Landscape-Level Patterns of Microbial Community Composition and Substrate Use in Upland Forest Ecosystems

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ABSTRACT

The composition and diversity of biotic communities are controlled by the availability of growth-limiting resources. Resource availability for microbial populations in soil is controlled by the amount and types of organic compounds entering soil from plant litter. Because plant communities differ in the amount and type of substrates entering soil, we reasoned that the composition and function of soil microbial communities should differ with the dominant vegetation. We tested this idea by studying two sugar maple (*Acer saccharum* Marsh.)-dominated and one oak (*Quercus* spp.)-dominated forest ecosystems in northern Lower Michigan that differ in rates of soil N cycling. We used phospholipid fatty acid (PLFA) analysis to gain insight into microbial community composition, and we used a subset of Biolog GN substrates found in root exudate to assess the metabolic capabilities soil microbial communities. Although microbial biomass did not differ among ecosystems, principal components analysis of bacterial, actinomycetal, and fungal PLFAs clearly separated the microbial communities of the three ecosystems. Similarly, principal components analysis separated microbial communities by differences in growth on carbohydrates, organic acids, and amino acids. Discrimination among microbial communities in the three ecosystems by PLFAs and substrate use occurred in spring, summer, and fall, but the individual PLFAs and substrates contributing to discrimination changed during the growing season. Our results indicate that floristically and edaphically distinct forest ecosystems also differ in microbial community composition and substrate use. This pattern was consistent across the growing season and repeatedly occurred across relatively large land areas.

Soil temperature and water potential influence microbial metabolism, but the production of organic substrates by plants most often limits microbial growth in soil. Plant litter can vary widely in its chemical constituents, presenting the possibility that landscape-level differences in plant community composition could influence microbial community composition. This probably occurs in the upper Lake States region where broad differences in leaf litter chemistry accompany a change in forest composition. For example, slow-decomposing white oak (*Quercus alba* L.) leaf litter contains relatively high quantities of lignin compared with the sugar maple leaf litter, which decomposes rapidly (McClaugherty et al., 1995). In forests dominated by these contrasting species, leaf litter should contain very different types of substrates for microbial growth, and it is plausible that differences in leaf litter chemistry among floristically distinct forest ecosystems could give rise to microbial communities that differ in composition and function. In northern Wisconsin, Christensen (1969) found evidence for such a relationship, wherein communities of soil microfungi were correlated with the occurrence of forest vegetation (also see Christensen et al., 1962; Tresner et al., 1954). Notwithstanding these observations, we have a limited understanding of the manner in which the broader soil microbial community varies with plant community composition at a landscape-level scale.

Temporal patterns of root and leaf litter production, combined with variation in the chemical composition of these tissues, could influence the composition and function of soil microbial communities. Root litter typically contains more N and lignin than leaf litter (Aber et al., 1990; Vogt et al., 1986). The fact that roots and leaves differ chemically could lead to changes in microbial community composition and function, depending on temporal variation in the proportion of leaf vs. root litter entering soil. In sugar maple–dominated forests, the greatest addition of root litter to soil occurs when fine-root mortality peaks in early autumn (Hendrick and Pregitzer, 1992), but substantial inputs also can occur throughout the growing season. Unlike the continuous input of dead fine roots to mineral soil, the majority of aboveground litter is deposited in late autumn. Seasonal variation in above- and belowground litter production could influence microbial community composition and function, depending on the types of substrates available for microbial metabolism.

Soil temperature and water potential directly influence microbial activity, presenting the possibility that different members of the soil microbial community may be more or less physiologically active as these environmental factors vary seasonally. Laboratory studies have demonstrated that microbial community composition and substrate use changed concomitantly with increasing soil temperature (Zogg et al., 1997), and it is likely that differences in soil water potential could influence microbial communities in a similar manner (Zak et al., 1999). These factors, in combination with temporal patterns of leaf- and root-derived substrate input to soil, could influence microbial community composition and function in an ecosystem-specific manner.

Our first objective was to determine whether microbial community composition and function differed among three floristically distinct forest ecosystems in northwestern Lower Michigan. We reasoned that differences in plant community composition are reflected in microbial community composition because plant litter provides the primary substrate for microbial growth in soil. A second objective was to investigate whether mi-

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Abbreviations: ANOVA, analysis of variance; FAME, fatty acid methyl ester; GC, gas chromatograph; MS, mass spectrometer; PCA, principal components axis; PLFA, phospholipid fatty acid.

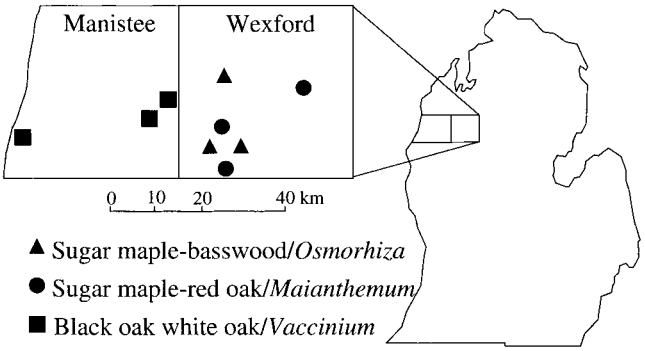

Fig. 1. Location of study sites in three upland forest ecosystems in Manistee and Wexford counties, northwestern Lower Michigan.

crobial community composition and function varied with seasonal changes in soil temperature, matric potential, and substrate input from leaf and root litter. To accomplish these objectives, we studied the seasonal dynamics of microbial community composition and substrate use in three upland forest ecosystems that differ in plant species composition, litter chemistry, and rates of soil N cycling.

