
Biogeochemistry **67:** 235–248, 2004.

Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions

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Received 22 July 2002; accepted in revised form 28 April 2003

Key words: ^{13}C-PLFA, Carbon cycling, Enzyme activities, Microbial community composition, Respiration, Temperature

Abstract. To determine if changes in microbial community composition and metabolic capacity alter decomposition patterns of young and old soil carbon pools, we incubated soils under conditions of varying temperature, N-availability, and water content. We used a soil from a pineapple plantation (CAM; δ^{13}C litter = −14.1‰) that had previously been under tropical forest (C3; δ^{13}C soil carbon = −26.5‰). Forest derived carbon represented 'old' carbon and plantation inputs represented 'new' carbon. In order to differentiate utilization of young (< 14 years) and old (> 14 years) soil carbon, we measured the δ^{13}C of respired CO_2 and microbial phospholipid fatty acids (PLFAs) during a 103 day laboratory incubation. We determined community composition (PLFA and bacterial intergenic transcribed spacer (ITS) analysis) in addition to carbon degrading and nutrient releasing enzyme activities. We observed that greater quantities of older carbon were respired at higher temperatures (20 and 35 °C) compared to the lower temperature (5 °C). This effect could be explained by changes in microbial community composition and accompanying changes in enzyme activities that affect C degradation. Nitrogen addition stimulated the utilization of older soil carbon, possibly due to greater peroxidase activity, but microbial community composition was unaffected by this treatment. Increasing soil moisture had no effect on the utilization of older SOM, but enzyme activity typically declined. Increased oxidative enzyme activities in response to elevated temperature and nitrogen additions point to a plausible mechanism for alterations in C resource utilization patterns.

Introduction

Understanding the controls on the dynamics of soil carbon is important because of the central role soil carbon plays in ecosystem sustainability, nutrient availability and the production of global greenhouse gases. The activity of the soil biota controls, in part, the release of soil carbon to the atmosphere. Relatively little is known regarding how changes in microbial community composition or metabolic capacity may alter the types or amounts of soil carbon consumed and respired (Sollins et al. 1996). Macdonald et al. (1995) and Zogg et al. (1997) found that changes in temperature alter the size of the available substrate pool, which was associated with changes in microbial community composition. They modeled the kinetics of microbial respiration (Y) using a

first order rate equation:

$$Y = A_o(e^{-kt}) \quad (1)$$

with a variable substrate pool size (A_o) and rate constant (k) where elevated temperature typically increases the rate constant (t is time). They found that the assumption of a constant pool size may be incorrect; increases in temperature appeared to increase the amount of available C for microbial utilization (Macdonald et al. 1995; Zogg et al. 1997). Research using free air CO_2 enrichment (FACE) sites has also shown that increases in temperature increase the utilization of older C (Andrews et al. 2000). Andrews et al. (2000) suggest that a possible mechanism for changing utilization patterns of soil organic carbon was alteration in microbial community composition, which may lead to changes in whole-community metabolic capacity. However, the mechanism that allows for greater access to older SOM was not demonstrated.

Extracellular macromolecular carbon degrading enzymes play a central role in the degradation of litter and soil organic matter and thus may provide a mechanistic link to alterations in resource utilization patterns. In some cases, enzymes are considered to be the rate-limiting step in decomposition (Sinsabaugh et al. 1994). Different microbial populations differ in the types of enzymes produced (Kirk and Farrell 1987). For example, fungi and actinomycetes produce phenol oxidase and peroxidase enzymes that are central to the degradation of phenolic compounds indicative of older, more recalcitrant organic matter (Kirk and Farrell 1987; Waldrop et al. 2003). Other important carbon degrading enzymes are hydrolytic cellulases (β-glucosidase, cellobiohydrolase), hemicellulases (β-xylosidase, N-acetyl-glucosaminidase), galactase, and α-glucosidase. Unlike oxidative enzymes, these hydrolytic enzymes are produced by a wide variety of microorganisms, and thus a change in community composition may not affect the activity of these enzymes. Extracellular enzyme activity has been observed to decrease with high soil moisture contents and greater N availability, possibly reducing microbial access to older C sources in SOM (Fog 1988; Freeman et al. 1996).

