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Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field

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Abstract The relationship between plant diversity and productivity has received much attention in ecology, but the relationship of these factors to soil microbial communities has been little explored. The carbon resources that support soil microbial communities are primarily derived from plants, so it is likely that the soil microbial community should respond to changes in plant diversity or productivity, particularly if the plant community affects the quality or quantity of available carbon. We investigated the relationship of plant diversity and productivity to the composition of the soil microbial community along a topographic gradient in a mid-successional old-field in southwestern Michigan. Soil moisture, soil inorganic N, and plant biomass increased from the top to the base of the slope, while light at ground level decreased along this same gradient. We characterized the changes in resource levels along this gradient using an index of productivity that incorporated light levels, soil N, soil moisture, and plant biomass. Average plant species richness declined with this productivity index and there were associated compositional changes in the plant community along the gradient. The plant community shifted from predominantly low-growing perennial forbs at low productivities to perennial grasses at higher productivities. Although there was variation in the structure of the soil microbial community [as indicated by fatty acid methyl ester (FAME) profiles], changes in the composition of the soil microbial community were not correlated with plant productivity or diversity. However, microbial activity [as indicated by Biolog average well color development and substrate-induced respiration (SIR)] was positively correlated with plant productivity. The similarity between patterns of plant biomass and soil microbial activity suggests that either plant productivity is driving microbial productivity or that limiting resources for each of these two communities co-vary.

Key words Soil microbial community · Diversity-productivity patterns · Old-field community · Fatty acid methyl ester · Biolog

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Introduction

A central question in ecology is why there are so many different organisms on the earth (Hutchinson 1959). Much of the work focusing on macroorganisms has emphasized the role of factors such as productivity, disturbance, energy, predation, resources, stochasticity, and colonization in determining the diversity of plant and animal communities (Rosenzweig 1995). Considerably less is known about what factors influence the abundance and diversity of microorganisms (Tiedje 1995). Microorganisms have rarely been incorporated into studies of mechanisms that may structure diversity-productivity relationships for plants and other macroorganisms (Ohtonen et al. 1997; Schläpfer and Schmid 1999). Although plant and soil communities are functionally linked, few studies have examined how patterns of diversity in plant and soil microbial communities co-vary.

Soil microbial communities are often limited by carbon (D.R. Zak et al. 1994) or nitrogen (Zak et al. 1990). Because the extant plant community is usually the main source for both of these resources, the composition and diversity of the soil microbial community may be closely associated with the plant community. It is difficult to assess composition and diversity of soil microbial communities. As a result, most investigations use techniques that assay different aspects of a subset of the microbial community. Two tools that are commonly used by ecolo-

gists to characterize the soil microbial community are Biolog assay and fatty acid methyl ester (FAME) profiles. Biolog assays sole-carbon-source utilization by the microbial community and provides an index of functional diversity. Biolog profiles have been successfully used to differentiate soil microbial communities associated with different plant communities (e.g., J.C. Zak et al. 1994; Goodfriend 1998), especially when used in concert with other techniques, like FAME profiles (e.g., Buyer and Drinkwater 1997). FAME profiles reflect the phenotypic composition of the soil microbial community (Tunlid and White 1992) and can be used to distinguish among microbial communities with different compositions (Haack et al. 1994; Cavigelli et al. 1995).

We investigated the relationship between the structure and activity of the soil microbial community and its relationship to the plant community within an ecologically variable site. We hypothesized that the structure of the soil microbial community would vary at this site in relation to: (1) soil characteristics, (2) plant productivity, and (3) plant diversity. We investigated the relationship between the soil microbial community and these three variates along a topographic productivity gradient in a mid-successional old-field in southwestern Michigan.

Methods

Site description

The study site was in a mid-successional abandoned field at the Lux Arbor Reserve of the W.K. Kellogg Biological Station (KBS) in southwestern Michigan. This site had been abandoned for approximately 25 years from agricultural production and during that period had not been grazed, burned, or otherwise managed. Successional fields in this area typically attain a stable species composition of herbaceous perennials 5–25 years after abandonment (Huberty et al. 1998). There has been no apparent change in the plant community at this site over the past 10 years (K.L. Gross, personal communication). The study site was located along a gentle slope, approximately 15° from the top to the bottom of the hill, along which there were apparent changes in plant species composition and productivity. The soil at the site is Kalamazoo sandy loam soil and does not vary across the study area.

Sampling design and characterization of the gradient

We established five parallel transects, 7.5 m apart, perpendicular to the slope of the hill and sampled soil and vegetation in seven 0.25-m^2 plots placed at 10-m intervals along each transect (n=7 per transect). To characterize the gradient, we measured light at ground level, soil moisture, soil inorganic nitrogen, and aboveground plant biomass.

