Compositional and Functional Shifts in Microbial Communities Due to Soil Warming

Gregory P. Zogg,* Donald R. Zak, David B. Ringelberg, Neil W. MacDonald, Kurt S. Pregitzer, and David C. White

ABSTRACT

Microbial decomposition processes are typically described using first-order kinetics, and the effect of elevated temperature is modeled as an increase in the rate constant. However, there is experimental data to suggest that temperature increases the pool size of substrate C available for microbial respiration with little effect on first-order rate constants. We reasoned that changes in soil temperature alter the composition of microbial communities, wherein dominant populations at higher temperatures have the ability to metabolize substrates that are not used by members of the microbial community at lower temperatures. To gain insight into changes in microbial community composition and function following soil warming, we used molecular techniques of phospholipid fatty acid (PLFA) and lipopolysaccharide fatty acid (LPS-OHFA) analysis and compared the kinetics of microbial respiration for soils incubated from 5 to 25°C. Substrate pools for microbial respiration and the abundance of PLFA and LPS-OHFA biomarkers for Gram-positive and Gram-negative bacteria differed significantly among temperature treatments, providing evidence for a shift in the function and composition of microbial communities related to soil warming. We suggest that shifts in microbial community composition following either large seasonal variation in soil temperature or smaller annual increases associated with global climate change have the potential to alter patterns of soil organic matter decomposition by a mechanism that is not considered by current simulation models.

ON A GLOBAL BASIS, terrestrial ecosystems are thought to be important sinks for increasing atmospheric CO_2 due to enhanced C fixation by plants (Tans et al., 1990). However, identifying the extent to which terrestrial ecosystems will function as a possible net C source or sink in response to climatic change also requires knowledge of microbial processes. Terrestrial ecosystems may be significant sources of CO_2 if elevated temperatures associated with climatic change accelerate the microbially mediated decomposition of soil organic matter (Jenkinson et al., 1991). Given that the pool of C stored in soils is twice as large as that found in the atmosphere (Post et al., 1982), and the potential for positive feedback between climatic warming and the flux of CO_2 from soils (Raich and Schlesinger, 1992), understanding the mechanisms controlling microbial respiration is critical to efforts to model C cycling at regional and global scales.

The kinetics of microbial decomposition processes can be described using a first-order rate equation (Stanford and Smith, 1972; Ellert and Bettany, 1988; Zak et al., 1993; MacDonald et al., 1995). The effect of elevated temperatures is typically modeled as an increase in the first-order rate constant (e.g., Stanford et al., 1973; Campbell et al., 1984), whereas the pool of labile substrate metabolized by soil microorganisms is assumed to be unaffected by temperature (Fig. 1a). However, MacDonald et al. (1995) have observed that the temperature dependence of microbial respiration is more complex than is commonly assumed and probably involves differential access to substrate pools with an increase in temperature, with little change in the first-order rate constant (Fig. 1b). One plausible mechanism for this response is a temperature-induced shift in microbial community composition, wherein dominant populations at higher temperatures have the ability to metabolize substrates that are not utilized by members of the microbial community at lower temperatures.

Although it is difficult to adequately characterize microbial community composition and diversity, molecular techniques of PLFA and LPS-OHFA analyses have been recently applied to soils and show considerable promise for differentiating among related groups of microorganisms (e.g., Zelles et al., 1992; Zak et al., 1996). In brief, the lipid content of cell membranes is extracted and the recovered PLFAs and LPS-OHFAs are used as biomarkers for soil microorganisms (Vestal and White, 1989; Tunlid and White, 1992; Zelles et al., 1995). To examine the effects of temperature on the composition and function of microbial communities, we used these molecular techniques in conjunction with an examination of the kinetics of microbial respiration. We hypothesized that changes in soil temperature would alter microbial community composition in forest soils, resulting in concomitant changes in microbial respiration (i.e., microbial function).

METHODS

Study Site

Our study was conducted in a sugar maple (*Acer saccharum* Marsh.) dominated northern hardwood ecosystem in western Lower Michigan. The soil is classified as a sandy, mixed, frigid Typic Haplorthod. Silt plus clay comprised 10.6% of the total mineral fraction, and organic C content was 1.9% (Randlett et al., 1992). Additional soil and vegetation characteristics for the stand have been previously reported elsewhere (Site 4a in MacDonald et al., 1991, 1992). In September 1993, we sampled the surface soil, including Oa, A, and upper E horizons, to a depth of 7 cm using polyvinyl chloride core samplers (5.4-cm i.d.). Six soil cores were collected from a randomly selected 0.5-m^2 area on the perimeter of each of three 30 by 30 m plots, for a total of 18 soil samples.

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Abbreviations: PLFA, phospholipid fatty acid; LPS-OHFA, lipopolysaccharide fatty acid; ANOVA, analysis of variance; PCA, principal component analysis.