METHODS

Study Area

Our study was conducted in the sugar maple–basswood/*Osmorhiza*, the sugar maple–red oak/*Maianthemum*, and the black oak–white oak/*Vaccinium* ecosystems located in northwestern Lower Michigan (Fig. 1). These upland forest ecosystems were classified by Host et al. (1988) by using an integrated approach that considers landform, soil, and vegetation. These ecosystems cover a large geographic extent in Michigan's northwestern lower peninsula, and they are named for the dominant overstory tree species and a common member of the groundflora vegetation. Mean annual temperature of the study area is 7.2°C, and mean annual precipitation is 81 cm (Albert et al., 1986). The sugar maple–basswood/*Osmorhiza* ecosystem occurs on the Interlobate moraine, which is composed of sandy glacial till. The sugar maple–red oak/*Maianthemum* ecosystem also occurs on the Interlobate moraine, but on slightly coarser sandy till. Soils forming beneath these forests are sandy Typic Haplorthods of the Kalkaska series. The black oak–white oak/*Vaccinium* ecosystem occurs on coarse, sandy, ice-contact topography; soils are Entic Haplorthods of the Rubicon series. A summary of overstory and soil properties of the three ecosystems is presented in Table 1. Hereafter, we refer to these ecosystems by their dominant overstory species (i.e., sugar maple–basswood).

Field Sampling

During the 1998 field season, we studied three randomly selected stands in each ecosystem type; the minimum distance between stands of one ecosystem type was 6 km (Fig. 1). In each stand, four 15 by 30 m plots were randomly located for soil sampling (Zak and Pregitzer, 1990). We collected two soil cores (2.2 cm in diam. and 10 cm deep) at four equally spaced locations along the long axis (north–south orientation) of each plot (i.e., eight cores per plot); cores collected within each plot were composited in the field. Each core was collected by removing the Oi horizon, and extracting a 10-cm-deep sample from the surface of the Oe horizon. Cores contained Oe, A, and E horizon material in all ecosystem types. We repeated this protocol in May, August, and October 1998 to gain insight into temporal changes in soil microbial community composition and substrate use. May samples were collected prior to canopy development and October samples were collected following leaf abcision. On each successive sampling date, soil cores were collected 2 m to the west of each previous sample location. Soil samples were placed in polyethylene bags, stored on ice, and were transported to our laboratory the following day. The sandy composite soil samples were homogenized by hand within each polyethylene bag prior to any laboratory analysis; sieving was unnecessary because these well-sorted, sandy soils contain <1% coarse fragments. Soil temperature for each stand was recorded on each sampling date using a hand-held digital thermometer. Measurements were made at a depth of 10 cm in each plot.

Microbial Nitrogen, Community Composition, and Substrate Use

Microbial N was determined using the $CHCl_3$ fumigation extraction method (Brooks et al., 1985). A 15-g subsample of

Table 1. Overstory and soil properties of three upland forest ecosystems located in northwestern Lower Michigan. (After Holmes and Zak, 1999; Zak et al., 1986, 1989; Zak and Pregitzer, 1990). Values are the means of three stands within each ecosystem.

	Black oak–white oak	Sugar maple–red oak	Sugar maple–basswood
Overstory			
Age, yr	86a†	79a	78a
Trees, ha^{-1}	864a	695a	791a
Overstory biomass, Mg ha^{-1}	151a	178a	209b
Overstory increment, Mg ha^{-1} yr^{-1}	2.2a	2.8ab	3.3b
Leaf litter, Mg ha^{-1} yr^{-1}	1.8a	3.2b	2.6b
Leaf litter N, kg N yr^{-1}	13.1a	30.4b	32.5b
Leaf litter C/N	133a	104b	80c
Mineral soil (0–3.8 cm)			
Texture, % coarse + medium sand	72a	56b	55b
Bulk density, Mg m^{-3}	0.69a	0.71a	0.70a
pH	3.9a	4.1a	5.6b
Organic C, g C kg^{-1}	44a	39a	55b
Total N, mg N kg^{-1}	1913a	1835a	3040b
C/N	23a	21b	18c
Gross N mineralization, ng g^{-1} d^{-1}‡	–	74a	91b
Microbial immobilization, ng g^{-1} d^{-1}‡	–	26a	20b
Net N mineralization, μg N g^{-1} yr^{-1}§	313a	382b	426b
Net nitrification, μg N g^{-1} yr^{-1}	18a	43a	364c

† Values followed by the same letter are not significantly different ($P \leq 0.05$).
‡ Determined by ^{15}N isotope dilution; see Holmes and Zak (1999) for details.
§ Determined by in situ field incubation; see Zak and Pregitzer (1990) for details.

homogenized soil was fumigated for 24 h with CH_3CH_2OH-free $CHCl_3$ in a vacuum desiccator. At the same time, another 15-g subsample was incubated in a moist desiccator with no $CHCl_3$. The fumigated soils were vacuumed and aerated eight times at the end of fumigation to remove excess $CHCl_3$. Fumigated and nonfumigated soil was extracted with 40 mL of 0.5 *M* K_2SO_4, and the extract was digested using concentrated H_2SO_4 and $CuSO_4$ as a catalyst. Total N in the extract was determined using automated colorimetry (Alpkem RFA 300, Alpkem, Clackmas, OR). Microbial biomass N was estimated by subtracting the amount of N in unfumigated samples from quantities in fumigated samples; this value was divided by a correction factor ($K_N = 0.68$) to estimate microbial N.