Above-ground plant biomass and species composition were sampled in June 1996 by clipping the plants at ground level (0 cm above the soil surface), sorting by species, drying at 60°C for 48 h, and weighing. To better estimate peak plant biomass, particularly at the more mesic end of the gradient which was dominated by warm-season grasses, the same plots were re-clipped in July 1996. Samples were processed and treated as before. Peak plant biomass was calculated as June biomass+July biomass (both living and standing dead).

Light availability at ground level was determined prior to clipping in June and July. Measurements were made at midday (1100–1400 hours EDT) using a Sunfleck PAR Ceptometer (Decagon Devices, Inc.). We measured photosynthetically active radiation (PAR) in full sun 1 m above the plots and took four measurements of PAR at ground level within each plot (cardinal directions). We averaged these four data points to obtain an estimate of the percentage of full sunlight penetrating to ground level.

Soils were also sampled in June and July. For the soil analyses, we aggregated five 2.5-cm-diameter by 10-cm-deep soil cores taken from each 0.25 m^2 plot in an X-shaped pattern. Samples were placed in sealed plastic bags and kept on ice for up to 6 h until they could be returned to the laboratory. There, they were passed through a 2-mm sieve and sub-sampled for gravimetric soil moisture and nitrogen content within 24 h of sampling. Samples were kept at 4°C until processed. Gravimetric soil moisture was determined by weight loss after drying 10–15 g soil at 105°C for 24 h. For the nitrogen assays, we extracted 20 g fresh soil in 100 ml 1M KCl. These samples were shaken for 1 min, settled for 24 h at room temperature, and filtered through a 1-μm Gelman glass filter. The NO_3^- and NH_4^+ concentrations of the extracts were determined using an Alpkem auto-analyzer. The remaining soil was used to characterize the soil microbial community. Soil for FAME analyses was kept at –20°C until the fatty acids were extracted.

Characterization of the soil microbial community

We characterized the soil microbial community from samples taken in July using a modified substrate-induced respiration (SIR) method, carbon-source utilization (Biolog) and FAME profiles. SIR assesses the microbial biomass of the soil microbial community and is a good indicator of microbial activity (Hassink 1993). For SIR microbial biomass, soil slurries were shaken with and without glucose in Erlenmeyer flasks sealed with parafilm, and the headspace CO_2 was measured. For the control, we combined 25 g soil and 25 ml water in 125-ml Erlenmeyer flasks, and for the glucose-addition we substituted 25 ml of 30 mg ml^{-1} glucose for the water. Both sets were shaken for 2 h at 22°C. After 2 h, we transferred 5 ml of the headspace gas to a serum vial and measured the initial CO_2 on an ADC series EGA infrared CO_2 gas analyzer (The Analytical Development Co. Ltd., Hoddesdon, Herts., UK). The flasks were shaken for another 38 h and headspace CO_2 again measured.

For the Biolog assay, 1 g of fresh, sieved soil was shaken with 99 ml of 1% phosphate buffer solution for 20 min and 150 μl of the solution was transferred into each well of a GN Biolog microtiter plate (95 Carbon sources; Biolog, Inc., Hayward, Calif., USA). Three replicate plates were inoculated for each plot. The plates were incubated at 25°C in the dark and optical densities were measured after 24 and 48 h using an Emax precision microplate reader (Molecular Devices Corp., Menlo Park, Calif., USA). It is well known that inoculation densities from a standard amount of soil can vary for samples taken from different environments (Konopka et al. 1998). Optical density measures are often standardized to account for differences in innoculation densities; however, the standardizations have been criticized for not accurately reflecting growth across samples with different compositions (Konopka et al. 1998). Therefore, instead of standardizing optical densities, we chose to take advantage of differences and used average well color development (AWCD) from Biolog (corrected within plate for water reading) as an index of microbial activity. Because profiles at 24 h and 48 h were similar only the results from the 48 h time point are presented here.

To obtain fatty acids for FAME analysis, we first extracted the lipids from whole soil samples for 2 h using a mixture of dichloromethane (DCM):methanol:phosphate buffer (1:2:0.8 v/v/v), following a modified Bligh-Dyer procedure (Bligh and Dyer 1959). We then saponified the samples using 1 ml NaOH (15% w/v) in methanol (50% v/v) at 100°C for 30 min and methylated the sample with 2 ml 6M HCl in methanol at 80°C for 10 min. We extracted the fatty acid methyl esters into 1.25 ml (1:1 v/v) methyl-*tert*-butyl ether-hexane for 10 min and washed the extract with 3 ml 1.2% NaOH. FAME analyses were carried out using a HP 5890 series II gas chromatograph (Hewlett Packard Co., Palo Alto, Calif., USA) equipped

with a 7673 autosampler and flame ionization detector (Microbial ID Inc., Newark, Del., USA). Peaks were identified by comparison with an external standard. We performed all analyses on the fatty acid proportions of the total peak area to correct for differences in overall peak area.