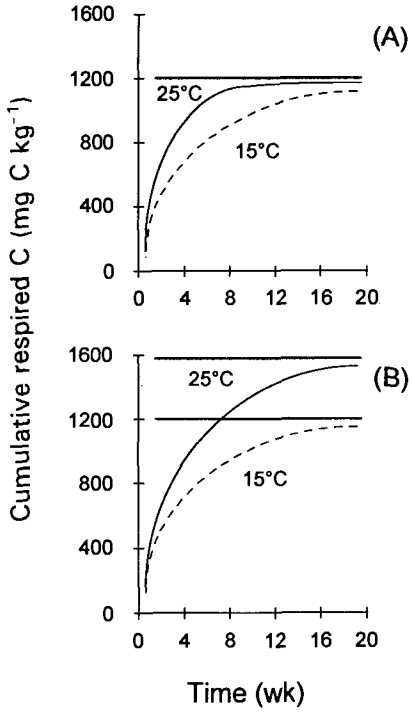

Fig. 1. Temperature effects on a first-order kinetic model of microbial respiration: $Y = A_0 (1 - e^{-kt})$, where Y is the cumulative respired C (mg C kg^{-1}) produced during time t (wk), A_0 is the amount of substrate C (mg C kg^{-1}) present at the start of the experiment, and k is the rate constant k (wk^{-1}): (A) Temperature-dependent changes in the rate constant k are reflected in a change in the rate at which the amount of cumulative respired C with time approaches some finite value, the pool size of readily mineralizable C (A_0, bold line). For example, these hypothetical data depict how an increase in soil temperature from 15 to 25°C leads to an approximate doubling of the rate constant ($k_{15°C}$ = 0.18, $k_{25°C}$ = 0.36 wk^{-1}), resulting in a more rapid depletion of what is assumed to be a relatively static pool of substrate C (A_0 = 1200 mg C kg^{-1}) available for microbial respiration. (B) Alternatively, temperature could have little effect on the rate constant ($k_{15°C} = k_{25°C}$ = 0.18 wk^{-1}) but might result in an increase in the pool size of substrate C respired ($A_{0\,15°C}$ = 1200, $A_{0\,25°C}$ = 1600 mg C kg^{-1}) due to some temperature-dependent constraint on microbial utilization of substrates (see MacDonald et al. [1995] for a more complete discussion of these two alternative scenarios).

Community Function

We quantified the kinetics of microbial respiration to gain insight into changes in microbial community function associated with soil warming. Two cores from each plot were incubated at 5, 15, or 25°C for 16 wk in air-tight, plastic incubation units (Zak et al., 1993, after Nadelhoffer, 1990). Although the 10 to 20°C differences between our incubation treatments were several orders of magnitude greater than predicted annual rises in global temperatures, they represent a normal seasonal range of surface soil temperatures at our study site. Carbon dioxide production for each incubation unit was measured at 1- or 2-wk intervals using gas chromatography (Zak et al., 1993). Following each CO_2 measurement, the soil was rinsed with 50 mL of $CaCl_2$ followed by 50 mL of a nutrient solution [0.002 *M* $CaCl_2$, 0.002 *M* $MgCl_2$, 0.005 *M* KCl, 0.005 *M* $Ca(H_2PO_4)_2$], and brought to approximate field capacity (−0.05 MPa) with a vacuum pump. Incubation units were subsequently flushed with about five headspace volumes of CO_2-free air and resealed.

Carbon dioxide production by soil microbes can be described using a first-order kinetic model (Zak et al., 1993, after Stanford and Smith, 1972):

$$Y = A_0 (1 - e^{-kt}) \quad [1]$$

where Y is the cumulative respired C (mg C kg^{-1}) produced at time t (wk). However, Ellert and Bettany (1988) have argued that modeling microbial decomposition processes using this basic, first-order rate equation is problematic, given that measures of Y are not independent. Therefore, any errors associated with the measurement of CO_2 production during a given time step will be magnified, recurring in all subsequent data points in the model (Eq. [1]) because CO_2 production is cumulatively summed.

We used an alternate, incremental model that eliminates this problem (after Ellert and Bettany, 1988, p. 1701):

$$Y_{it} = A_0 e^{-kt} (e^{ki} - 1) \quad [2]$$

where Y_{it} is the incremental amount of CO_2 respired (mg C kg^{-1}) during an interval of length i (wk) preceding time t (wk). Nonlinear least squares regression (Wilkinson, 1990) was used to simultaneously estimate the parameters A_0, the amount of substrate C (mg C kg^{-1}) present at the start of the experiment, and the rate constant k (wk^{-1}) for microbial respiration. Kinetic parameters were estimated for each individual incubation unit. For purposes of comparison, product accumulation curves were developed using the basic first-order rate equation (Eq. [1]), but with parameter estimates (A_0, k) provided by the incremental model (Eq. [2]).

Community Composition

We used molecular techniques of lipid analysis at the termination of the 16-wk incubation period to gain insight into the composition of the soil microbial communities. Fatty acids in freeze-dried soil from each incubation unit were extracted using a single-phase, phosphate-buffered $CHCl_3$–CH_3OH solvent system (White et al., 1979). Water and chloroform were added to separate aqueous and organic phases that contained LPS-OHFAs and PLFAs, respectively (Ringelberg et al., 1994). The LPS-OHFAs in the aqueous residue were recovered after acid hydrolysis and esterification (Mayberry and Lane, 1993). The PLFAs in the organic phase were fractionated using silicic acid column chromatography and derivitized in a mild alkaline system to form fatty acid methyl esters (White et al., 1979). The identification and abundance of individual PLFAs and LPS-OHFAs were determined using a coupled gas chromatograph–mass spectrometer (Ringelberg et al., 1994).

