Changes in Soil Microbial Community Structure in a Tallgrass Prairie Chronosequence

Victoria J. Allison,* R. Michael Miller, Julie D. Jastrow, Roser Matamala, and Donald R. Zak

ABSTRACT

Increasing the abundance of fungi relative to bacteria should favor C accrual, because fungi use C more efficiently, and are composed of more recalcitrant C compounds. We examined changes in soil microbial community structure following cessation of tillage-based agriculture and through subsequent succession in a C-accumulating tallgrass prairie restoration chronosequence. We predicted that the relative abundance of fungi would increase following conversion from tillage-based agriculture. Soil microbial community structure was assessed as relative abundances of phospholipid fatty acids (PLFAs). Cessation of tillage-based agriculture did initially lead to an increase in the abundance of fungi, particularly arbuscular mycorrhizal fungi (AMF), relative to bacteria. We suggest this is primarily due to reduced disturbance when tillage ceases. Vegetation characteristics also appear to be important, with high cyclopropyl/precursor PLFA ratios indicating bacterial communities under stress in agricultural soils, probably due to low C, and possibly to low C relative to N inputs. A secondary gradient in soil microbial community structure was related to successional time, and tied to soil characteristics, particularly bulk density (Db), pH, and soil organic C and N. However, while the fungi/bacteria (F/B) ratio was high in early succession plots, it declined later in succession. In addition, although the F/B ratio increased with SOC in the agricultural soils, it decreased with SOC in prairie soils. We conclude that increased community metabolic efficiency due to higher relative abundances of fungi is not the primary mechanism leading to enhanced C storage in these soils.

The activity and size of microbial subgroups is an important regulator of mineralization and immobilization of plant and microbe derived residues in soil (e.g., Bardgett and McAlister, 1999; Frey et al., 1999; Guggenberger et al., 1999). In general, fungi metabolize substrates more efficiently than can bacteria (Holland and Coleman, 1987; Griffith and Bardgett, 2000). In addition, the complex chitin and melanin residues that make up fungal biomass are more difficult to decompose than bacterially derived peptidoglycans (e.g., Nakas and Klein, 1979; Malik and Haider, 1982; Guggenberger et al., 1999). Hence, increasing fungal abundance should favor the accrual of soil C in more recalcitrant pools.

Understanding the mechanisms that regulate microbial community structure and activity is important if we are to manage C and N stocks in soils. In general, fungi become more important determinates of decomposition in soils returned to a more natural state (e.g., Bardgett et al., 1993; Cambardella and Elliot, 1994; Beare, 1997; Bardgett and McAlister, 1999; Stahl et al., 1999; Zeller et al., 2001; Bailey et al., 2002), whereas soil bacteria dominate decomposition and soil nutrient cycling in more intensively managed soils that are tilled and fertilized (e.g., Moore and de Ruiter, 1991; Lovell et al., 1995). However, the plant and environmental factors that mediate these management impacts are poorly understood (Bardgett et al., 1993; Grayston et al., 2001; Zeller et al., 2001). Conversion from native vegetation to cropland leads to rapid depletion of soil organic matter, and reduced microbial biomass (Steenworth et al., 2002). Tilling may directly affect AMF, by disrupting hyphal networks (Drijber et al., 2000). Increasing C inputs to the soil, either directly by application of C-rich organic material, or indirectly via increased plant production, increases total microbial biomass (Broughton and Gross, 2000; Yao et al., 2000). In perennial systems, increasing C inputs can also increase the F/B biomass ratio (Yeates et al., 1997; Bailey et al., 2002). Bardgett et al. (1999) also found higher F/B in spring; a shift in community structure correlated with increased soil N and moisture in this perennial forage system. However, in some cases N fertilizer can increase the relative abundance of fungi in spite of decreased root biomass. Bardgett et al. (1999) suggest that this process is mediated through changes in plant species composition, perhaps due to differences in the quantity or type of root exudates among plant species (Grayston et al., 2001).

An important step toward identifying factors that influence the microbial community in soils has been the application of PLFA analysis (Vestal and White, 1989; Tunlid and White, 1992). Phospholipids are integral components of cell membranes, and are metabolized rapidly on cell death. As a result, PLFAs reflect viable biomass (Frostegård and Bååth, 1996). Further, specific signature PLFAs are associated with subsets of the microbial community, including gram-positive and gram-negative bacteria, actinomycetes, AMF, and saprophytic fungi (Vestal and White, 1989; Tunlid and White, 1992; Frostegård and Bååth, 1996; Olsson, 1999). The marker PLFAs allow detection of changes in microbial community structure, that can, in turn, be related to soil and vegetation dynamics (e.g., Frostegård and Bååth, 1996; Grayston et al., 2001; Pennanen, 2001). In addition,

Abbreviations: AMF, arbuscular mycorrhizal fungi; CA, correspondence analysis; D, bulk density; F/B fungi/bacteria; FID, flame ionization detector; GC, gas chromatograph; GS, growing seasons; MBC, microbial biomass carbon; PLFA, phospholipid fatty acid; SOC, soil organic carbon; TN, total nitrogen.
PLFAs can indicate the physiological status of the community. For example, the ratio of cyclopropyl PLFAs (cy17:0 and cy19:0) to their precursors (16:1ω7c and 18:1ω7c) can be used as an indicator of the physiological status of gram-negative bacterial communities. In these bacteria the precursors are increasingly converted to cyclopropyl fatty acids as bacterial growth moves from logarithmic to stationary-phase growth, in response to stresses including limitation by C (Guckert et al., 1986; Bossio and Scow, 1998; Bossio et al., 1998) and O2 (Calderón et al., 2001; Jackson et al., 2003).