We describe fatty acids using standard nomenclature where the total number of carbon atoms appears before the colon and the total number of C-C double bonds appears after it. Cyclo-propane analogs are indicated by "cyclo," and the location of the epoxy bond is indicated by a "c" followed by two numbers. If the cis or trans configuration is unknown, the word "at" is used. The number following "cis", "trans" or "at" indicates the location of the double bond in relation to the carboxyl end of the molecule. A number before "OH" indicates the location of the hydroxyl group in relation to the carboxyl end of the molecule. Those fatty acids with the same retention time are grouped as "sum in feature" and given a unique number designation.

Statistical analyses

To obtain an index of productivity along the gradient we performed a principal components analysis (PCA) on those variates expected to be closely related to productivity:light at ground level, gravimetric soil moisture, soil inorganic N, and peak plant biomass. To examine the productivity-diversity patterns, we regressed plant species diversity, number of carbon sources metabolized (Biolog), AWCD (from Biolog), and SIR microbial biomass against this index of productivity. Changes in plant community composition along the gradient were evaluated with indirect gradient correspondance analysis on species-specific above-ground plant biomass. To visually compare the plant and soil community patterns, we performed *K*-means cluster analysis on plant-species-specific biomass data, carbon source utilization profiles (Biolog), and fatty acid methyl ester profiles (FAME). Multidimensional scaling (MDS) was used to predict the number of clusters expected for the Biolog and FAME profile data. As there were more parameters than samples for the Biolog data, we randomly split the parameters into two subgroups that were run through all analyses independently. The results of these two independent analyses were consistent, so the data from only one are presented.

Results

Characterization of the gradient

Light at ground level, gravimetric soil moisture, soil inorganic N, and peak plant biomass all co-varied along the topographic gradient. Light availability at ground level (%PAR) decreased from 85% at the crest of the hill to 3% at the base of the hill. Gravimetric soil moisture increased from 15% to 36%, soil inorganic N increased from 3.29 to 18.58 μg N g^{-1} dry soil, and above-ground plant biomass increased from 89.2 to 309.6 g m^{-2} along the slope.

We performed a PCA to obtain an index of productivity that incorporated these measures of resource availability plus above-ground plant biomass. One sample point at the bottom of the hill was excluded from the analysis because of an abnormally high inorganic soil N value (10-fold higher than the median). The first principal component (PC1) based on resource levels measured in June and peak plant biomass (June+July) accounted for 64.8% of the variation in the data set (λ=2.594). Soil moisture, nitrogen, and peak plant biomass were positively correlated with PC1, whereas light at ground level was negatively correlated with PC1 (Fig. 1A). PC2 accounted for an additional 17.1% of the variation, but showed no pattern in relation to the gradient. Therefore, we used PC1 as an index of productivity in the remaining analyses (r^2=0.50, Fig. 1B). A PCA performed on the same variates from the July sampling was indistinguishable from the PCA on the June data, so we will present and use only the June results here.

Plant and soil microbial community relationships with productivity

Plant species richness declined with increasing productivity (PC1) at this site, but productivity accounted for little of the variation in diversity (r^2=0.17, Fig. 2A). This