Our objective was to quantify the utilization of young and old soil C under altered environmental conditions by monitoring the $\delta^{13}C$ of respired CO_2 and microbial biomarkers (PLFAs), and relating these data to changes in the microbial community composition and extracellular C-degrading enzyme activities. We hypothesized that increases in soil temperature would increase the utilization of old soil C and that high soil moisture and soil N would reduce the utilization of older soil C. We also hypothesized that utilization of older C would be associated with increases in oxidative enzyme activities.

Methods

Soils were obtained from pineapple plantations and adjacent forests on the island of Tahiti (17°30′S, 149°50′W) in French Polynesia. The maximum mean

monthly temperature was 27 °C (January–March) and the minimum was 24 °C (July–August). The forest (C3 physiology consisting of *Hibiscus tillaceus*, *Nephrolepis* and *Gleichenia linearis*, *Psidium guajava*, *Pistache*, and *Eugenia cumini*) was clearcut, forest litter burned, and pineapple plantations were established within 1 year (CAM physiology; *Bromilacea ananas comelsus* var. *quentii*). Thirty thousand plants per hectare were grown for 14 years. Every 3–4 years the plants became unproductive and were pulverized and incorporated into the surface soil. Each plant was given approximately 80 g fertilizer per year (30:10:30:10 N:P:K:S). Soil cores (12.5 cm long × 4.5 cm diameter) were taken every 3 m along three transects in the plantation and along five transects in the adjacent forest. Plantation soil was homogenized and stored for approximately 9 months at −4 °C before the incubation began. The conversion from forest to plantation shifted bulk soil $\delta^{13}C$ from $-26.5 \pm 0.1‰$ to $-23.0 \pm 0.1‰$ in the pineapple plantation (pineapple litter $\delta^{13}C = -14.1 \pm 0.5‰$ ($n = 3$)).

The experiment was organized in a factorial design with control, water, and N additions within each temperature treatment using three replicate soil samples. Thus we had 27 total samples. Fifty grams of oven dry equivalent soil were placed into 1.0 L mason jars and the soil water content adjusted to 0.43 g g^{-1}. The water content of the '+ water' treatment was increased until there was standing water (0.59 g g^{-1}). The '+N' treatment received NH_4NO_3 equivalent to 30 μg N g^{-1} and was at the same water content as the control. Soil temperature was altered by incubating soils at 5, 20 or 35 °C. Mason jars were kept sealed but opened after gas sampling to allow re-equilibration of the headspace gas. We used new lids with silicone-lined septa to minimize leakage problems. Headspace gas samples were taken using a syringe through a septum in the mason jar lid. CO_2 concentrations were measured on a Schimadzu gas chromatograph with a thermal conductivity detector. Subsamples of headspace CO_2 were stored in Hungate glass vials with septa until analyzed for $\delta^{13}C$ values (relative to V-PBD) on a Finnigan MAT (Bremen Germany) Delta Plus XL isotope ratio mass spectrometer (IRMS).

All microbial community analyses (enzyme activities, PLFA, DNA analysis) were conducted at the end of the incubation experiment (day 103). β-glucosidase, α-1,4-glucosidase, galactase, β-xylosidase, cellobiohydrolase, NAGase, phosphatase, and sulfatase were assayed using MUB-linked substrates in 5 mM pH 8 bicarbonate buffer. Aliquots of a 1:100 (w/v) soil slurry were added to substrate solutions and assayed on microplates (8 analytical replicates) with appropriate quenching controls using a Fluorolog 3 spectrofluorometer with a Micromax plate reader (ISA instrumetents) with the excitation wavelength set at 360 nm. Plates were incubated at 27 °C for 1–2 h. Emission (450 nm) was measured at the beginning and end of the incubation. Enzyme activities were expressed as μmol substrate converted h^{-1} g^{-1}.

Phenol oxidase and peroxidase enzyme substrate was 10 mM L-dihydroxyphenyalanine (LDOPA). Phenol oxidase activity was measured as the increase in absorbance at 469 nm after 1 h using a Spectramax plus spectrophotometer (Molecular Devices). Controls were 100 μl soil mixture and 100 μl buffer.

Peroxidase activity the difference between samples reacted with and without 3 μl 3% H_2O_2. Units for phenol oxidase and peroxidase are μmol LDOPA converted h^{-1} g^{-1}. Following the enzyme assays, dissolved organic carbon content was measured on the bicarbonate buffer soil solution filtered through a Whatman number 1 filter. Soil extracts were analyzed on a 1010 total organic carbon analyzer (OI analytical).