Fatty acid nomenclature is as follows: The number before the colon identifies the number of C atoms in the fatty acid (i.e., the C chain length). The number after the colon designates the number of C=C double bonds and their location relative to the methyl end (ω) of the molecule. For example, 18:2ω6 is an 18-C fatty acid with two double bonds beginning at the sixth C from the methyl end. The letters *c* and *t* indicate the *cis* and *trans* isomers of the double bond. Methyl branching in the first (*iso*) or second (*antesio*) C (relative to the ω end of the molecule) are designated by *i* or *a*, respectively; branches in other positions are simply identified by a number indicating the position of the methyl branch relative to the carboxylic end of the molecule. For example, *i*16:0 has a methyl branch on the first C from the ω end and 10Me16:0 has a methyl branch on the 10th C from the carboxylic end. The prefix *cy* denotes a cyclopropane fatty acid. The presence and location of hydroxyl groups in LPS-OHFAs is designated by a prefix that indicates the position of the hydroxyl group relative to the carboxyl end of the fatty acid. For example, 3OH14:0 is

a 14-C LPS-OHFA with the hydroxyl group on the third C from the carboxyl end.

Individual PLFAs and LPS-OHFAs are found in a wide variety of microorganisms. However, lipid profiles can provide insight into microbial community composition because the relative abundance of certain PLFAs and LPS-OHFAs differs considerably among specific groups of microorganisms. For example, although monoenoic PLFAs can occur in both Gram-negative and Gram-positive bacteria, their relative contribution to total PLFA content in Gram-positive bacteria is typically very small (e.g., <20%); thus monoenoic PLFAs can be used as general biomarkers for Gram-negative bacteria (Ratledge and Wilkinson, 1988). Cyclopropane PLFAs also are common in Gram-negative bacteria (Wilkinson, 1988). Branched, saturated PLFAs are abundant in Gram-positive bacteria (O'Leary and Wilkinson, 1988). Polyenoic, unsaturated PLFAs indicate the presence of eukaryotic organisms such as fungi (Federle, 1986). Finally, LPS-OHFAs are found primarily in the outer membranes characteristic of Gram-negative bacteria (Wilkinson, 1988). We used these relationships to determine changes in microbial community composition within the range of incubation temperatures.

Statistical Analyses

Differences in the kinetic parameters (A_0, k) for microbial respiration (i.e., microbial function) among temperature treatments were determined using one-way ANOVA and Fisher's least significant difference procedure. We compared overall patterns of community composition among temperature treatments using PCA of the mole fraction (percentage) of the most abundant PLFAs; any PLFAs comprising <1% of the total mole percent were dropped from the analyses. Differences in the relative proportion of Gram-positive bacteria, Gram-negative bacteria, and fungi among incubation temperatures were determined by one-way ANOVAs of the mole fraction of select PLFA biomarkers. Similarly, temperature-induced changes within the Gram-negative bacterial community were determined by one-way ANOVAs of the mole fraction of select LPS-OHFA biomarkers. Differences in total PLFA content (pmol kg^{-1}) among temperature treatments were also determined using a one-way ANOVA. Values reported are the mean of six samples (two per plot) at each temperature. Significance for all statistical tests was accepted at $\alpha = 0.05$.

RESULTS

Community Function

Microbial respiration increased dramatically with soil warming due to an apparent increase in the pool size of C metabolized by soil microbes at higher temperatures (Fig. 2). Simultaneous estimation of A_0 and k revealed a threefold increase in the substrate pools for microbial respiration (A_0) from 5 to 25°C; k decreased by 10% but this decline was not significant (Table 1). Goodness-of-fit values (r^2) for individual incubation units ranged from 0.82–0.93.

Community Composition

Monoenoic and cyclopropane unsaturated PLFAs, characteristic of Gram-negative bacteria (Wilkinson, 1988), comprised approximately 40.0% of total PLFAs in all three temperature treatments (data not shown).

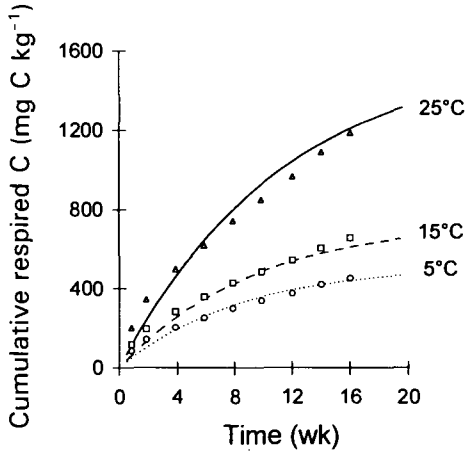

Fig. 2. Cumulative respired C for soils incubated at 5, 15, and 25°C. Symbols are mean values for six samples at each temperature; lines are product accumulation curves developed using the basic first-order rate equation (Eq. [1]), but with parameter estimates provided by the incremental model (Eq. [2]). Note how the data do not approach a single asymptote, indicating a temperature-dependent change in the pool size of respirable C (compare with Fig. 1).