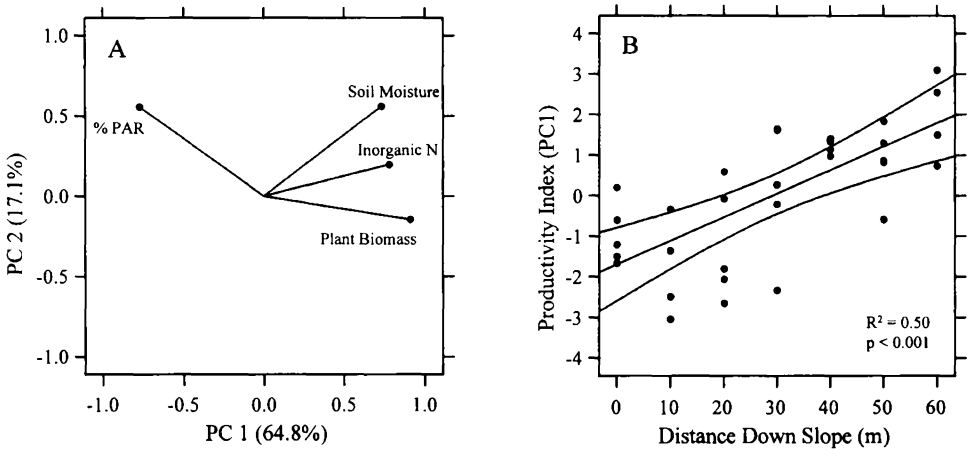

Fig. 1 **A** Factor loadings plot for principal components (PCs) 1 and 2 from the principal components analysis (PCA) of the June 1996 sample for light at ground level, soil moisture, soil inorganic N, and peak plant biomass; PC 1 and 2 account for 64.8% and 17.1% of the variation, respectively. Correlations between factor 1 and light (r=–0.77), moisture (r=0.73), N (r=0.80), and biomass (r=0.91) were significant at P<0.001; correlations between factor 2 and light (r=0.56) and moisture (r=0.56) were significant at P<0.005. **B** The relationship between PC 1 and distance down slope for 34 of the 35 sampling points, y=-1.69+0.058x; *lines* depict regression and 95% confidence intervals

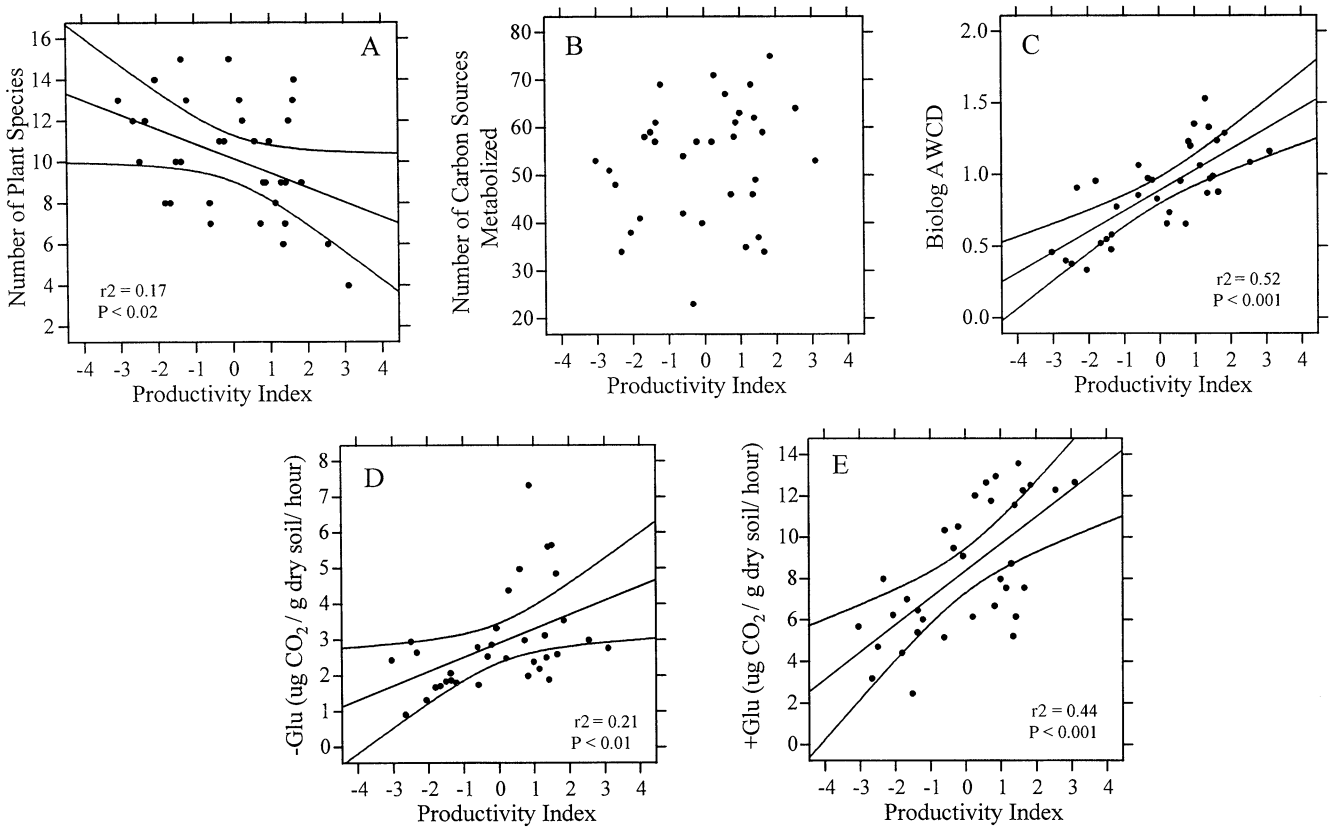

Fig. 2A–E The relationship between productivity index and several variates describing the plant and microbial communities at this site. **A** Plant species richness, y=10.18-0.71x. **B** The number of carbon sources metabolized by the soil microbes after 48 h. **C** Biolog average well color development (*AWCD*) after 48 h, y=0.89+0.14x. **D** C evolution (μg CO_2-C g^{-1} dry soil h^{-1}) from substrate-induced respiration (SIR) control (*–glu*) after 40 h, y=0.54x-1.55. **E** C evolution (μg CO_2-C g^{-1} dry soil h^{-1}) from SIR glucose addition treatment (*+glu*) after 40 h, y=0.33x-2.80. *Lines* depict regression and 95% confidence intervals, n=34