Investigating the factors that influence microbial community structure requires broad environmental gradients. In the chronosequence at the Fermi National Accelerator Laboratory (Fermilab), an agroecosystem soil under continuous tillage-based cultivation for the last century is being transformed to a prairie soil dominated by rhizospheric processes. The most dramatic effect of prairie reconstruction at Fermilab is an increase in above- and belowground plant production (Jastrow, 1987). In addition, the cessation of tillage results in litter accumulation on the soil surface, with the amount of litter accumulating depending on vegetation production and burn regime implemented. In previous investigations, we have demonstrated that as this system recovers from agricultural disturbance, aggregate formation and stabilization is promoted by development of AMF hyphae and fibrous root growth (Jastrow, 1987; Miller and Jastrow, 1990; Jastrow et al., 1998). These studies also indicate a close relationship between the formation of macroaggregated soil structure and the accumulation of soil organic C, which should in turn favor the development of fungal-dominated food webs (Beare et al., 1992; Bardgett et al., 1993).

In this study, we ask whether shifts in microbial community structure occur in an aggrading soil system, and how these shifts are related to environmental gradients. More specifically, we predict that the relative abundance of fungi should increase following conversion from tillage-based agriculture to prairie, and continue to increase with time as soil C increases in restored prairie soils.

**MATERIALS AND METHODS**

**Site Description**

Samples were collected at Fermilab, Batavia, IL, from sites with Drummer series soil (fine-silty, mixed, mesic Typic Hapludoll); a deep, poorly drained soil that is very typical of the soils of the Prairie Peninsula of Illinois. Nearly 80% of this landscape was prairie before European settlement, but has been in cultivation since the 1840s. The site was in continuous corn since at least 1969 (when Fermilab was established). Beginning in 1992, the agricultural fields have been rotated between corn and soybean. Although exact management practices have varied, the agricultural fields are chisel-plowed most of the time, and fertilizer is applied when required. Since 1975, between 2 and 25 ha have been restored to tallgrass prairie annually (Betz, 1986; Jastrow, 1987). These restorations represent a chronosequence, which allows us to examine successional processes by substituting space for time: substituting similar soils (space) to obtain a chronosequence of different restoration ages (time). Space for time substitution can be problematic because of the potential for site differences to obscure trends due to time, or even generate unrelated patterns (Pickett, 1989). In this system, the past history of sites is very similar, time at which succession began is accurately documented, restoration procedures were similar, and we ensured that sampling occurred on the same soil type, thus keeping site variation to a minimum.

**Sampling**

Nine restored prairie plots and two tillage-based agricultural fields were sampled. The prairie plots were planted in spring 1975 (25 growing seasons [GS], Plot 1D), spring 1977 (23 GS, Plot 3D), fall 1978 (21 GS, Plot 5D), fall 1981 (18 GS, Plot 8D), spring 1984 (16 GS, Plot 11D), spring 1985 (15 GS, Plot 14D), spring 1992 (8 GS, plot 18D), summer 1993 (7 GS, Plot 13D), and spring 1997 (3 GS, Plot PLD). The two agricultural fields used in the study were planted to soybean (BD) and corn (CD).

All samples were collected over a 3-wk period starting the third week in August and ending the first week in September 1999. Aboveground biomass and litter were collected from circular quadrats with an area of 0.1 m2 by using a sickledrake (Kennedy, 1972). Five quadrats were located in each plot, except for the 21-yr-old plot, which had 10. In the prairie plots, these quadrats were randomly distributed. In the agricultural fields, three quadrats were located within rows, and two quadrats were placed between rows to representatively capture the variation of a cultivated field. In each quadrat, three soil cores (diameter 4.8 cm, depth 5 cm) were taken and combined. The shallow sampling depth was chosen to avoid diluting the influence of surface litter on community composition. At the end of each day, biomass samples were refrigerated, and soil cores were frozen at −20°C.

**Laboratory Analyses**

Aboveground vegetation samples were sorted into grasses, forbs, dead standing material, and litter (picked from the ground surface), then dried to constant weight at 65°C. Dried tissue was ground in a standard model 3 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA), and a subsample was ground more finely with a Spex mill (Spex-Certiprep Inc., Metuchen, NJ). Finely ground tissue was analyzed for C and N contents and fibrous root growth (Jastrow, 1987; Miller and Jastrow, 1990; Jastrow et al., 1998). These studies also indicate a close relationship between the formation of macroaggregated soil structure and the accumulation of soil organic C, which should in turn favor the development of fungal-dominated food webs (Beare et al., 1992; Bardgett et al., 1993).

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Table 1. Assignment of signature phospholipid fatty acids (PLFAs) to soil microbial groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Signature PLFAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces</td>
<td>10Me16:0†, 10Me18:0†</td>
</tr>
<tr>
<td>Bacteria</td>
<td>i17:0†, a15:0‡, i16:0†</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td>cy17:0†, 18:1ω7c§</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td>16:1ω7c§, 16:1ω7c§, 16:1ω7c§</td>
</tr>
<tr>
<td>Fungi</td>
<td>18:2ω6c†, 18:2ω6c†</td>
</tr>
<tr>
<td>AMF</td>
<td>16:1ω5c§§</td>
</tr>
</tbody>
</table>


Table 2. Soil and plant environmental characteristics of plots in the Fermilab chronosequence (mean and standard deviation).†