Branched, saturated PLFAs, characteristic of Gram-positive bacteria (O'Leary and Wilkinson, 1988), comprised 18.5% of total PLFAs. Polyenoic unsaturated PLFAs (17.7% of total) indicated the presence of eukaryotic organisms that we assume to be fungi (Federle, 1986). In general, PLFA profiles had decreasing unsaturation, greater chain length, and larger numbers of cyclopropyl fatty acids at higher temperatures. Total PLFA content declined significantly with increasing soil temperature: PLFA content was 95 pmol kg^{-1} at 5°C, 61 pmol kg^{-1} at 15°C, and 44 pmol kg^{-1} at 25°C.

Principal components analysis of the 30 most abundant PLFAs revealed clear differentiation among the temperature treatments (Fig. 3). The soils incubated at 25°C were particularly well separated from the rest of the samples, although there was also good discrimination between the soils incubated at 15 and 5°C (Fig. 3). Monoenoic, unsaturated PLFAs and several saturated PLFAs were heavily weighted along the first PCA axis (16:1ω5*c*, 16:1ω7*c*, 16:1ω9*c*/*a*16:0, *i*19:0, 22:0, and 22:1). Saturated PLFAs were heavily weighted along the second PCA axis (*i*15:0, 16:0, *i*16:0, a17:0, *i*17:0, and 18:0).

We found significant differences among temperature treatments in the mole fraction of select PLFAs and LPS-OHFAs. Some general PLFA biomarkers (*i*17:0

Table 1. Substrate C pools (A_0) and first-order rate constants (k) for microbial respiration for soils incubated at 5, 15, and 25°C. Values are the means ($n = 6$), with standard errors in parentheses.

Incubation temperature	A_0	k
°C	mg C kg^{-1}	wk^{-1}
5	514 a† (55)	0.120 a (0.011)
15	742 b (109)	0.107 a (0.011)
25	1607 c (304)	0.087 a (0.015)

† Means with the same letter in a column are not significantly different ($P < 0.05$) as determined by Fisher's least significant difference test.

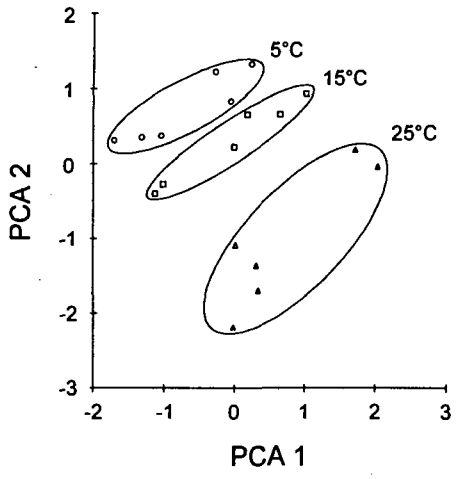

Fig. 3. Principal components analysis (PCA) of the mole percentage distribution of 30 phospholipid fatty acids (PLFAs) (in 18 soil samples) after 16 wk of incubation at 5, 15, or 25°C. Variance explained by PCA axis 1 (PCA1) and axis 2 (PCA2) were 32.9 and 26.1%, respectively.

and cy19:0/19:1ω6*c*) for Gram-positive and Gram-negative bacteria (Wilkinson, 1988; O'Leary and Wilkinson, 1988) increased with temperature, whereas others (16:1ω5*c*, 16:1ω7*t*, 16:1ω9*c*/*a*16:0, 18:1ω5*c*, 16:1ω7*c*, and 18:1ω7*c*) decreased (Fig. 4). Certain LPS–OHFAs (3,2OH14:0, 3OH16:0, and 3OH14:0), characteristic of Gram-negative bacteria (Wilkinson, 1988), also differed significantly among temperature treatments (Fig. 5).

DISCUSSION

The changes in membrane lipid profiles and kinetic parameters for microbial respiration that we observed, taken together, provide some evidence for a shift in microbial community composition and function related to soil warming. Our results indicate that an increase in soil temperature led to an apparent increase in the pool size of C respired by soil microbes, with little or no effect on the first-order rate constant. This result is in dramatic contrast to what one might predict given previous studies of other decomposition processes (e.g., N mineralization) in which rate constants consistently varied with temperature (Stanford et al., 1973; Campbell et al., 1984). However, one problem with the analyses conducted in these prior studies, discussed recently by MacDonald et al. (1995), is that they assume, a priori, that substrate pool size remains constant. With substrate pool size in the first-order kinetic model fixed, estimation procedures will by default yield temperature-dependent changes in the rate constant (MacDonald et al., 1995).