relationship was clearly driven by two low diversity points at the high end of the productivity gradient (Fig. 2A). However, averaging over transects there was clear decline in species richness across the gradient: an average of 13 species per plot (0.25 m^2) were found at the top of the hill, where productivity was lowest, while as few as 4 species per plot were found at the bottom of the hill, where productivity was highest (Fig. 2A). Although the number of carbon sources metabolized by the microbial community varied from 23 to 75 across this gradient, there was no relationship between the number of carbon sources metabolized and productivity (Fig. 2B). However, AWCD at 48 h, which could be indicative of either microbial activity or biomass, increased from 0.4 to 1.4 as productivity increased (r^2=0.52, Fig. 2C). Similarly, SIR basal activity rate after 40 h also increased along the gradient (r^2=0.21, Fig. 2D), as did the rate at which glucose was consumed between 2 and 40 h (r^2=0.44, Fig. 2E). We used the SIR control and experimental treatments separately in our analysis as measures of microbial activity because the SIR time course was insufficient to determine microbial biomass.

Compositional shifts in the plant and soil microbial communities

To visually compare patterns in the plant and microbial communities, we performed separate *K*-means cluster analyses on the plant species biomass data and the Biolog and FAME profiles of the soil microbial communities. A plot of above-ground plant biomass across the study site clearly shows the topographic-productivity gradient (Fig. 3A) and allows visual comparisons to plant diversity and microbial community measures (Fig. 3B–D). There were compositional changes in the plant community along the productivity gradient. MDS on the species biomass data indicated three to seven valid clusters: we chose to illustrate seven in order to more completely portray the variation in plant composition. The cluster analysis revealed an inverse relationship between plant community diversity and productivity. More specifically, the cluster analysis showed a shift in the plant community from the top of the slope, where there was a mixed community of forbs and no clearly dominant species (Table 1, cluster 1), to a mid-slope region dominated by perennial herbs (Table 1, clusters 3–4) to a low diversity community dominated by *Agropyron repens* near the bottom of the slope (Table 1, clusters 5–7, Fig. 3B). Plots at the bottom of the hill with highest soil moisture and productivity were dominated by *Polyg-*