Phospholipid fatty acid analysis (PLFA) was conducted on 5 g samples of freeze dried soil using the procedure by White and Ringelberg (1998) and peaks were identified on a MIDI-FAME system (MIDI, Inc, Newark, DE). Isotope ratios of microbial PLFAs were measured on an isoprime isotope ratio mass spectrometer linked through a combustion interface to an HP 1530A gas chromatograph with a 25 m × 0.2 mm × 0.33 μm Ultra 2 (5%-phenyl)-methylpolysiloxane column (Hewlett Packard). The GC analyzed a 2 μl splitless injection, at an initial temperature of 45 °C, ramped up to 145 °C at 20 °C per minute, then slowed to 0.5 °C per minute until 180 °C, and finally ramped up to 250 °C at 20 °C per minute. We corrected the isotope ratios of PLFAs for the additional carbon moiety added during transesterification using the equation by Abraham et al. (1998).

DNA for bacterial intergenic transcribed spacer (ITS) region analysis was extracted using a BIO 101 DNA extraction kit. Initial vortexing was increased to 30 s and heating of the sample was increased to 7.5 min at 37 °C. This was repeated three times. Bacterial 16-23S ITS regions were amplified using primer set 1406F and 115R (Borneman and Triplett 1997). Thermocycling was performed on a Perkin Elmer GeneAmp 2400 PCR system and consisted of 30 cycles at 92 °C melting temperature, 55 °C annealing temperature, and a 72 °C extension temperature. The PCR product was run on a 5% polyacrylamide gel at 170 V for 4.5 h, stained with ethidium bromide and visualized by UV transillumination. ITS banding patterns were compared using similarity analysis and based upon a Pearson's correlation (GelCompar software, Applied Maths).

The kinetics of microbial respiration were modeled using the first order rate equation (Eq. 1). Calculation of A_o and k were made using an iterative best-fit technique that allowed both A_o and k to vary (JMP software, SAS). Isotope ratios of respired CO_2 were compared using analysis of covariance (ANCOVA) with cumulative respired CO_2 as the covariate. Two-way analysis of variance (ANOVA) was to compare the effect of temperature and treatment on measured variables. Mean separation was performed using Tukey's HSD test. Significance level was $P < 0.05$ unless otherwise noted.

Results

Temperature

Cumulative respired CO_2 was highest at 35 °C, intermediate at 20 °C, and lowest at 5 °C over the 103 day incubation period (Figure 1). Respired CO_2

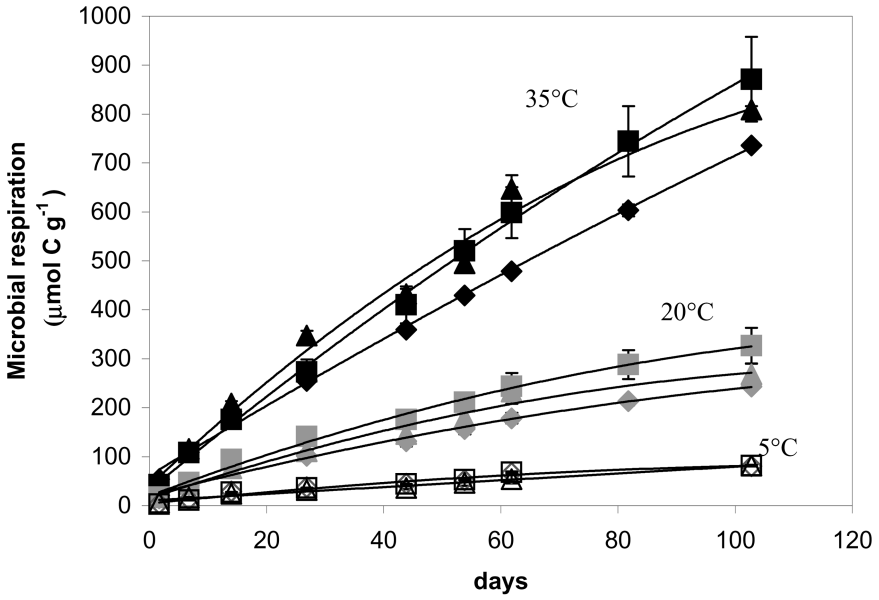

Figure 1. Cumulative respired CO_2 over time. Black, grey, and white symbols represent 35, 20, and 5 °C treatments, respectively. Triangles, squares, and diamonds represent +N, +water, and control treatments, respectively. Bars are standard errors of the mean; $n = 3$.