Microbial community composition was determined using phospholipid fatty acid (PLFA) analysis (Vestal and White, 1989). After thoroughly homogenizing the composite sample collected in each plot, we removed and freeze-dried a 2-g subsample for PLFA analysis. We used a single phase, phosphate buffered $CHCl_3$-CH_3OH solution to extract PLFAs from the freeze-dried soil (White et al., 1979). Phospholipid fatty acids were separated by silicic acid chromatography, and they were derivitized in an alkaline system to form fatty acid methyl esters (FAMEs; White et al., 1979). Fatty acid methyl esters were analyzed using a Hewlett-Packard 5890 Series 2 capillary gas chromatograph equipped with a 50-m non-polar column (0.2-mm i.d., 0.11-μm film thickness) and a flame ionization detection (Agilent, Sunnyvale, CA). Column temperature was held at 60°C for the first 2 min of the analysis; it was then increased to 150°C at 10°C min^{-1}, and subsequently increased to 312°C at 3°C min^{-1}. The injector and detector were maintained at 270 and 290°C, respectively. Preliminary peak identification was accomplished by comparing retention times with known standards.

We used a Hewlett-Packard 5890 series 2 GC interfaced to a Hewlett-Packard 5971 mass-selective detector to confirm peak identifies on a subset of samples; chromatographic conditions were identical to those described above. Mass spectra were determined by electron impact at 70 eV; methyl nonadecanonate (c19:0) was used as the internal standard. Double-bond position within monounsaturated FAMEs was determined by the GC-MS analysis of dimethyl disulfide adducts (Nichols et al., 1986). Mid-chain branching locations of FAMEs were determined by electron impact spectra (Apon and Nicolaides, 1975); a library of mass spectra for known FAMEs was used to identify all compounds (D.C. White, 1999, unpublished data).

Biolog GN microplates (Biolog Inc., Hayward, CA) were used to gain insight into difference in substrate use among microbial communities in the three forest ecosystems. A 10-g subsample of soil from each plot was placed in 100 mL of sterile saline. Garland (1996) demonstrated that differences in inoculum density can influence Biolog analysis. To avoid this possibility, samples from the sugar maple–basswood and the sugar maple–red oak ecosystems, which had a large microbial biomass, were diluted to 1:100, whereas samples from the black oak–white oak ecosystem, which had a smaller microbial biomass, were diluted to 1:10. The dilutions were used to inoculate Biolog GN microplates, which have 95 wells containing a buffered nutrient medium, a unique C source, and tetrazolium chloride. The microplates were scanned using an ELx800 automated microplate reader (Bio-Tek Instruments, Winooski, VT) at 595 nm; absorbance was determined immediately following inoculation and after 72 h of incubation at 25°C. Because many substrates on Biolog GN microplates are not found in forest soils, using a selected set of substrates is thought to give a more accurate representation of differences in substrate use (Haack et al., 1995; Insam, 1997; Hitzl et al., 1997). For our analysis, we used 26 substrates on Biolog GN plates that commonly occur as components of root exudates (Smith, 1976; Table 2). Campbell et al. (1997) demonstrated that these compounds can be used to detect differences in substrate use among microbial communities.

Table 2. Substrates on Biolog GN microplates found in root exudates (after Campbell et al., 1997 and Smith, 1976). We used these compounds to assess differences in substrate use among microbial communities beneath three floristically distinct forest ecosystems in northern Lower Michigan.

Carbohydrates	Carboxylic acids	Amino acids
α-D-glucose	acetic acid	γ-amino butyric acid
arabinose	α-hydroxy butyric acid	hydroxy L-proline
D-fructose	α-keto valeric acid	L-alanine
D-galactose	citric acid	L-alanyl-glycine
D-raffinose	malonic acid	L-asparagine
L-rhamnose	propionic acid	L-aspartic acid
maltose	succinic acid	L-leucine
sucrose		L-ornithine
		L-phenylalanine
		L-serine
		L-threonine

Statistical Analyses

A repeated-measures one-way nested analysis of variance (ANOVA) was used to investigate temporal patterns in microbial biomass; ecosystems and stands nested within ecosystem were fixed factors. For every Biolog microplate, the absorbance value of the control well was subtracted from the absorbance value of all other wells. Using these corrected values, we subtracted the initial absorbance values from absorbance after the 72-h incubation to calculate overall color development, a measure of substrate use. Principal component analysis of the Biolog substrates common in root exudates was performed on color development. If differences between stands are observed when the first two factors from the principal components analysis are plotted, those differences indicate differences in C utilization (Garland, 1996). Twenty-three PLFAs indicative of broad taxonomic groups of soil microorganisms were chosen a priori for our analysis of microbial community composition. We reasoned that they would provide clear indication of differences in microbial community composition among ecosystems. We used a repeated-measures one-way ANOVA to investigate whether there were significant differences in individual bacterial, actinomycetal, fungal, and protozoan PLFAs among sampling dates and ecosystems. We also used principal components analysis to investigate differences in PLFA composition among the three ecosystem types. Significance for all statistical analyses was accepted at $\alpha = 0.05$.