<table>
<thead>
<tr>
<th>Plot</th>
<th>GS</th>
<th>Ds</th>
<th>pH</th>
<th>SOC</th>
<th>TN</th>
<th>Soil C/N</th>
<th>Root C/N</th>
<th>Litter C/N</th>
<th>Roots</th>
<th>Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>0</td>
<td>1.11 (0.11)</td>
<td>0.11 (0.24)</td>
<td>0.26 (0.05)</td>
<td>0.21 (0.04)</td>
<td>12.37 (0.16)</td>
<td>33.63 (2.02)</td>
<td>35.34 (9.42)</td>
<td>53.0 (53.0)</td>
<td>355.2 (94.4)</td>
</tr>
<tr>
<td>CD</td>
<td>0</td>
<td>1.15 (0.15)</td>
<td>0.18 (0.13)</td>
<td>0.33 (0.01)</td>
<td>0.08 (0.01)</td>
<td>11.69 (0.15)</td>
<td>62.97 (11.75)</td>
<td>28.68 (6.42)</td>
<td>89.5 (142.8)</td>
<td>63.5 (13.9)</td>
</tr>
<tr>
<td>PLD</td>
<td>3</td>
<td>1.15 (0.07)</td>
<td>0.18 (0.15)</td>
<td>0.33 (0.05)</td>
<td>0.08 (0.01)</td>
<td>13.33 (0.04)</td>
<td>72.54 (9.38)</td>
<td>31.05 (3.98)</td>
<td>143.3 (85.2)</td>
<td>299.8 (33.6)</td>
</tr>
<tr>
<td>N3D</td>
<td>7</td>
<td>1.10 (0.14)</td>
<td>0.18 (0.12)</td>
<td>0.33 (0.07)</td>
<td>0.08 (0.01)</td>
<td>13.17 (0.55)</td>
<td>106.49 (15.59)</td>
<td>69.27 (11.51)</td>
<td>428.4 (250.8)</td>
<td>925.8 (145.5)</td>
</tr>
<tr>
<td>18D</td>
<td>1</td>
<td>1.07 (0.08)</td>
<td>0.19 (0.15)</td>
<td>0.34 (0.02)</td>
<td>0.08 (0.01)</td>
<td>12.79 (0.19)</td>
<td>100.99 (20.36)</td>
<td>50.38 (11.71)</td>
<td>370.5 (146.9)</td>
<td>113.4 (29.3)</td>
</tr>
<tr>
<td>14D</td>
<td>1</td>
<td>1.07 (0.08)</td>
<td>0.19 (0.15)</td>
<td>0.32 (0.01)</td>
<td>0.08 (0.01)</td>
<td>13.53 (0.41)</td>
<td>121.21 (21.40)</td>
<td>92.33 (11.46)</td>
<td>582.3 (539.4)</td>
<td>743.6 (96.1)</td>
</tr>
<tr>
<td>11D</td>
<td>1</td>
<td>0.91 (0.17)</td>
<td>0.17 (0.17)</td>
<td>0.28 (0.02)</td>
<td>0.08 (0.01)</td>
<td>12.59 (0.22)</td>
<td>117.59 (23.30)</td>
<td>45.16 (4.67)</td>
<td>695.2 (322.8)</td>
<td>735.7 (43.4)</td>
</tr>
<tr>
<td>8D</td>
<td>1</td>
<td>0.91 (0.06)</td>
<td>0.17 (0.17)</td>
<td>0.26 (0.04)</td>
<td>0.08 (0.01)</td>
<td>13.75 (0.38)</td>
<td>81.60 (26.49)</td>
<td>68.85 (16.44)</td>
<td>626.5 (194.2)</td>
<td>1101.4 (130.9)</td>
</tr>
<tr>
<td>5D</td>
<td>2</td>
<td>0.89 (0.07)</td>
<td>0.16 (0.16)</td>
<td>0.30 (0.03)</td>
<td>0.08 (0.01)</td>
<td>12.92 (0.62)</td>
<td>94.52 (10.29)</td>
<td>92.17 (20.61)</td>
<td>1033.0 (273.1)</td>
<td>905.3 (266.1)</td>
</tr>
<tr>
<td>3D</td>
<td>2</td>
<td>0.84 (0.10)</td>
<td>0.23 (0.12)</td>
<td>0.38 (0.05)</td>
<td>0.08 (0.01)</td>
<td>12.55 (0.16)</td>
<td>85.27 (21.25)</td>
<td>50.93 (12.07)</td>
<td>440.6 (293.5)</td>
<td>869.2 (197.0)</td>
</tr>
<tr>
<td>1D</td>
<td>2</td>
<td>0.96 (0.07)</td>
<td>0.37 (0.37)</td>
<td>0.48 (0.43)</td>
<td>0.30 (0.04)</td>
<td>14.43 (0.91)</td>
<td>92.99 (15.30)</td>
<td>70.62 (26.29)</td>
<td>1052.0 (538.4)</td>
<td>450.5 (42.9)</td>
</tr>
</tbody>
</table>

† GS, growing season; Ds, bulk density; SOC, soil organic carbon; TN, total nitrogen; CD, corn crop; BD, soybean crop.

Data Analysis

Although a number of generally distributed PLFAs were extracted (most notably 16:0 and 18:0), we excluded these from analyses to avoid obscuring changes in microbial groups (Zelles, 1999). The composition of the soil microbial community was summarized using a correspondence analysis (CA) on the relative mole abundances of PLFAs in each sample. In CA, samples are sorted so that the distance between samples is related to their similarity. The axes along which samples are positioned are not necessarily representative of actual environmental variables but are artificially created variables that explain the maximum amount of variation (Leps and Smilauer, 1999). The ordination shows both the positions of samples relative to one another, and the average position of each PLFA along each axis. As a result, comparison of ordination plots showing sample position to ordination plots showing average position of PLFAs enables interpretation of sample responses in terms of changes in community composition. We used Pearson product-moment correlations to relate positions of samples along the ordination axes to other measured environmental variables. In addition, we calculated a fungal/bacterial (F/B) ratio from the sum of saprophytic fungi and AMF, and all bacterial microbial group signatures (Frostegård and Báth, 1996). We compared the F/B of agricultural and prairie plots with a t test, and the F/B ratio of each plot with a one-way ANOVA, using a Bonferroni post-hoc test to assess differences among plots. Correspondence analysis was performed in PC-ORD (McCune and Mefford, 1999), whereas correlations and ANOVAs were performed with SAS (SAS Institute, 1994). Data were transformed as necessary to meet assumptions of normality and homogeneity of variance, and tests were considered significant at p ≤ 0.05.
Table 3. Microbial community characteristics of plots in the Fermilab chronosequence (mean and standard deviation).†