data were closely fit by the first-order kinetic model ($r^2 > 0.98$). Modeling the kinetics of microbial respiration using a variable substrate pool size and rate constant indicated that the available substrate pool size was five times larger at 35 °C compared to 5 °C (Table 1). The respiration rate constant (k) was higher at 20 °C than at either 35 or 5 °C (Table 1). Hydrolytic enzyme activities and dissolved organic carbon concentrations were generally lower in the higher temperature treatments. On the other hand, oxidative enzyme activities were not significantly different among the temperature treatments, and phenol oxidase tended to increase (Table 1). Thus the ratio of oxidative enzyme activity to hydrolytic enzyme activity increases as temperature increases.

The $\delta^{13}C$-CO_2 values became more negative over time confirming that young carbon is easily metabolized, and more forest derived C is respired as the young C is depleted (Figure 2). The respired $\delta^{13}C$-CO_2 values were used to quantify differences in young and old carbon utilization among treatments. A direct comparison of the mean $\delta^{13}C$-CO_2 values over the entire experiment cannot be made among the three temperature treatments because microbes at high temperatures utilized older carbon by virtue of the fact that they respired more total carbon. To overcome this dilemma, we compared the $\delta^{13}C$-CO_2 values using ANCOVA using cumulative respiration as the covariate. Using the ANCOVA, the isotope ratio of microbial respiration displayed a significant temperature effect ($F = 36.92$, $p < 0.0001$). Analysis of the $\delta^{13}C$-CO_2

Table 1. Available C pool (A_o), first-order rate constant (k) of microbial respiration, and hydrolytic and oxidative soil enzyme activities in treated soils. Letters indicate differences among column means for either the three incubation temperatures or the three soil treatments.

	Incubation temperature (°C)			Treatment		
	5	20	35	Control	+Water	+N
A_o[1]	323[c]	496[b]	1538[a]	666[x]	721[x]	924[x]
k[1]	1.9[a]	3.7[b]	2.8[a]	2.8[x]	3.0[x]	2.8[x]
$\delta^{13}C\text{-}CO_2$						
Least square means	−17.43[b]	−19.08[a]	−19.27[a]	−18.41[y]	−18.21[y]	−19.17[x]
DOC (mg C kg^{-1})	698[a]	573[b]	592[ab]	604[x]	607[x]	652[x]
β-glucosidase[2]	552[a]	387[ab]	231[b]	421[x]	385[x]	340[x]
α-glucosidase[2]	240[a]	175[ab]	108[b]	176[x]	165[x]	172[x]
Galactase[2]	247[a]	173[ab]	107[b]	191[x]	156[x]	170[x]
Cellobiohydrolase[2]	258[a]	178[ab]	118[b]	410[x]	311[x]	315[x]
Xylosidase[2]	471[a]	315[ab]	270[b]	201[x]	197[x]	143[x]
NAGase[2]	389[a]	294[ab]	195[b]	342[x]	235[x]	290[x]
Phosphatase[2]	1198[a]	787[a]	681[a]	1002[x]	688[x]	946[x]
Sulfatase[2]	255[a]	123[ab]	154[b]	194[x]	166[x]	166[x]
Phenol oxidase[3]	41[a]	34[a]	48[a]	51[x]	30[y]	40[y]
Peroxidase[3]	55[a]	52[a]	49[a]	57[y]	33[y]	70[x]

[1] A_o and k were calculated using an iterative best-fit technique ($n=9$, $p<0.05$). Units for A_o are mg C kg^{-1} and units for k are $year^{-1}$.
[2] Hydrolytic enzyme activites using MUB-substrates are in units of nmol h^{-1} g^{-1} ($n=9$, $p<0.10$).
[3] Oxidative enzyme units are μmol LDOPA h^{-1} g^{-1}.

values revealed that the isotope ratios of respired CO_2 were depleted in ^{13}C at 20 °C compared to 5 °C, and there was no change in $\delta^{13}C\text{-}CO_2$ values between the 20 and 35 °C treatments (Table 1, Figure 2). The isotope ratios of the respired CO_2 indicated that as temperatures warmed above 5 °C, the microbial community shifted to the utilization of older, forest-derived C3 carbon.