Our approach to modeling the kinetics of microbial respiration, which involves simultaneous estimates of both substrate pool size and rate constant, assumes nothing about the initial state or temperature dependence of either parameter. Thus, our calculations showed that temperature had a greater effect on apparent substrate pool size than on the rate constant. However, our approach is subject to some of the same inherent limitations of prior studies that use kinetic models; i.e., substrate

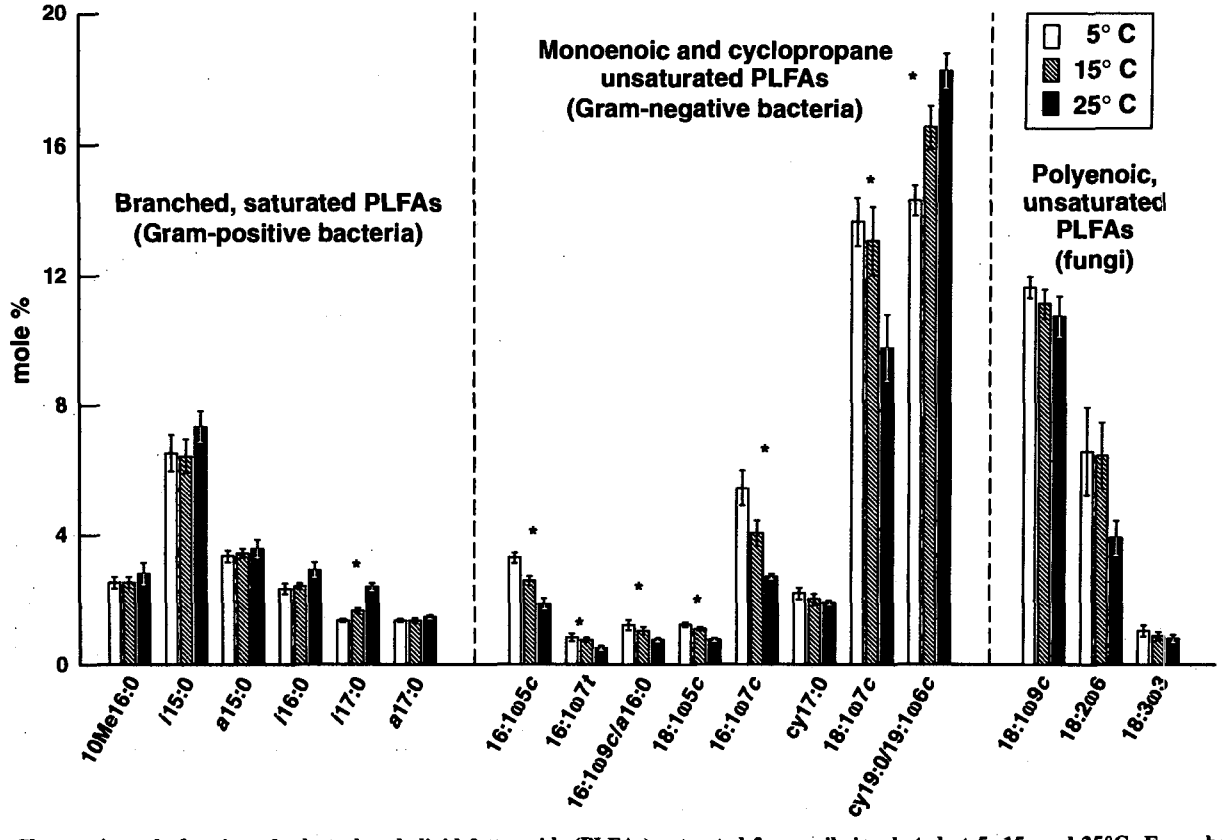

Fig. 4. Changes in mole fraction of select phospholipid fatty acids (PLFAs) extracted from soils incubated at 5, 15, and 25°C. Error bars are one standard error of the mean of six samples. An asterisk (*) denotes a significant ($P < 0.05$) temperature effect on PLFA abundance, as determined by a one-way analysis of variance.

pool size is not quantified directly, but rather inferred from microbial respiration based on the assumption that a given kinetics model (first-order kinetics in this case) adequately describes the decomposition process. Nonetheless, we suggest that our results provide some evidence for a temperature-dependent constraint on microbial utilization of substrate C. If temperature had simply increased the metabolic activity of soil microorganisms and substrate pools did not change, then one would expect to see the type of response shown in Fig. 1a. Clearly our data (Fig. 2) do not resemble such a response. MacDonald et al. (1995), using the same estimation procedure, reported a similar pattern in kinetic parameters for microbial respiration in several northern hardwood forests, including our study site.

One plausible explanation for the apparent increase in substrate pool size at higher temperatures is a shift in microbial community composition associated with soil warming. It is widely recognized that certain groups of soil microorganisms are well adapted to particular temperature regimes. Furthermore, soil microbes can vary considerably in their affinity for different substrates. If temperature elicits changes in community composition such that dominant populations favored at higher temperatures have the ability to metabolize substrates that are not used by members of the microbial community at lower temperatures, then the substrate pool size for microbial respiration could potentially increase.

Alternately, soil warming might lead to an apparent increase in the pool of substrate C metabolized by microbes by some other mechanism such as a temperature-dependent change in growth efficiency or diffusional processes (Ellert and Bettany, 1988; MacDonald et al., 1995), without a change in community composition. However, we are unaware of experimental evidence demonstrating that the growth efficiency of specific microorganisms varies along a temperature gradient. Furthermore, it seems likely that temperature-induced changes in diffusional processes would only increase the rate at which a given pool of substrate is utilized (i.e., reflected in larger rate constants), but would have little or no effect on the types or amount of substrate metabolized (i.e., total pool size). Nonetheless, we cannot rule out either of these possibilities, because we did not evaluate growth efficiencies or substrate use patterns for individual soil microbes.

