The Netherlands. Biology and Fertility of Soils 26, 258–267.

Bever, J.D., 1994. Feedback between plants and their soil communities in an old field community. Ecology 75, 1965–1977.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.

Corkidi, L., Rowland, D.L., Johnson, N.C., Allen, E.B., 2002. Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. Plant and Soil 240, 299–310.

Coyne, M.S., 1999. Soil Microbiology: An Exploratory Approach. Delmar Publishers, Albany, NY.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32, 1419–1430.

Egerton-Warburton, L.M., Allen, E.B., 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecological Applications 10, 484–496.

Elvert, M., Boetius, A., Knittel, K., Jørgensen, B., 2003. Characterization of specific membrane fatty acids as chemotaxonomic markers for sulfate-reducing bacteria involved in anaerobic oxidation of methane. Geomicrobiology Journal 20, 403–419.

Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196, 159–171.

Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.

Graham, J.H., Hodge, N.C., Morton, J.B., 1995. Fatty acid methyl ester profiles for characterization of Glomalean fungi and their endomycorrhizae. Applied and Environmental Microbiology 61, 58–64.

Grigal, D.F., Chamberlain, L.M., Finney, H.R., Wroblewski, D.W., Gross, E.R., 1974. Soils of the Cedar Creek Natural History Area. University of Minnesota Agriculture Experiment Station, St. Paul.

Guerin, L., Stroup, W.W., 2000. A simulation study to evaluate PROC MIXED analysis of repeated measures data. In: Proceedings of the 12th Annual Conference on Applied Statistics in Agriculture. Kansas State University, Manhattan, KS.

Gustafson, D.J., Casper, B.B., 2004. Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. Plant and Soil 259, 9–17.

Harte, J., Kinzig, A.P., 1993. Mutualism and competition between plants and decomposers: implications for nutrient allocation in ecosystems. American Naturalist 141, 829–846.

Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., Hogberg, P., 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs—a hypothesis based on field observations in boreal forest. New Phytologist 160, 225–238.

Hogervorst, R.F., Dijkhuis, M.A.J., van der Schaar, M.A., Berg, M.P., Verhoef, H.A., 2003. Indications for the tracking of elevated nitrogen levels through the fungal route in a soil food web. Environmental Pollution 126, 257–266.

Johnson, N.C., 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecological Applications 3, 749–757.

Johnson, N.C., Tilman, D., Wedin, D., 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73, 2034–2042.

Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warburton, L.M., Allen, E.B., 2003a. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84, 1895–1908.

Johnson, N.C., Wolf, J., Koch, G.W., 2003b. Interactions among mycorrhizae, atmospheric CO_2 and soil N impact plant community composition. Ecology Letters 6, 532–540.

Johnson, C.K., Wienhold, B.J., Doran, J.W., Drijber, R.A., Wright, S.F., 2004. Linking microbial-scale finding to farm-scale outcomes in a dryland cropping system. Precision Agriculture 5, 311–328.

Kroppenstedt, R.M., 1985. Fatty acid and menaquinon analysis of actinomycetes and related organisms. In: Goodfellow, M., Minnikin, D.E. (Eds.), Chemical Methods in Bacterial Systematics. Academic Press, London, pp. 173–199.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915–1925.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC 633pp.

Littell, R.C., Stroup, W.W., Freund, R.J., 2002. SAS for Linear Models. SAS Institute Inc., Cary, NC 466pp.

Lundquist, E.J., Scow, K.M., Jockson, L.E., Uesugi, S.L., Johnson, C.R., 1999. Rapid response of soil microbial communities from conventional low input, and organic farming systems to a wet/dry cycle. Soil Biology & Biochemistry 31, 1661–1675.

Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscpe-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65.

Naeem, S., Hahn, D.R., Schuurman, G., 2000. Producer–decomposer co-dependency influences biodiversity effects. Nature 403, 762–764.

Nazih, N., Finlay-Moore, O., Hartel, P.G., Fuhrmann, J.J., 2001. Whole soil fatty acid methyl ester (FAME) profiles of early soybean rhizosphere as affected by temperature and matric water potential. Soil Biology & Biochemistry 33, 693–696.

Nichols, P.D., Smith, G.A., Antworth, C.P., Hanson, R.S., White, D.C., 1985. Phospholipid and lipopolysaccharide normal and hydroxy fatty acids as potential signatures for methane-oxidizing bacteria. FEMS Microbiology Ecology 31, 327–335.

Nichols, P., Stulp, B.K., Jones, J.G., White, D.C., 1986. Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria *Vitreoscilla, Flexibacter, Filobacter*. Archives of Microbiology 146, 1–6.

Olsson, P.A., Johansen, A., 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycological Research 104, 429–434.

Olsson, P.A., Bååth, E., Jakobsen, I., Soderstrom, B., 1995. The use of phospholipid and neutral lipid fatty-acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99, 623–629.

Olsson, P.A., Thingstrup, I., Jakobsen, I., Bååth, E., 1999. Estimation of biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biology & Biochemistry 31, 1879–1887.

Ritchie, N.J., Schutter, M.E., Dick, R.P., Myrold, D.D., 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology 66, 1668–1675.

SAS Institute Inc, 1999. SAS/STAT® User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Schutter, M.E., Dick, R.P., 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64, 1659–1668.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.

Stahl, P.D., Klug, M., 1996. Characterization and differentiation of filamentous fungi based on fatty acid composition. Applied and Environmental Microbiology 62, 4136–4146.

Tilman, D., 1987. Secondary successional and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs 57, 189–214.

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379, 718–720.

Treseder, K.K., Allen, M.F., 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytologist 155, 507–515.

van Aarle, I.M., Olsson, P.A., 2003. Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 69, 6762–6767.

Wedin, D.A., Tilman, D., 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274, 1720–1723.

Yu, Q., Lui, B., Zhang, J.Y., Huang, Z.H., 1989. Location of double bonds in fatty acids of fish oil and rat testis lipids: gas chromatography–mass spectrometry of the oxazoline derivatives. Lipids 24, 79–83.

Zelles, L., Bai, G.Y., Beck, T., Beese, F., 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology & Biochemistry 24, 317–323.