<table>
<thead>
<tr>
<th>Plot age</th>
<th>MBC (µg g⁻¹)</th>
<th>Total Gram⁻ PLFA (n mol g⁻¹)</th>
<th>Gram⁺ PLFA (n mol g⁻¹)</th>
<th>Actino PLFA (n mol g⁻¹)</th>
<th>Bacteria PLFA (n mol g⁻¹)</th>
<th>AMF PLFA (n mol g⁻¹)</th>
<th>Fungi PLFA (n mol g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS</td>
<td>478 (17)</td>
<td>80.40 (30.64)</td>
<td>18.13 (7.60)</td>
<td>14.84 (5.13)</td>
<td>9.63 (2.98)</td>
<td>25.22 (9.96)</td>
<td>3.78 (1.62)</td>
</tr>
<tr>
<td>BD</td>
<td>478 (86)</td>
<td>74.71 (27.72)</td>
<td>14.26 (5.83)</td>
<td>14.15 (4.83)</td>
<td>8.15 (3.04)</td>
<td>24.21 (8.39)</td>
<td>3.60 (1.88)</td>
</tr>
<tr>
<td>CD</td>
<td>629 (95)</td>
<td>89.96 (15.40)</td>
<td>17.09 (2.96)</td>
<td>16.45 (3.03)</td>
<td>9.50 (1.67)</td>
<td>28.28 (5.66)</td>
<td>7.19 (1.16)</td>
</tr>
<tr>
<td>7</td>
<td>929 (96)</td>
<td>86.19 (29.81)</td>
<td>19.65 (6.84)</td>
<td>12.66 (4.13)</td>
<td>10.31 (3.35)</td>
<td>23.52 (6.97)</td>
<td>9.55 (4.60)</td>
</tr>
<tr>
<td>8</td>
<td>1007 (82)</td>
<td>138.94 (11.35)</td>
<td>28.70 (2.98)</td>
<td>18.17 (6.55)</td>
<td>16.56 (0.93)</td>
<td>42.33 (2.56)</td>
<td>12.45 (0.99)</td>
</tr>
<tr>
<td>15</td>
<td>797 (125)</td>
<td>120.49 (13.61)</td>
<td>28.64 (1.78)</td>
<td>17.35 (2.21)</td>
<td>14.41 (2.26)</td>
<td>33.69 (4.91)</td>
<td>9.45 (1.36)</td>
</tr>
<tr>
<td>16</td>
<td>1620 (120)</td>
<td>209.37 (28.08)</td>
<td>38.92 (8.20)</td>
<td>33.36 (5.86)</td>
<td>30.25 (4.68)</td>
<td>58.74 (18.43)</td>
<td>13.78 (1.24)</td>
</tr>
<tr>
<td>18</td>
<td>1697 (227)</td>
<td>177.32 (26.11)</td>
<td>39.12 (5.12)</td>
<td>25.53 (1.94)</td>
<td>24.44 (4.39)</td>
<td>55.60 (10.85)</td>
<td>12.65 (0.99)</td>
</tr>
<tr>
<td>21</td>
<td>1499 (93)</td>
<td>164.68 (62.20)</td>
<td>38.15 (14.22)</td>
<td>22.86 (8.82)</td>
<td>25.10 (9.75)</td>
<td>44.29 (17.63)</td>
<td>13.30 (4.99)</td>
</tr>
<tr>
<td>23</td>
<td>1939 (307)</td>
<td>185.44 (42.02)</td>
<td>40.66 (10.58)</td>
<td>27.60 (3.90)</td>
<td>27.85 (5.93)</td>
<td>57.02 (14.02)</td>
<td>11.97 (2.49)</td>
</tr>
<tr>
<td>25</td>
<td>1230 (109)</td>
<td>138.62 (54.87)</td>
<td>29.44 (11.83)</td>
<td>18.34 (10.56)</td>
<td>16.40 (6.83)</td>
<td>46.63 (18.74)</td>
<td>9.61 (4.11)</td>
</tr>
</tbody>
</table>

† GS, growing season; MBC, microbial biomass C; PLFA, phospholipid fatty acid; CD, corn crop; BD, soybean crop.

RESULTS

Agricultural plots tended to have higher bulk density than the older restored prairie plots (Table 2). The agricultural soils also had lower SOC and TN, lower litter and root C/N, and lower root and litter biomass than prairie soils (Table 2). Microbial biomass, assessed as either MBC or total PLFA, was generally higher in prairie than agricultural plots (Table 3). As a result of higher total microbial biomass, in general prairie plots also had higher amounts of each of the microbial groups (Table 3).

Axis 1 of the CA explained 54% of the variation in microbial community structure, and Axis 2 explained an additional 18% (Fig. 1). Axis 3 explained 13% of the variation in the microbial community, but could not be related to any measured environmental variable. As a result, this axis is not discussed further. Agricultural plots (soybean and corn rotations) fell to the negative side of Axis 1 of the CA, whereas prairie plots generally fell to the positive side (Fig. 1). The agricultural plots were associated with high root N concentrations, while prairie plots had high root C/N ratios, high litter C concentrations, and higher pH (Fig. 1).