We did find that changes in microbial respiration, which can be viewed as a measure of community function, were associated with concomitant changes in PLFA profiles. Principal components analysis revealed considerable differences among temperature treatments in total PLFA composition (Fig. 3). These results, in combination with the differences we observed among temperature treatments in the relative abundance of specific PLFA biomarkers (Fig. 4), provide some evidence for a shift in microbial community composition with changes in soil warming. However, microorganisms (e.g., pure cultures of bacteria) have some ability to adjust the PLFA

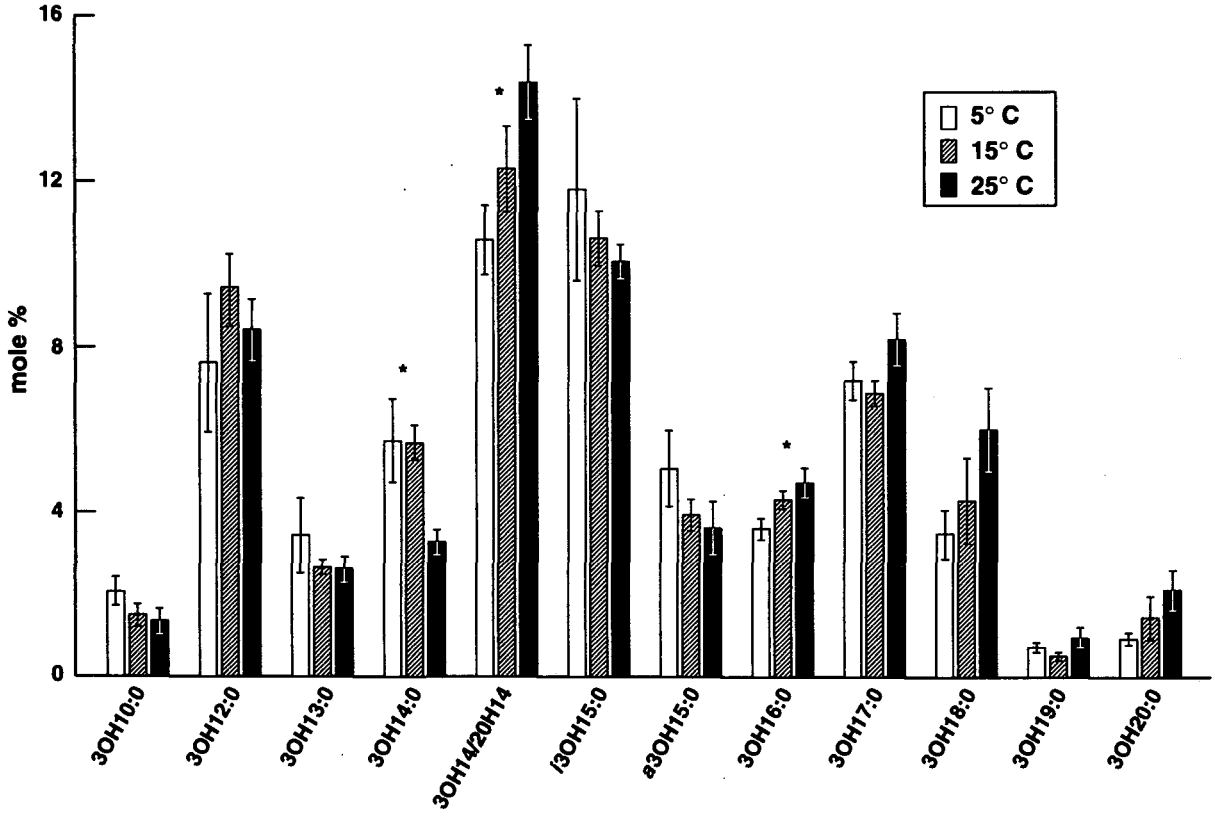

Fig. 5. Temperature effects on the mole fraction of select lipopolysaccharide fatty acids (LPS-OHFAs), characteristic of Gram-negative bacteria, derived from soils incubated at 5, 15, and 25°C. Error bars are one standard error of the mean of six samples. An asterisk (*) denotes a significant ($P < 0.05$) temperature effect on LPS-OHFA abundance, as determined by a one-way analysis of variance.

content of cellular membranes in response to environmental changes in temperature (Rose, 1989). If the changes in PLFA composition we observed resulted from thermoadaptation without a change in community composition, then the proportions of certain PLFAs and their molecular precursors should change predictably with temperature. For example, cy17:0 is synthesized from 16:1ω7*c*, and an increase in the former should be reflected in a decrease in the latter. We found no significant change in the mole fraction of cy17:0 ($P > 0.390$), despite a significant decrease in 16:1ω7*c* ($P < 0.001$), suggesting that the changes in PLFA content were not solely the result of a thermal response in membrane function.

Differences in the relative abundance of select LPS-OHFA, characteristic of the Gram-negative bacteria, provide further evidence for a temperature-induced shift in community composition (Fig. 5). It is unlikely that Gram-negative bacteria adjust LPS-OHFA composition in response to temperature, since the role of LPS-OHFAs are to provide structural integrity to microbial cells and to protect the inner membrane and intracellular components (Russell, 1989). Nonetheless, there is limited evidence that temperature influences LPS-OHFA profiles in several bacterial strains (Kropinski et al., 1987), and some of the changes we observed in LPS-OHFA composition may have been due to thermoadaptation.