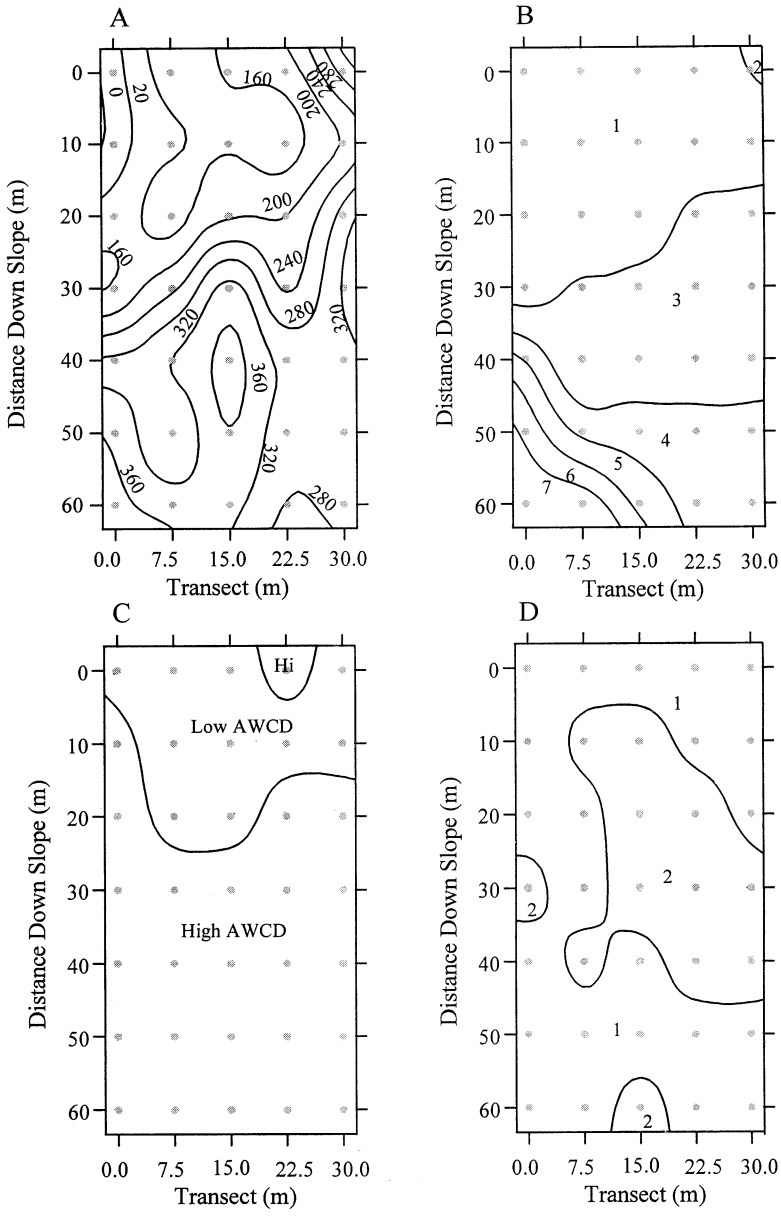

Fig. 3A–D Results from *K*-means cluster analysis evaluating the changes in production and composition of the plant and soil communities in relation to transect position at this site. **A** Contour plot of aboveground plant biomass, values are g m^{-2}; **B** Plant species composition: cluster 1 has no dominant species, cluster 2 is dominated by *Rubus* sp., cluster 3 by *Solidago canadensis*, cluster 4 by *S. canadensis* and *Poa pratense*, cluster 5 by *Agropyron repens*, cluster 6 by *Poa pratense*, and cluster 7 by *Polygonum ambibium* var. *emersum* (see Table 1 for species lists). **C** Biolog carbon source utilization profiles separated into two clusters based on AWCD; and **D** Fatty acid methyl ester (FAME) profiles: cluster 2 had smaller proportions of 18:1 cis 9, 16:0, and summed in feature 9 (18:2 cis 9, 12 and 18:0 anteiso, Table 2) than cluster 1

onum amphibium var. *emersum* (water smartweed) (Fig. 3B).

MDS of the Biolog profiles indicated two strong clusters; however, cluster formation relied solely on AWCD and not number or type of carbon sources. This is consistent with the soil microbial community-productivity relationship (Fig. 2B,C). The number of carbon sources was not related to the productivity index (Fig. 2B), while AWCD was significantly related to the productivity index (Fig. 2C). Similarly, in the k-means cluster analysis, sites located at the top of the slope were characterized by lower AWCD, while the sites at the base of the hill had higher AWCD (Fig. 3C). There was no difference in the number or types of carbon sources metabolized across the gradient (data not shown).

Although there was variation in the FAME profiles from these samples, MDS showed no distinct clusters in this data set along the productivity-diversity gradient. Fatty acids used in MDS and cluster analyses are listed in Table 2. When we forced the cluster analysis to create two clusters, soils from cluster 1 had larger proportions of fatty acids 18:1 cis 9, 16:0, and summed in feature 9 (18:2 cis 9, 12 and 18:0 anteiso, Table 2) than soils from cluster 2 (Fig. 3D). However, the cluster-based FAME profiles did not show any pattern condordant with peak plant biomass (Fig. 3A), plant diversity (Fig. 3B), or Biolog AWCD patterns (Fig. 3C).

Discussion

We had hypothesized that the structure of the soil microbial community at this site would be related to plant community diversity, plant productivity, or soil charac-

Table 1 Plant species composition in the clusters from the *K*-means cluster analysis shown in Fig. 3B. Plant species are listed from most common (by biomass) to least common. Only species that have a total biomass >1 g for the cluster are listed. Nomenclature follows Gleason and Cronquist (1991)