RESULTS

Microbial Nitrogen and Total Phospholipid Fatty Acid

In all three ecosystems, microbial N, an indicator of dead and living microbial biomass, was greatest in spring and steadily declined during summer and autumn (Fig. 2A). However, this temporal change in microbial N was not significant within an individual ecosystem (Fig. 2A), nor was it significant among stands nested within an individual ecosystem. When averaged across ecosystems and stands, there was a statistically significant difference in microbial N over time (time main effect), wherein mean microbial N in May (1.5 N g m^{-2}) was greater

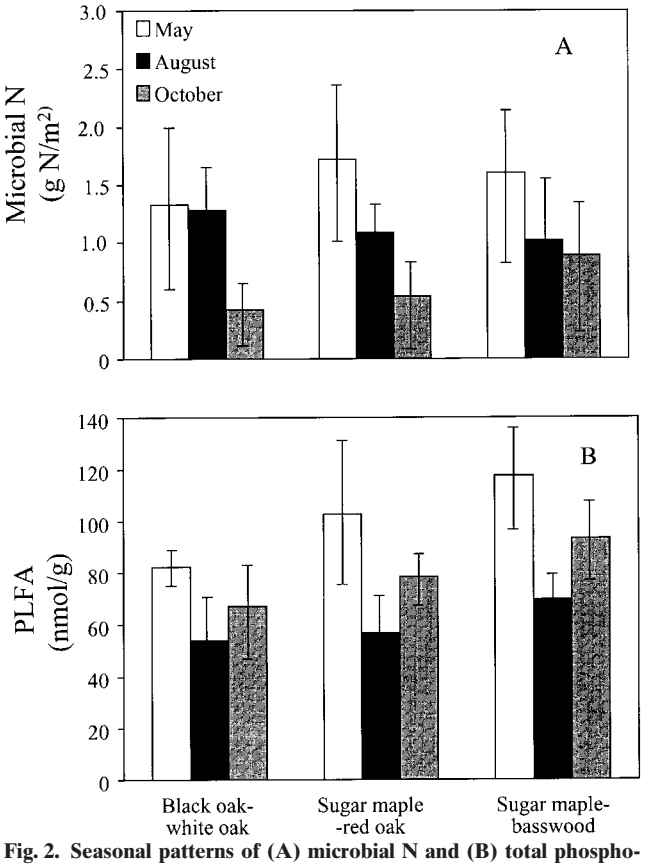

Fig. 2. Seasonal patterns of (A) microbial N and (B) total phospholipid fatty acid (PLFA) in three forest ecosystems in Manistee and Wexford Counties, northwestern Lower Michigan. Values given are ecosystem means; one standard error is one-half the length of each error bar.

than that measured in August (1.12 N g m^{-2}) or October (0.61 N g m^{-2}). Total PLFA is an indicator of living microbial biomass, and it varied throughout the growing season. Amounts were generally high in spring and fall, and low in midsummer (Fig. 2). However, total PLFA did not vary significantly over time within an individual ecosystem (Fig. 2B), nor did it differ among stands nested within an ecosystem. Averaged across ecosystems and stands, there was a significant difference in total PLFA among sampling dates; the mean amount of PLFA in May (101 nmol g^{-1}) was significantly greater than the mean amounts on the other sampling dates (Aug. = 60 nmol g^{-1}; Oct. = 79 nmol g^{-1}). Mean soil temperature varied from 10°C in May, to 18°C in August, to 9.2°C in October, a pattern not well reflected in seasonal patterns of microbial N or total PLFA. Soil temperature did not significantly differ among ecosystems, or among stands nested within individual ecosystems.

Microbial Community Composition

There were significant differences in mole fraction of most PLFAs extracted from the soil of each ecosystem (Fig. 3). Fungal (18:2ω6 and 18:1ω9c) PLFAs made up the largest portion of the microbial community in the black oak–white oak ecosystem, whereas the sugar maple–basswood soil contained a relatively higher proportion of nonspecific bacterial PLFAs (16:1ω7c, 16:1ω9c, and 16:1ω5c) than the oak ecosystem. The sugar maple–basswood ecosystem also had the largest amount of tuberculostearic acid (10Me18:0), which is indicative of soil actinomycetes. The sugar maple–red oak ecosystem had a microbial community that primarily was dominated by Gram-negative PLFAs, especially cy19:0.

The first two principal components for the May principal components analysis accounted for 69% of the total variance (Table 3). Fungal PLFAs (18:2ω6 and 18:1ω9c) and several nonspecific bacterial PLFAs (15:0, 18:0, and 16:1ω7t) received high positive weights on Principal Components Axis (PCA) 1. Actinomycetal (10Me18:0), Gram-negative (18:1ω7t and cy19:0), and a Gram-positive (10Me16:0) PLFAs received high negative weights on PCA 2 (Table 3). The first two PCAs accounted for 78% of the total variance in the August analysis. Phospholipid fatty acids that received high positive or negative weights on PCA 1 included those that are indicative of nonspecific bacteria (16:1ω5c, 16:1ω9c, and a17:0), Gram-positive bacteria (a15:0), and Gram-negative bacteria (16:1ω7c; Table 3). Gram-negative (10Me16:0,18:1ω7t) and nonspecific bacterial (15:0, 17:0, and 18:0) PLFAs received negative weights on PCA 2. For the October principal components analysis, the first two PCAs accounted for 77% of the total variance, with nonspecific bacterial and fungal PLFAs receiving high positive weights on PCA 1 (Table 3). Nonspecific bacterial, Gram-negative bacterial (cy19:0 and 18:1ω7t), actinomycetal, and fungal (20:1ω9c) PLFAs received high positive weights on PCA 2; negative weights were assigned to two bacterial PLFAs (Table 3).