Interestingly, the largest pool of C respired in our experiment (at 25°C) was metabolized by the smallest active microbial biomass. We found that total PLFA content, which reflects active microbial biomass (Vestal and White, 1989; Tunlid and White, 1992), declined from 95 pmol kg^{-1} at 5°C to 44 pmol kg^{-1} at 25°C. This decline in biomass probably resulted from greater metabolic stress at higher temperatures and is consistent with the changes we observed in PLFA profiles. Changes in lipid composition, particularly decreases in unsaturation, typically occur only as a result of biosynthesis of new lipids; since biosynthesis is energetically expensive it is probable that portions of the microbial community will experience metabolic stress and death as temperature increases (Petersen and Klug, 1994). If the dead microbial cells were simply replaced by members of the same populations without a change in community composition, then one would expect elevated temperatures to increase metabolic rates, leading to a larger rate constant for microbial respiration. However, our data indicate that temperature influenced the amount of substrate for microbial respiration to a greater extent than it influenced the first-order rate constant.

Furthermore, the increase in substrate C pools at higher temperatures is not due to the decomposition of the dead microbial biomass alone. Because PLFA content and microbial biomass C are highly correlated, we estimate that microbial biomass decreased from approximately 1095 mg C kg^{-1} at 5°C to 473 mg C kg^{-1} at 25°C (microbial biomass = PLFA × 0.012 − 64.125, $r^2 = 0.974$, $P < 0.01$; data from Zelles et al., 1992). Growth efficiencies for bacteria and fungi, the dominant organisms in our soils, can range from 20 to 75% (Chalal and Wagner, 1965; Hernandez and Johnson, 1967; Harley, 1971; Griffin, 1972; Behera and Wagner, 1974). Even if we assume a very low, uniform growth efficiency of 20% for the entire microbial community (i.e., 80% conversion of dead microbial cells into CO_2), the CO_2 produced by the metabolism of the dead biomass (497 mg CO_2–C kg^{-1}) can only account for 50% of the apparent increase in the pool of substrate C from 5 to 25°C (1091 mg C kg^{-1}). The fact that the lowest biomass utilized the largest substrate pool and produced the greatest amount of respired C further indicates that some significant changes in patterns of substrate utilization occur at higher temperatures.

We suggest that the changes in lipid profiles and apparent substrate pools for microbial respiration that we observed with an increase in temperature provide some evidence for a shift in microbial community composition and function related to soil warming. At a minimum, our results underscore some fundamental gaps in our understanding of temperature effects on microbial communities, which could have important implications for the development of predictive models of soil C flux under various climate change scenarios. Temperature effects on soil organic matter turnover are typically modeled as an increase in the rate constants of decomposition (Jenkinson, 1990; Parton et al., 1987). However, results from this study and others (MacDonald et al., 1995; Ellert and Bettany, 1992) indicate that decomposition kinetics are more complex and probably involve differential utilization of substrate pools with a change in temperature.

It appears that shifts in microbial community composition associated with soil warming have the potential to significantly alter the kinetics of microbial respiration. This may have important implications for predicting the flux of CO_2 from soil because such a mechanism is not considered in current simulation models. Additional research on the effects of temperature on patterns of substrate utilization among microbial populations and communities is clearly necessary in order to confirm our conclusions and to better understand the role of microbial respiration in soil C flux under future climate warming scenarios.

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REFERENCES

Behera, B., and G.H. Wagner. 1974. Microbial growth rates in glucose amended soils. Soil Sci. Soc. Am. Proc. 38:591-594.

Campbell, C.A., Y.W. Jame, and G.E. Winkleman. 1984. Mineralization rate constants and their use for estimating nitrogen mineralization in some Candian prairie soils. Can. J. Soil Sci. 64:333-343.

Chalal, K.S., and G.H. Wagner. 1965. Decomposition of organic

matter in Sanborn field soils amended with glucose. Soil Sci. 52: 1692–1702.

Ellert, B.H., and J.R. Bettany. 1988. Temperature dependence of net sulfur and nitrogen mineralization. Soil Sci. Soc. Am. J. 52: 1692–1702.

Ellert, B.H., and J.R. Bettany. 1992. Comparison of kinetic models for describing net sulfur and nitrogen mineralization. Soil Sci. Soc. Am. J. 56:1133–1141.

Federle, T.W. 1986. Microbial distribution in soil – New techniques. p. 493–498. *In* F. Megusar and M. Gantar (ed.) Perspectives in microbial ecology. Slovene Soc. for Microbiol., Ljublijana.

Griffin, D.M. 1972. Ecology of soil fungi. Syracuse Univ. Press, Syracuse, NY.

Harley, J.L. 1971. Fungi in ecosystems. J. Anim. Ecol. 41:1–16.

Hernandez, E., and M.J. Johnson. 1967. Energy supply and cell yield in aerobically grown microorganisms. J. Bacteriol. 94:996–1001.

Jenkinson, D.S. 1990. The turnover of organic carbon and nitrogen in soil. Phil. Trans. R. Soc. Lond. B 329:361–368.

Jenkinson, D.S., D.E. Adams, and A. Wild. 1991. Model estimates of CO_2 emissions from soil in response to global warming. Nature (London) 351:304–306.