Cluster (no. plots)	Plant species
1 (13)	*Centaurea maculosa, Rubus occidentalis, Hieracium* sp., *Achillea millifolium, Rubus allegheniensis, Rumex acetosella, Poa compressa, Panicum* sp., *Agropyron repens, Solidago canadensis, Potentilla recta, Solidago graminifolia, Poa pratense, Aster pilosus, Plantago lanceolata, Phleum pratense, Cerastium vulgatum, Daucus carota, Trifolium pratense, Lespedeza capitata, Dactylis glomerata*
2 (2)	*Rubus occidentalis, Poa pratense, Solidago canadensis, Phleum pratense, Hieracium* sp., *Rumex acetosella, Agropyron repens, Polygonum amphibium* var. *emersum, Panicum* sp.
3 (9)	*Solidago canadensis, Rubus occidentalis, Poa pratense, Achillea millifolium, Monarda fistulosa, Phleum pratense, Poa compressa, Rumex acetosella, Agropyron repens, Daucus carota, Potentilla recta, Solidago graminifolia, Cornus racemosa, Apocynum cannabinum, Hieracium* sp., *Lespedeza capitata, Rubus allegheniensis, Centaurea maculosa, Trifolium pratense, Cerastium vulgatum, Taraxacum officinale, Rumex crispus, Hypericum perforatum*
4 (6)	*Poa pratense, Solidago canadensis, Agropyron repens, Achillea millifolium, Monarda fistulosa, Phleum pratense, Aster strigosa, Potentilla recta, Taraxacum officinale, Galium aparine, Daucus carota, Rumex crispus, Solidago graminifolia, Rumex acetosella*
5 (2)	*Agropyron repens, Solidago canadensis, Monarda fistulosa, Polygonum amphibium* var. *emersum, Rubus occidentalis, Solidago graminofolia, Galium aparine, Poa pratense, Achillea millifolium*
6 (1)	*Poa pratense, Agropyron repens, Solidago canadensis, Achillea millifolium, Polygonum amphibium* var. *emersum*
7 (2)	*Polygonum amphibium* var. *emersum, Agropyron repens, Polygonum persicaria, Solidago canadensis, Rumex acetosella, Poa pratense, Poa compressa*

Table 2 Fatty acids used in *K*-means cluster analysis of fatty acid methyl ester (FAME) profiles

Fatty acid	
12:0	17:0 anteiso
11:0 iso 3OH	17:1 cis 10
C9 dicarboxylic acid	17:0 cyclo
14:0	18:3 cis 6, 12, 14
15:0 iso	18:1 cis 9
15:0 anteiso	18:0
15:1 cis 7	19:0 cyclo C11–12
15:0	18:0 2OH
16:0 iso	20:4 cis
16:1 cis 9	20:0
16:1 cis 11	22:0
16:0	23:0
iso 17:1 G	22:0 2OH
anteiso 17:1 at 9	24:0
17:0 iso	23:0 2OH
Summed Feature 9	Summed Feature 10:
18:2 cis 9, 12; 18:0 anteiso	18:1 cis 11; 18:1 trans 9; 18:1 trans 6

teristics. Because these three factors covaried at our site (Fig. 1), we combined them into an index of productivity, but still could not detect any relation to the soil microbial community structure. Neither Biolog nor FAME assays of the soil microbial community were strongly related to variation in productivity. There were changes in the diversity and composition of the plant community associated with soil fertility and plant biomass; however, these differences in plant community composition had no detectable effect on the composition of the soil microbial community.

We did find evidence, however, that suggested the activity (or biomass) of the soil microbial community varied in relation to plant productivity, paralleling the edaphic gradient. We have two lines of evidence that support the idea that soil microbial community activity (or biomass) increases with plant productivity at this site. First, we detected an increase in AWCD of the Biolog plates in relation to productivity (Fig. 2C). Although Biolog AWCD is not a direct measure of activity, it is strongly related to innoculum density (Garland and Mills 1991; Haack et al. 1995) and, as such, can be interpreted as an indicator of total number of bacteria (biomass). Conversely, two wells with same inoculation density may differ in AWCD because of differences in microbial activity (Konopka et al. 1998). In either case, the higher AWCD in the sites at the base of the hill indicates a more productive microbial community and this corresponds to areas along the gradient where the plant community is also the most productive. This is consistent with the higher amounts of N, moisture, and plant biomass at the base of the hill, which should make more C available to the microorganisms. Secondly, the modified SIR analysis indicates higher rates of activity at the base of the hill where productivity was highest (Fig. 2D,E).

Most studies that have reported changes in soil microbial community composition across community types have sampled sites that differed in plant species composition, productivity, and soil type. From these studies, it is unclear whether the plant community or the underlying edaphic factors are influencing the soil microbial community structure. For example, J.C. Zak et al. (1994) used Biolog to investigate changes in functional diversity of the soil microbial community from grasslands lo-

cated along an elevational and moisture gradient in New Mexico. They found differences in the Biolog profiles of the soil microbial community from six distinct plant communities along this gradient. However, because soil characteristics also varied among these sites, it is not clear whether the differences in Biolog profiles were due to changes in plant community composition, edaphic factors, or some other variable. Similarly, Goodfriend (1998) found that Biolog distinguished among the soil microbial communities of eight sites representing a variety of wetlands in the southwestern United States. However, it was not clear whether plant community composition or edaphic characteristics were more important in influencing the grouping of those Biolog profiles into habitat types.