Position on Axis 2 appeared to be loosely determined by successional age, with the most recently restored soils (3 yr) high on Axis 2, and older plots (>16 yr) low on Axis 2, except for several of the oldest plots (25 yr), which fell high on Axis 2 (Fig. 1). The young plots had high bulk density, and low TN, SOC, MBC, and pH. Low pH in younger fields may be a residual effect of fertilizer application to agricultural fields. Older plots had high TN, SOC, MBC, and pH, with the exception of several quadrats from the oldest plot, which had microbial community composition and physical properties more similar to the youngest restored plots (Fig. 1, Table 2). Agricultural plots generally fell between the old and young restored plots on Axis 2.

The average positions of signature PLFAs were plotted in the same ordination space used to summarize microbial community structure (Fig. 2). Plots found on the right side of Axis 1 (prairie) were associated with...
high relative abundances of the fungal signature 18:2ω6, and the AMF signature 16:1ω5c. In contrast, plots occurring on the left side of Axis 1 (agricultural) had high relative abundances of bacterial signature PLFAs (Fig. 2). Plots high on Axis 2 (young restored plots) had high relative abundances of the fungal signature 18:2ω6 and of the bacterial signature 14:0, while plots low on Axis 2 (older restored plots, with the exception of the oldest plot) had high relative abundances of the actinomycetes signature 10Me16:0, and the gram-negative bacterial signatures cy17:0 and 18:1ω7c (Fig. 2).

To evaluate how relative abundance of major microbial taxonomic groups changed along Axis 1, we plotted the relative amounts of marker PLFAs for all fungi (saprophytic fungi + AMF) and for bacteria against Axis 1. Bacteria decreased and fungi increased with increasing position on Axis 1 (Fig. 3). When fungal signatures were separated into saprophytic and AMF markers, the relative abundance of AMF had a stronger positive slope than that for saprophytic fungal markers, indicating that much of the increase in fungi measured along the gradient was AMF (Fig. 4).

As predicted, prairie plots had significantly higher saprophytic fungi/bacteria ratios than did the tillage-based agricultural plots ($t_{1,56} = 2.0171, p = 0.0485$), and also significantly higher AMF/bacteria ratios ($t_{1,56} = 6.6106, p = 0.0001$). Using a one-way ANOVA, we found that the saprophytic F/B ratio differed significantly among plots ($F_{10,47} = 7.1282, p = 0.0001$). Using a Bonferroni post-hoc test, we found that while the saprophytic F/B ratio differed between the soybean plot and the early successional plots, it did not differ between the soybean plot and the older restored plots, and there was no significant difference between the corn plot and any of the restored plots (results not shown). The AMF/bacteria biomass ratio increased with time for the first seven growing seasons, then declined as succession continued (Fig. 5). Using a one-way ANOVA, we found that the AMF/bacteria ratio differed significantly among plots ($F_{10,47} = 20.6796, p = 0.0001$). The agricultural plots had lower AMF/bacteria ratios than all except the 23 yr old plot (Fig. 5).

We determined the correlation between positions of samples on the ordination axes, and environmental variables as a means of identifying the environmental characteristics most likely to be driving changes in community structure. Position on CA Axis 1 was positively correlated with root biomass ($R^2 = 0.55, p = 0.0001$) and with litter C ($R^2 = 0.42, p = 0.0001$) concentration; prairie plots had higher plant biomass, with higher C concentrations than did agricultural plots (Fig. 1, Table 4). Position on Axis 1 was negatively correlated with N concentrations in root ($R^2 = 0.50, p = 0.0001$) and litter ($R^2 = 0.27, p = 0.0001$) (Table 4). Position on Axis 2 was related more strongly to changes in soil characteristics; most notably, samples high on Axis 2 were associated with high bulk density ($R^2 = 0.29, p = 0.0001$), and with low SOC ($R^2 = 0.30, p = 0.0001$) and TN ($R^2 = 0.42, p = 0.0001$) (Fig. 1, Table 4). The only variable associated with both axes was pH, increasing with position.
on axis 1 \((R^2 = 0.38, p \leq 0.0001)\) (conversion from agriculture to prairie) and decreasing with position on Axis 2 \((R^2 = 0.31, p \leq 0.0001)\) (Fig. 1, Table 4).

The saprophytic F/B ratio was negatively correlated with N concentration of both roots \((R^2 = 0.41, p \leq 0.0001)\) and litter \((R^2 = 0.10, p \leq 0.0171)\) (Table 4), and increased with increasing root biomass \((R^2 = 0.15, p \leq 0.0025)\) and pH \((R^2 = 0.10, p \leq 0.0183)\) (Table 4). The relationship with SOC was less straightforward: when agricultural and prairie plots were analyzed simultaneously, there was no relationship between SOC and the saprophytic F/B ratio \((R^2 = 0.02, p \leq 0.3637)\) (Table 4). However, when the agricultural and prairie soils were analyzed separately, we found a strong positive correlation \((R^2 = 0.82, p \leq 0.0001)\) between SOC and the saprophytic F/B ratio in agricultural soils (Fig. 6A) and a weaker negative correlation \((R^2 = 0.28, p \leq 0.0001)\) in prairie soils (Fig. 6B). There was no relationship between the AMF/B ratio and SOC in agricultural soils \((R^2 = 0.01, p \leq 0.4751)\), but a weak negative correlation \((R^2 = 0.19, p \leq 0.0011)\) in prairie soils.