Our ordination of stands by microbial PLFAs indicates that all three ecosystems differed in their microbial community composition throughout the growing season (Fig. 4). The difference in microbial communities among ecosystems mainly resulted from amounts of bacterial PLFAs. In our principal components analysis, nonspecific bacterial PLFAs, including 15:0, 18:0, a17:0, and 17:0, received high weights in the May, August, and October (Table 3). There was a seasonal shift in the microbial community composition in the sugar maple–basswood and black oak–white oak ecosystems between May and August (Fig. 4). In the sugar maple–basswood ecosystem, this seasonal shift in composition resulted from a change in the amount of Gram-negative bacterial (16:1ω7c), nonspecific bacterial (17:0 and 18:0), fungal (18:1ω9c), and protozoan (20:4ω6) PLFAs, whereas the seasonal shift in the black oak–white oak ecosystem occurred from changes in the amount of PLFAs that were representative of Gram-positive bacteria (a15:0), nonspecific bacteria (16:1ω9c and 16:1ω5c), fungi (18:2ω9c), and protozoan (20:4ω6) PLFAs.

Substrate Use

Microbial growth on substrates contained in root exudate differed among the three ecosystems (Fig. 5). The first two PCAs of the May analysis accounted for 67% of the total variance in our data. Carbohydrates and amino acids received high positive weights on PCA 1,

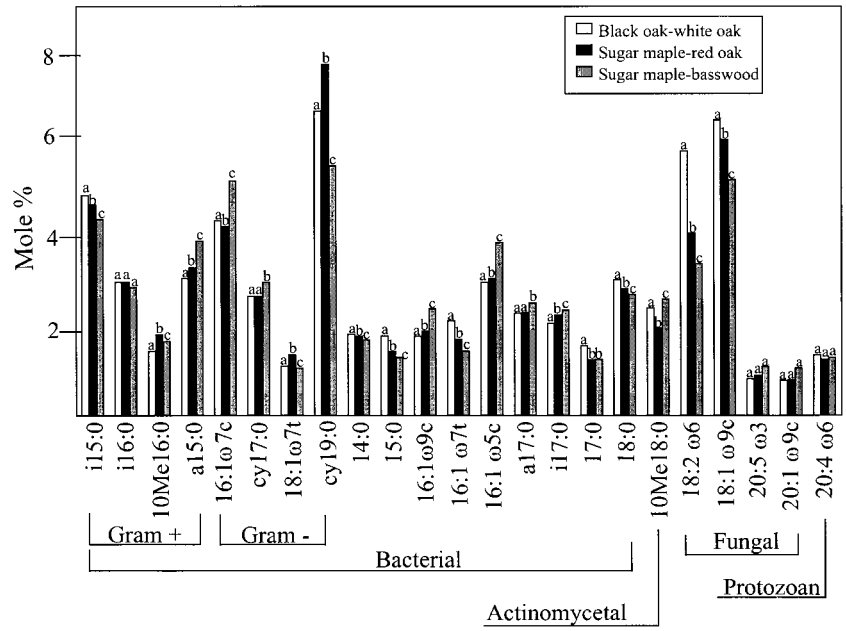

Fig. 3. The mole fraction of bacterial, actinomycetal, fungal, and protozoan phospholipid fatty acids in three upland forest ecosystems in northwestern Lower Michigan. Values given are ecosystem means averaged across sampling dates. For each fatty acid, ecosystem means that have the same letter are not significantly different at $\alpha = 0.05$.

whereas several carboxylic and amino acids received high positive weights on PCA 2 (Table 4). Weak negative weights (i.e., −0.25 to −0.50) were assigned to L-threonine and L-phenylalanine on PCA 1; fructose and L-aspartic acid received weak negative weights on PCA 2. PCA 1 and 2 of the August analysis accounted for 73% of the total variance; carbohydrates, carboxylic acids, and amino acids received high positive weights on PCA 1 (Table 4). Substrates receiving high positive weights on PCA 2 included amino (L-ornithine and L-alanyl glycine) and carboxylic acids (acetic acid and α-keto valeric acid). Only acetic acid received a negative weight on PCA 1, but L-leucine, maltose, and D-galactose all received weak negative weights on PCA 2. The first two PCAs of the October analysis accounted for 69% of the variance, with amino acids (L-threonine), carbohydrates (D-galactose and D-fructose), and carboxylic acids (α-keto valeric acid and α-hydroxy butyric acid) receiving high positive weights on PCA 1 (Table 4). Ornithine was given a negative weight on this PCA axis. Amino acids (γ-amino butyric acid, L-ornithine, and L-alanine) received high positive weights on PCA 2 (Table 4), whereas acetic and citric acids received weak negative weights.

Ordination of stands by substrate use demonstrated a clear separation of black oak–white oak stands from those in the two sugar maple–dominated ecosystems on each sampling date (Fig. 5). However, discrimination was minimal among stands in the two sugar maple–dominated ecosystems in May and August, but differences in substrate use separated stands of the two sugar maple–dominated ecosystems in October (Fig. 5). Com-

Table 3. Microbial PLFAs receiving large positive (>0.80) or negative (<−0.80) weights on the first and second principal components axis for the May, August, and October sampling dates.