Several authors have argued that phospholipid fatty acids (PLFAs), a subset of fatty acids present in the phopholipid membrane, may provide a more sensitive indicator to distinguish among microbial communitities. Phospholipids break down easily in the soil and are thus thought to represent the active soil microbial community (Bossio and Scow 1998). Zelles et al. (1992) used PLFA profiles to compare soil microbial community patterns in grassland and agricultural fields under different management regimes and found that profiles differed among the different fields, but they did not distinguish between plant community and edaphic effects. Bossio et al. (1998) concluded that soil type has stronger effects on the soil microbial community structure than plant community type. They found that the addition of a cover crop (an increase in plant community diversity over time) was less influential in changing PLFA profiles than soil type. The differences in edaphic characteristics at our site, although substantial, were not as striking as differences between soil types would be.

There are several possible reasons why we did not detect changes in the soil microbial community composition along this gradient: (1) there is no connection between the structure of the soil microbial community and the soil characteristics, plant diversity, or plant productivity; (2) the soil microbial community structure is very stable and affected mainly by factors like long-term plant community composition or historical C inputs to the soil; (3) the spatial scale or time of year we sampled was inappropriate for detecting differences in the soil microbial community; or (4) the techniques we used to assay the soil microbial community were not specific enough to detect what differences were there.

The first two reasons seem unlikely because there should be a linkage between the microbial (consumer) community and the resources (plant carbon) that they utilize (Paul and Clark 1996). Much of the carbon available to soil microbes is being provided to the soil microbes each year by the extant plant community, and although this is a successional community, the plant community composition at this site has remained stable for the past decade (K.L. Gross, personal communication). Even if soil microbial community structure is not affected by plant community composition, increasing plant diversity or productivity should provide additional resources to the extant soil microbial community and thus influence soil microbial community composition. Additionally, past agricultural use at this site likely would have depleted soil C (Drinkwater et al. 1998; Robertson et al. 1993), and therefore made the current community inputs of C important in determining the structure and activity of the soil microbial community.

It is difficult to know if sampling at a different time of year or spatial scale would have revealed associations between the plant and microbial communities at this site. Both temporal and spatial scales are important in the observation of ecological phenomena. We chose to sample in mid-summer on the assumption that at this time of year both the plant and soil microbial communities would be most active. Other researchers have revealed associations between plant and microbial communities using soil sampled in mid-summer. Bossio et al. (1998) detected differences in PLFA patterns of soil sampled in July from different agricultural treatments in California. Similarly, using carbon source utilization patterns, Westover et al. (1997) differentiated among rhizosphere soils sampled in August from several grass species in Washington.

It is possible that if our sampling had been done at smaller, more fine-grained scale we might have detected associations between microbes and specific plant species. Westover et al. (1997) detected differences among soil microbial communities of rhizosphere soils of several grass species in both the field and greenhouse. Grayston and Campbell (1996) used Biolog to differentiate between the microbial communities of rhizosphere soils from two tree species, *Larix eurolepis* and *Picea sitchensis*. However, others have found associations between plant and microbial communities at spatial scales similar to the scale used in our study. Plant community composition is more likely to affect soil microbial community composition than plant diversity or productivity. A recent experimental study by Wardle et al. (1999) did detect differences in PLFA composition of the soil microbial community that were significantly related to the plant removal treatments. This suggests that 3–4 years of abandonment is sufficient to detect changes in the soil microbial community. L.C. Broughton, K.L. Gross, and A. Hector (unpublished work) saw a similar relationship between plant community composition and PLFA patterns of the soil microbial community at the Silwood Park BIODEPTH site after 3 years.

The inadequacy of tools to assess microbial diversity has been a long-standing limitation to our understanding of soil microbial communities (Tiedje 1995). While there are clearly limitations to the ability of functional tools such as Biolog and PLFA to distinguish among microbial communities, as noted above, a number of studies have used these tools to successfully differentiate among communities (Zelles et al. 1992, 1995; J.C. Zak et al. 1994; Goodfriend 1998). The development of molecular techniques more sensitive to shifts in composition may reveal natural shifts from one closely related microbe to another along a gradient, just as there are shifts among

closely related plant species along gradients. Additionally, molecular techniques may allow us to better address the roles of dominance and plasticity in structuring soil microbial communities.

The correlative nature of our study does not allow us to determine what factors may underlie the observed variation in the soil microbial community at this site. Despite our expectation that there should be a close association between the plant community and the soil microbial community, at this spatial scale (within a site), using these tools, we were not able to detect any association between plant community composition and soil microbial community composition. The similarity between patterns of plant biomass and soil microbial activity is intriguing, however, and suggests that the resources that limit each of these communities co-vary. In contrast, the differences between patterns of plant diversity and soil microbial community structure suggest that different mechanisms are responsible for structuring diversity in these associated communities.

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