There was a significant positive linear relationship between microbial biomass determined by fumigation-extraction (MBC) and total PLFA \((R^2 = 0.47, p \leq 0.0001)\) (Table 4). Microbial biomass C was very highly correlated with time since restoration began \((R^2 = 0.69, p \leq 0.0001)\). Microbial biomass was most strongly correlated with soil characters: MBC increased with increasing SOC \((R^2 = 0.61, p \leq 0.0001)\), TN \((R^2 = 0.50, p \leq 0.0001)\), and pH \((R^2 = 0.29, p \leq 0.0001)\), but decreased with increasing bulk density \((R^2 = 0.21, p \leq 0.0001)\). Microbial biomass C was also positively correlated with plant community characters, increasing with increasing litter C concentration \((R^2 = 0.49, p \leq 0.0001)\), and with root biomass \((R^2 = 0.34, p \leq 0.0001)\) and litter mass \((R^2 = 0.36, p \leq 0.0001)\) (Table 4).

The cyclopropyl/precursor ratio (calculated as the sum of \text{cy}17:0 and \text{cy}19:0 divided by the sum of their precursors, \text{16:1}\text{o}7\text{c} and \text{18:1}\text{o}7\text{c}) declined as position on Axis 1 increased \((R^2 = 0.80, p \leq 0.0001)\) (Fig. 7). The agricultural soils demonstrated the highest ratios, suggesting that the microbial communities in these soils are under the highest stress. This analysis was repeated with the \text{cy}17:0 to \text{16:1}\text{o}7\text{c} and \text{cy}19:0 to \text{18:1}\text{o}7\text{c} ratios calculated independently, and revealed the same trend as found for the summed ratio (results not shown).

**DISCUSSION**

Organic matter decomposition and associated nutrient cycling are regulated by the soil microbial community (e.g., Van Veen et al., 1984; Wardle, 1992; Scow, 1997). The size and activity of this microbial community

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**Table 4. Correlations between environmental variables and microbial community measures (correlation coefficients and significance).†**

<table>
<thead>
<tr>
<th>MBC, µg g⁻¹</th>
<th>Total PLFA, n mol g⁻¹</th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>F/B</th>
<th>Cyclo/precursor</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg g⁻¹</td>
<td>µmol g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.000</td>
<td>0.688 (0.0001)</td>
<td>0.404 (0.0018)</td>
<td>-0.460 (0.0003)</td>
<td>0.046 (0.7371)</td>
<td>-0.391 (0.0027)</td>
</tr>
<tr>
<td>0.688 (0.0001)</td>
<td>1.000</td>
<td>0.294 (0.0264)</td>
<td>-0.357 (0.0064)</td>
<td>0.107 (0.4290)</td>
<td>-0.219 (0.1017)</td>
</tr>
<tr>
<td>0.541 (0.0001)</td>
<td>0.365 (0.0048)</td>
<td>0.111 (0.4130)</td>
<td>0.537 (0.0001)</td>
<td>0.231 (0.0843)</td>
<td>0.226 (0.0905)</td>
</tr>
<tr>
<td>0.777 (0.0001)</td>
<td>-0.467 (0.0002)</td>
<td>0.614 (0.0001)</td>
<td>-0.560 (0.0001)</td>
<td>0.312 (0.0183)</td>
<td>-0.604 (0.0001)</td>
</tr>
<tr>
<td>0.541 (0.0001)</td>
<td>0.624 (0.0001)</td>
<td>0.464 (0.0003)</td>
<td>-0.406 (0.0017)</td>
<td>0.075 (0.5803)</td>
<td>0.500 (0.0001)</td>
</tr>
<tr>
<td>0.830 (0.0001)</td>
<td>0.646 (0.0003)</td>
<td>0.087 (0.5182)</td>
<td>-0.646 (0.0001)</td>
<td>-0.221 (0.0981)</td>
<td>0.124 (0.3564)</td>
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<tr>
<td>0.706 (0.0001)</td>
<td>0.238 (0.0749)</td>
<td>0.549 (0.0001)</td>
<td>-0.123 (0.3637)</td>
<td>-0.274 (0.0389)</td>
<td>0.488 (0.0001)</td>
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<td>0.498 (0.0001)</td>
<td>0.340 (0.0097)</td>
<td>0.336 (0.1107)</td>
<td>0.488 (0.0001)</td>
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<tr>
<td>0.213 (0.1121)</td>
<td>-0.302 (0.0224)</td>
<td>-0.705 (0.0001)</td>
<td>0.137 (0.3085)</td>
<td>-0.638 (0.0001)</td>
<td>0.444 (0.0005)</td>
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<td>-0.454 (0.0004)</td>
<td>-0.392 (0.0003)</td>
<td>-0.295 (0.2568)</td>
<td>0.013 (0.9219)</td>
<td>0.303 (0.0216)</td>
<td>0.241 (0.0710)</td>
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<tr>
<td>0.209 (0.1365)</td>
<td>0.252 (0.0576)</td>
<td>0.120 (0.4162)</td>
<td>0.584 (0.0001)</td>
<td>0.193 (0.4071)</td>
<td>0.393 (0.0007)</td>
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<td>0.534 (0.0001)</td>
<td>0.393 (0.0005)</td>
<td>0.641 (0.0001)</td>
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<tr>
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<td>0.315 (0.0171)</td>
<td>0.529 (0.0001)</td>
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<td>0.651 (0.0001)</td>
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<td>0.316 (0.0165)</td>
<td>0.611 (0.0001)</td>
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<td>0.351 (0.0074)</td>
<td>0.301 (0.0216)</td>
<td>0.620 (0.0001)</td>
<td>-0.249 (0.0615)</td>
<td>0.352 (0.0072)</td>
<td>0.622 (0.0001)</td>
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<tr>
<td>0.582 (0.0001)</td>
<td>0.471 (0.0002)</td>
<td>0.744 (0.0001)</td>
<td>0.080 (0.5551)</td>
<td>0.393 (0.0025)</td>
<td>0.641 (0.0001)</td>
</tr>
<tr>
<td>0.598 (0.0001)</td>
<td>0.360 (0.0044)</td>
<td>0.510 (0.0001)</td>
<td>-0.410 (0.0015)</td>
<td>0.016 (0.9065)</td>
<td>-0.760 (0.0001)</td>
</tr>
</tbody>
</table>