	May		August		October	
	PCA 1	PCA 2	PCA 1	PCA 2	PCA 1	PCA 2
Positive weights						
Bacteria	14:0 15:0 i15:0 16:1ω7t 17:0 18:0		a15:0 16:1ω7c 16:1ω9c 16:1ω5c a17:0 cy17:0	17:0 15:0 18:0	15:0 i15:0 16:1ω9c 17:0 18:0	a15:0 16:1ω5c a17:0
Actinomycetes		10Me18:0				10Me18:0
Fungi	18:2ω6 18:1ω9c2		20:5ω3		18:2ω6 18:1ω9c2	20:1ω9c
Negative weights						
Bacteria	a15:0 16:1ω9c i17:0	10Me16:0 18:1ω7t cy19:0		10Me16:0 18:1ω7t	i15:0 10Me16:0	cy19:0 18:1ω7t
Actinomycetes						
Fungi			18:1ω9c			

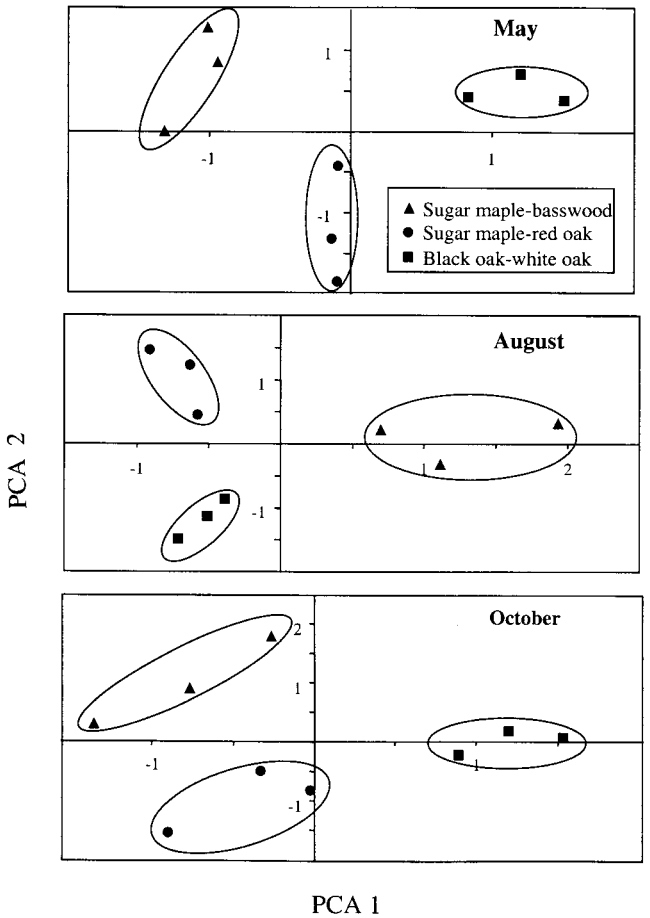

Fig. 4. Ordination of stands within each ecosystem type using microbial phospholipid fatty acids. Microbial community composition was distinct among ecosystems, evidence by the unique principal component space occupied by stands of each ecosystem type on the May, August, and October sampling dates.

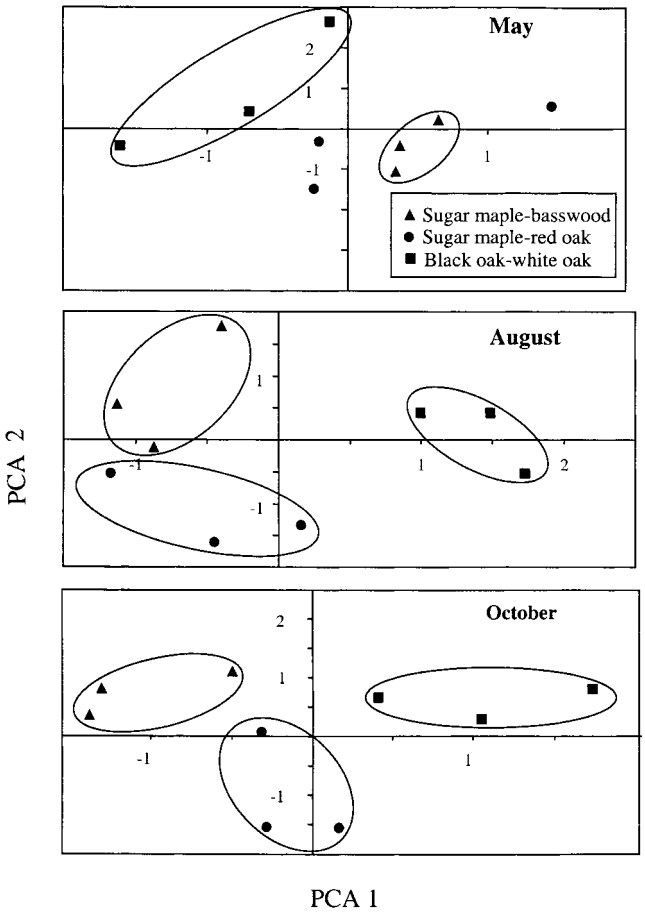

Fig. 5. Ordination of stands within each ecosystem type using root-derived substrates on Biolog GN plates. Substrate use was distinct between the sugar maple–dominated and black oak–white oak ecosystem in May and August. In October, stands within each ecosystem type occupy unique principal components axis (PCA) space, suggesting that microbial communities in each ecosystem differ in their ability to grow on simple substrates.