Kropinski, A.M.B., V. Lewis, and D. Berry. 1987. Effect of growth temperature on the lipids, outer membrane proteins, and lipopolysaccharides of *Pseudomona aeruginosa* PAO. J. Bacteriol. 169: 1960–1966.

MacDonald, N.W., A.J. Burton, M.F. Jurgensen, J.W. McLaughlin, and G.D. Mroz. 1991. Variation in forest soil properties along a Great Lakes air pollution gradient. Soil Sci. Soc. Am. J. 55:1709–1715.

MacDonald, N.W., A.J. Burton, H.O. Liechty, J.A. Witter, K.S. Pregitzer, G.D. Mroz, and D.D. Richter. 1992. Ion leaching in forest ecosystems along a Great Lakes air pollution gradient. J. Environ. Qual. 21:614–623.

MacDonald, N.W., D.R. Zak, and K.S. Pregitzer. 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. Soil Sci. Soc. Am. J. 59:233–240.

Mayberry, W.R., and J.R. Lane. 1993. Sequential alkaline saponification/acid hydrolysis/esterification: A one-tube method with enhanced recovery of both cyclopropane and hydroxylated fatty acids. J. Microbiol. Methods 18:21–32.

Nadelhoffer, K.J. 1990. Microlysimeter for measuring nitrogen mineralization and microbial respiration in aerobic soil incubations. Soil Sci. Soc. Am. J. 54:411–15.

O'Leary, W.M., and S.G. Wilkinson. 1988. Gram-positive bacteria. p. 117–202. *In* C. Ratledge and S.G. Wilkinson (ed.) Microbial lipids. Vol. 1. Academic Press, London.

Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci. Soc. Am. J. 51:1173–1179.

Petersen, S.O., and M.J. Klug. 1994. Effects of sieving, storage and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Appl. Environ. Microbiol. 60:2421–2430.

Post, W.M., W.R. Emanuel, P.J. Zinke, and A.G. Stangenberger. 1982. Soil carbon pools and world life zones. Nature (London) 298:156–159.

Raich, J.W., and W.H. Schlesinger. 1992. Global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44B:81–99.

Randlett, D.L., D.R. Zak, and N.W. MacDonald. 1992. Sulfate adsorption and microbial immobilization in northern hardwood forests along an atmospheric deposition gradient. Can. J For. Res. 22:1843–1850.

Ratledge, C., and S.G. Wilkinson (ed.). 1988. Microbial lipids. Vol. 1. Academic Press, London.

Ringelberg, D.B., G.T. Townsend, K.A. DeWeerd, J.M. Suflita, and D.C. White. 1994. Detection of the anaerobic dechlorinating microorganism *Desulfomonile tiedjei* in environmental matrices by its signature lipopolysaccharide branched-long-chain hydroxy fatty acids. FEMS Microbiol. Ecol. 14:9–18.

Rose, A.H. 1989. Influence of the environment on lipid composition. p. 255–278. *In* C. Ratledge and S.G. Wilkinson (ed.) Microbial lipids. Vol. 2. Academic Press, London.

Russel, N.J. 1989. The role of microbial lipids. p. 279–366. *In* C. Ratledge and S.G. Wilkinson (ed.) Microbial lipids. Vol. 2. Academic Press, London.

Stanford, G., M.H. Frere, and D.H. Schwaninger. 1973. Temperature coefficient of soil nitrogen mineralization. Soil Sci. 115:321–23.

Stanford, G., and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. Proc. 36:465–472.

Tans, P.P., I.Y. Fung, and T. Takahashi. 1990. Observational constraints on the global atmospheric CO_2 budget. Science (Washington, DC) 247:1431–1438.

Tunlid, A., and D.C. White. 1992. Biochemical analysis of biomass, community structure, nutrient status, and metabolic activity of microbial communities in soil. p. 229–262. *In* J. Bollag and G. Slotsky (ed.) Soil biochemistry. Marcel Dekker, New York.

Vestal, J.R., and D.C. White. 1989. Lipid analysis in microbial ecology. BioScience 39:535–541.

White, D.C., W.M. Davis, J.S. Nickels, J.C. King, and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51–62.

Wilkinson, L. 1990. SYSTAT: The system for statistics. SYSTAT, Evanston, IL.

Wilkinson, S.G. 1988. Gram-negative bacteria. p. 299–408. *In* C. Ratledge and S.G. Wilkinson (ed.) Microbial lipids. Vol. 1. Academic Press, London.

Zak, D.R., D.F. Grigal, and L.F. Ohmann. 1993. Kinetics of microbial respiration and nitrogen mineralization in Lake States forests. Soil. Sci. Soc. Am. J. 57:1100–1106.

Zak, D.R., D.B. Ringelberg, K.S. Pregitzer, D.R. Randlett, D.C. White, and P.S. Curtis. 1996. Soil microbial communities beneath *Populus grandidentata* Michx. grown under elevated atmospheric CO_2. Ecol. Appl. 6:257–262.

Zelles, L., Q.Y. Bai, and F. Beese. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem. 24:317–323.

Zelles, L., Q.Y. Bai, R. Rackwitz, D. Chadwick, and F. Beese. 1995. Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structure in soils. Biol. Fertil. Soils 19:115–123.