† MBC, microbial biomass carbon; TN, total nitrogen; SOC, soil organic carbon; PLFA, phospholipid fatty acid; F/B, fungal to bacteria ratio.
Fig. 7. Relationship between the ratio of the sum of cyclopropyls (cy17:0 and cy19:0) to the sum of their precursors (16:1o7c and 18:1o7c) and positions on ordination axis 1, in row crop soils (open symbols) and prairie chronosequence soils (filled symbols).

depends on site differences in climate (e.g., Insam et al., 1989; Wardle and Parkinson, 1990; McCulley and Burke, 2004), topography, and parent material (e.g., Schimel et al., 1985; Anderson and Domsh, 1993), mediated through their impacts on primary productivity and plant litter quality. However, these site factors can be altered by land management practices, including tillage, fertilizer, and amendment applications, grazing, and agricultural deintensification (e.g., Bardgett et al., 1993, 1997; Beare et al., 1993; Zelles et al., 1995; Hedlund, 2002). The mechanisms by which management alters soil microbial community structure are not well understood, but they are presumably due to changes in disturbance or in the quantity and quality of inputs, with reduced disturbance and mineral nutrient inputs commonly increasing the relative abundance of fungi (Bardgett et al., 1993; Cambardella and Elliot, 1994; Beare, 1997; Bardgett and McAlister, 1999; Stahl et al., 1999; Zeller et al., 2001; Bailey et al., 2002). Increased fungi in aggrading soil systems may be a mechanism for increasing whole-soil C storage, because fungi (i) are unique in their ability to translocate and utilize spatially separated nutrient resources (e.g., Frey et al., 2003), (ii) are believed to respire more efficiently than bacteria (e.g., Holland and Coleman, 1987), and (iii) are composed of more complex recalcitrant compounds (e.g., Guggenberger et al., 1999). In this study, we examined changes in soil microbial community structure in a range of reconstructed tallgrass prairies, and predicted an increase in the F/B ratio with time since restoration began.

Conversion from tillage-based agriculture to prairie increased total microbial biomass (Table 3). As a result, biomass of all microbial groups was generally higher in prairie than in agricultural plots. In addition, as predicted, conversion from agriculture to prairie increased the abundance of fungi relative to bacteria (Fig. 3). As previously demonstrated (e.g., Johnson et al., 1991; Hedlund, 2002), abandoning tillage-based agriculture increases fungal dominance. Further, AMF responded more strongly than did saprophytic fungi (Fig. 4). The rapid increase in AMF once tillage ceases is possible because there are abundant viable hyphal fragments and spores that can act as inoculum even in disturbed agricultural soils. The increase in AMF corroborates previous findings from the Fermilab site, which demonstrate the importance of extraradical hyphae of AMF and fibrous roots in the stabilization of soil aggregate structure (Jastrow et al., 1998; Miller and Jastrow, 1990, 1992), and the subsequent accumulation of SOC (Jastrow, 1987; 1996; Jastrow et al., 1996) after conversion from tillage-based agriculture to prairie.

Cessation of disturbance rather than changes in vegetation or soil characteristics appears to be primarily responsible for the increase in the AMF/bacteria ratio that immediately follows conversion from tillage-based agriculture to prairie. While vegetation and soil characteristics tend to change fairly linearly with successional time (Table 2), the initially high AMF/bacteria ratios subsequently decline to levels similar to those seen in the agricultural fields (Fig. 5). As a result, the AMF/bacteria ratio is not strongly related to successional time. Several other studies have suggested that higher F/B ratios early in secondary succession are due largely to the cessation of disturbance rather than vegetation changes. Hedlund et al. (2003) found that experimental manipulation of the plant community had very little effect on the microbial community relative to changes after cessation of agriculture, suggesting that reduced disturbance and mineral fertilizer inputs were more important than changes in the plant community. Further, even when plant community composition is similar, reduced agricultural disturbance can increase fungal dominance. For example, Drijber et al. (2000) found that PLFA signatures for AMF and fungi were higher in relative abundance in untilted than in tilled fallow fields, both sowed to wheat.

The relationship between microbial community structure and soil organic C further suggests that relative abundances of fungi do not increase with successional time. Initially, it appeared that microbial community composition was only weakly related to SOC (Table 4). However, when examined for agricultural and prairie soils individually, we found that while F/B was strongly positively correlated with SOC in the tillage-based agricultural soils (Fig. 6A), it was negatively correlated with SOC in prairie soils (Fig. 6B). This pattern suggests that improved community metabolic efficiency due to increased relative abundance of active fungi is not a mechanism promoting C storage in abandoned agricultural soils. Instead, fungi may contribute to soil organic C sequestration through the formation of a stable soil structure (Jastrow et al., 1998; Miller and Jastrow, 2000; Zhu and Miller, 2003) and the buildup of recalcitrant residues (e.g., Gleixner et al., 2002). These mechanisms by which fungi promote C sequestration are not reflected in PLFA patterns, as PLFAs only assess active biomass. The prevalence of recalcitrant fungal biomass was demonstrated by Klein et al. (1995) in another secondary successional system. These authors found increased hyphal development with time, initially sug-
suggesting that fungi were the primary decomposers later in succession. However, only 10% of hyphae were active as measured by FDA, and Klein et al. (1995) suggest that bacteria were the dominant decomposers even in later succession.