ponents of the microbial community in the sugar maple–basswood ecosystem consistently grew on L-ornithine and L-alanine (i.e., high absorbance values; data not shown), whereas the components of the microbial community in the sugar maple–red oak ecosystem grew to a greater extent on α-hydroxy butyric acid and maltose. Growth on α-keto valeric acid and L-threonine was consistently high in the black oak–white oak ecosystem, compared with the other two ecosystems. Moreover, we observed a seasonal shift in substrate use between the May and August sampling dates in the black oak–white oak and the sugar maple–basswood ecosystem (Fig. 5). The seasonal shift in the black oak–white oak ecosystem resulted from differences in microbial growth on carbohydrates (D-galactose, arabinose, L-rhamnose, D-fructose, and maltose), carboxylic (α-hydroxy butyric acid) and amino acids (L-phenylalanine), which received strong weights in the principal components analysis. Substrates that accounted for seasonal differences in substrate use in the sugar maple–basswood ecosystem (i.e., high absorbance and strong weighting on PCA axes) included carboxylic (malonic acid, succinic acid, and α-keto valeric acid) and amino acids (L-threonine and L-aspartic acid) and carbohydrates (maltose, D-raffinose, D-fructose, and arabinose).

DISCUSSION

Microbial metabolism in soil is limited by the availability and types of organic substrates, and it is plausible that ecosystems that differ floristically will produce litter with chemically distinct substrates that will differentially foster microbial growth. The three ecosystems we studied were floristically distinct (Host and Pregitzer, 1991), and they differ in litter quality (Table 1). Although we did not directly measure the chemical constituents of litter, several lines of evidence suggest that the ecosystems we studied substantially differ in the types of organic substrates contained in leaf litter. For example, leaf litter C/N varied from 80 in the sugar maple–basswood ecosystem to 133 in the black oak–white ecosystem (Table 1), implying that broad differences in litter chemistry exist among ecosystems that influence gross and net rates of soil N transformations. Furthermore, it is well established that sugar maple leaf litter decomposes more rapidly than oak leaf litter (McClaugherty et al., 1985; Aber et al., 1990), indicating that it contains a higher proportion of labile compounds that can be rapidly metabolized by soil microorganisms. The ecosys-

Table 4. Compounds found in root exudate that received large positive (>0.80) weights on the first and second principal components axis. In our analyses, no compound received large negative weights on any PCA.

	May		August		October	
	PCA 1	PCA 2	PCA 1	PCA 2	PCA 1	PCA 2
Positive weights						
Carbohydrates	L-rhamnose L-arabinose D-galactose D-fructose		D-galactose L-arabinose maltose sucrose		D-fructose	
Carboxylic acids	succinic acid	α-keto valeric acid α-hydroxy butyric acid	malonic acid citric acid	acetic acid α-keto valeric acid	D-galactose α-keto valeric acid α-hydroxy butyric acid	
Amino acids	L-alanine L-asparagine L-aspartic acid γ-amino butyric acid hydroxy L-proline L-ornithine	L-threonine L-phenylalanine	L-asparagine L-serine	L-ornithine L-alanyl glycine	L-threonine L-phenylalanine	γ-amino butyric acid L-ornithine L-alanine

tems we studied varied in the proportion of these overstory species, suggesting that floristic differences among ecosystems could foster compositional and functional differences in soil microbial communities. The consistent differences we observed in microbial community composition and substrate use supports this contention.

Microbial community composition varied among the three ecosystems as evidenced by PLFA analysis (Fig. 3 and 4). Soil bacteria are the primary decomposers of simple carbohydrates, organic acids, and amino acids, whereas soil fungi are the primary decomposers of recalcitrant compounds (Sylvia et al., 1998). The sugar maple–basswood and the sugar maple–red oak ecosystems, which had litter that probably contained large amounts of labile compounds, were found to have microbial communities dominated by bacterial PLFAs. In contrast, the black oak–white oak ecosystem, which probably contained litter with a larger percentage of more recalcitrant compounds, had a microbial community dominated by fungal PLFAs. Soil fungi are tolerant of low soil matric potentials (Paul and Clark, 1996), and the relatively coarse-textured, xeric soils of the oak ecosystem probably further contribute to the abundance of fungi in this ecosystem. The compositionally distinct microbial communities we observed among ecosystems support the idea that plant and microbial community composition are linked at a landscape-level scale.

Phospholipid fatty acid analysis demonstrated seasonal differences in microbial community composition in the sugar maple–basswood ecosystem (Fig. 4). Temporal change resulted from a shift from a large mole percentage of bacterial PLFA in May to a greater portion of fungal PLFA in August, a trend that reversed in October. In this ecosystem, total bacterial PLFA was greatest in the spring when warm soil temperatures probably allowed soil bacteria to metabolize labile substrates in leaf and root litter that entered soil the previous autumn. The seasonal decline in total bacterial PLFA may have arisen from a decline in substrates for which bacteria are effective competitors (i.e., carbohydrates, organic acids, amino acids) or through a midsummer decline in soil matric potential. There was an overall change in microbial biomass in the sugar maple–basswood ecosystem, wherein biomass was greatest in spring, declined in August, and then increased in October (Fig. 2B), albeit this trend was not statistically significant. These changes in microbial biomass help support the idea that there was a pulse of microbial growth in spring when warm soil temperatures allowed the microbial community to metabolize litter, a dieback in the summer as the labile components of the litter were depleted and matric potential became more negative, and a subsequent pulse of growth in October as new litter entered the soil.