In a number of other successional systems F/B ratios continue to increase with time. Pennanen et al. (2001) found increased relative abundance of fungi with time in a primary successional system, and suggest that fungal biomass increased in response to the accumulation of organic matter and increasing C/N ratio of the plant material. Similarly, Ohtonen et al. (1999) found an increase in microbial biomass and in the F/B ratio with time in a glacial sere. In addition, the authors found that the respiration rate per unit biomass was lower in late than early successional communities, suggesting more efficient C use by these more fungal-dominated, late successional communities. We suggest that high F/B ratios are not maintained during succession at Fermilab because high ratios early in succession are driven primarily by increased biomass of AMF rather than saprophytic fungi. The biomass of AMF is limited by root availability that, like AMF, may be approaching equilibrium in the surface horizons of our older plots (Table 2). A second possible explanation is that a high proportion of C inputs in this prairie system are from easily metabolizable rhizodeposition rather than from more recalcitrant litter, and thus promote bacterial proliferation rather than growth of saprophytic fungi (Zak et al., 1996; Buyer et al., 2002).

Although reduced disturbance appears to be the primary mechanism by which conversion from tillage-based agriculture to prairie influences microbial community structure, changes in vegetation characteristics, such as tissue nutrient concentrations, are also important. For example, agricultural fields have higher cyclopropyl to precursor ratios than do prairie fields (Fig. 7). This indicates that the bacterial component of the microbial community is under stress, and that a higher proportion of the bacterial community is in the stationary rather than logarithmic growth phase (Guckert et al., 1986; Bossio and Scow, 1998; Bossio et al., 1998; Calderón et al., 2001; Jackson et al., 2003). The agricultural fields at this site have high inputs of N, as a result of the fertilizer inputs and soybean rotation. However, they have low litter and root biomass (Table 2) and thus inputs of C relative to N are low compared with that in the prairie sites.

The strongest gradient in microbial community structure is the disturbance response, summarized by ordination axis one (Fig. 1). A secondary gradient appears to be more closely related to time since conversion to prairie, and is tied to soil variables (Fig. 1). The youngest prairie plots have higher relative abundances of fungi (Fig. 2) and high soil bulk density (Fig. 1), while the older plots have higher relative abundances of actinomycetes and gram-negative bacteria, and high SOC and TN. Actinomycetes are known to be important decomposers of the fungal wall compound chitin (Krsek and Wellington, 2001; Metcalfe et al., 2002), and we suggest actinomycetes increase in response to a build up of recalcitrant fungal material. Younger prairie plots also have higher relative abundance of the putative bacterial PLFA 14:0 (Fig. 2). Although we identified PLFA 14:0 as bacterial (Table 1), it is actually found in a broad range of microbial functional groups (Zelles, 1999). This illustrates a limitation of PLFA analysis: the functional group to which PLFAs are assigned is based on current knowledge, and many PLFAs are non-specific (Pennanen, 2001).

Our finding that soil factors were of secondary importance in determining the microbial community structure following the cessation of tillage-based agriculture initially appears to contradict previous research at Fermilab and other sites. For example, Bailey et al. (2002) compared an agricultural field to a 21-yr-old restored prairie site and found that fungal metabolic activity was most strongly correlated with SOC. The authors suggest that either fungi increase in soils with high C concentrations, or that fungi favor the storage of C in soils. Similarly, Bardgett et al. (1999) found increased fungal biomass in less intensively managed grasslands and demonstrated that this change was strongly related to changes in soil characteristics, particularly soil mineral N and moisture. In contrast to this research, our conclusions are based on changes in relative rather than total abundance of microbial groups, and thus do not confound changes in composition with changes in total microbial biomass.

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Our conclusions rely on the assumption that we can substitute sites of different restoration ages to represent a time effect. Sparling et al. (2003) tested this assumption on a chronosequence of landslips, which were resampled 14 yr after the original survey. They found that for most measures, a single sampling of a chronosequence was as effective as following individual sites through time. In secondary successional systems, results are likely to be less consistent due to differences in site history. At Fermilab, for example, there is considerable variation in pH among sites of different ages (Table 2), and we suggest that this is due to historical differences in farming practices. Variation in current land management practices among sites may also influence our findings. For example, several plots from the oldest restored site have microbial community composition and soil bulk density similar to the earliest restored sites (Fig. 1). A possible explanation is soil compaction resulting from mechanical seed harvesting at this site. However, successional patterns determined from resampling (1985 and 1999) were very similar to those predicted from our chronosequence (Jastrow, 1987; Jastrow, 1996; Jastrow et al., 1998; Miller and Jastrow, 1990; R. Matamala, unpublished data), and we suggest that our system effectively identifies large-scale successional patterns, in spite of plot-level historical artifacts.
In conclusion, cessation of tillage-based agriculture and reconstitution of prairie at the Fermilab site increases total microbial biomass, and initially increases abundance of fungi, particularly AMF, relative to bacteria in the surface soil layer. We suggest this is due primarily to reduced disturbance when tillage ceases, with early changes reversed later in succession. Vegetation characteristics also appear to be important, with high cyclopropyl to precursor ratios indicating bacterial communities under stress in agricultural but not prairie soils, possibly due to low C relative to N inputs. Although the strongest gradient is the response to cessation of agriculture, there is a secondary gradient related to successional time. This gradient is more strongly tied to soil characteristics, particularly soil bulk density, SOC, and TN. Although F/B increases with SOC in agricultural soils, it decreases with SOC in prairie soils and with successional time. As a result, we suggest that improved metabolic efficiency due to increased relative abundances of fungi is unlikely to be a mechanism enhancing C storage in these soils. Instead, we suggest that fungi contribute to C sequestration through their role in soil structure and inputs of recalcitrant compounds.

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