Seasonal changes in microbial community composition also occurred in the black oak–white oak ecosystem (Fig. 4). The microbial community changed from fungal dominance in spring to a greater percentage of bacterial PLFAs in August and an increase in fungal PLFAs in October. One possible explanation for the high proportion of bacterial PLFAs in August may be related to a temporary increase in soil water content. Sampling in this ecosystem occurred following a rainstorm and an increase in soil water content could have caused a temporary increase in soil bacteria. Microbial biomass N was high in August in this ecosystem and was similar to the microbial biomass N in May, whereas total PLFA in August was twofold lower than in May (Fig. 2). Taken together, these data indicate a decline in the proportion of living microbial biomass in August and a shift to bacterial dominance.

Microbial community function differed among the three ecosystems, evidenced by different rates of soil N cycling (Zak and Pregitzer, 1990; Holmes and Zak, 1999) and different patterns of substrate use in the Biolog assay (Fig. 5). Dissimilarities in microbial substrate use among a range of soils that varied in soil water regime, elevation, and vegetation was documented by Zak et al. (1994). Campbell et al. (1997) used the same substrates in our study (Table 2) and found differences in their use by microbial communities among three distinct grassland ecosystems. This approach also has been used to discern differences in microbial communities exposed to a range of fertilizer additions, which probably altered the production and chemistry of plant litter (Fliebbach and Mader, 1997; Sharma et al., 1997). These studies demonstrate that variation in substrate input to the soil, due to differences in plant litter chemistry, alters the

function of soil microbial communities in manner that is detectable by the Biolog assay.

There was a seasonal shift in the microbial community function of the sugar maple–basswood and the black oak–white oak ecosystems, evidenced by changes in the patterns of substrate use (Fig. 5). Bossio and Scow (1995) found that microbial community function of a rice (*Oryza sativa* L.) agroecosystem changed with time as different types of substrates were incorporated into soil during residue management. Similarly, Buyer and Drinkwater (1997) observed a seasonal shift in microbial community function within three different agricultural systems, as well as broad differences among them, due to crop litter inputs and management history. It is possible that temporal changes in substrate input or abundance resulted in seasonal shifts in microbial community function within the sugar maple–basswood and the black oak–white oak ecosystems. It is not clear why in October there was a shift in microbial community composition in these two ecosystems, with no change in substrate use. Previous studies (Buyer and Drinkwater, 1997) were able to detect shifts in microbial community composition independent from any change in substrate use.

There were no detected seasonal changes in microbial community composition (Fig. 5) or function (Fig. 4) in the sugar maple–red oak ecosystem. It is possible that either our methods were not able to resolve seasonal changes in microbial community composition and substrate use in this ecosystem, or that the microbial community in this ecosystem remained relatively constant throughout our study. Although the microbial community in the sugar maple–red oak ecosystem did not have a detectable seasonal shift, it was clearly distinct in composition and partially distinct in substrate use from the other two ecosystems.

It could be argued that differences in the use of substrates contained on the Biolog GN plates provide relatively little insight into changes in soil microbial communities that would substantially alter the flow of energy or N through soil food webs. For example, substrates contained on the Biolog GN plates fall into three classes (i.e., carbohydrates, organic acids, and amino acids), all of which are rapidly metabolized upon entering soil. Therefore, it is unlikely that shifts in the use of carbohydrates, organic acids, or amino acids among microbial communities would reflect broad differences in physiology that would alter soil C or N cycling. Notwithstanding this criticism, the consistent differences in substrate use among the sugar maple–dominated and oak-dominated forests, as well as the seasonal shifts within them, suggest that the types of labile organic substrates used by soil microbial communities vary predictably in time and space. Understanding whether these patterns result from differences in substrate input, temperature, or matric potential cannot be determined from our study and requires further investigation.

The fact that two different methods, PLFA and Biolog analyses, allowed us to discriminate among the microbial communities of three ecosystems suggests that differences in microbial composition and function do indeed exist among them. It cannot be definitively determined whether these changes directly result from differences in litter chemistry among the ecosystems or due to differences in the physical environment. Because microbial growth is determined by the organic substrates in plant litter, it is likely that at least part of the differences in microbial community composition and substrate use between the three ecosystems resulted from differences in litter chemistry among ecosystems. The PLFA and Biolog analyses also indicate that a seasonal shift in microbial community composition and substrate use did occur in the sugar maple–basswood and the black oak–white oak ecosystems, but this pattern did not occur in the sugar maple–red oak ecosystem. These seasonal shifts in the microbial communities probably result from a variation in substrate availability, soil temperature, matric potential, or a combination of these factors. Because these factors covaried in our study, we cannot draw conclusions regarding their individual or combined influence on microbial community composition or substrate use. Nevertheless, our results suggest that compositionally and functionally distinct soil microbial communities exist in ecosystems that differ in plant community composition. Moreover, these relationships were consistent across a large land area in northern Lower Michigan, suggesting that landscape-level patterns of plant community composition give rise to landscape-level patterns of microbial community composition and function.

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