PLFA analysis and bacterial ITS analysis revealed that the microbial community differed among the three temperature treatments (Table 2, Figures 3 and 4), although there was no change in the overall size of the microbial community, based upon the sum of total moles of PLFA. Interestingly, the total and relative abundance of fungal and actinomycete biomarkers were significantly lower at higher temperatures (Table 2). Some microbial PLFAs are known to be temperature sensitive (Petersen and Klug 1994), thus these changes in fatty acid composition may be partially due to thermoadaptation. ITS analysis, on the other hand, is not temperature sensitive, and thus may be a more robust measure of community composition in this experiment. ITS patterns were compared among the control soils in the three temperature treatments. ITS patterns and similarity analysis reveal that there is a marked shift in community composition among the temperature treatments (Figure 4). The isotope ratios of microbial PLFA biomarkers ranged from −22.3 to −32.4‰.

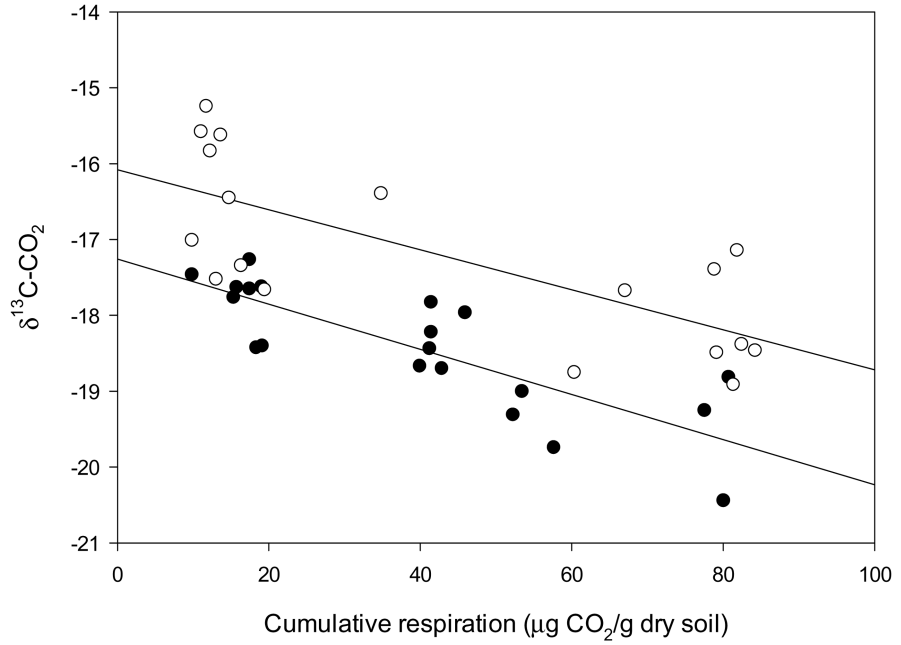

Figure 2. Carbon isotope ratio of microbial respiration ($\delta^{13}C$-CO_2) over the 103 day incubation experiment in the 20 (black circle), and 5 °C (white circle) treatments. Isotope ratios of respired CO_2 were depleted in ^{13}C at 20 °C compared to 5 °C.

Several PLFA biomarkers were depleted in ^{13}C in the 5 °C treatment relative to 35 °C. These included PLFA biomarker 18:0, 'Gram +' biomarkers i16:0, i17:0I, i17:0, and the fungal biomarker 18:2ω6 (Table 2).

Water addition

Increasing soil water content from 0.43 to 0.59 g g^{-1} had no significant effect on the size of the available substrate pool (Table 1), the utilization of young and old soil carbon (Table 1), or microbial community composition (Table 2). However, increasing water content decreased phenol oxidase activity by 41% (Table 1). Increasing soil water content also reduced peroxidase activity by 42%, and tended to reduce all other enzyme activities, but the effect was not significant (Table 1).

Nitrogen addition

N addition also did not significantly affect the available pool size of C or the rate constant (k) of microbial respiration (Table 1). However, ANCOVA of

Table 2. PLFA relative abundance and $\delta^{13}C$ values from soils incubated at three temperatures. Letters indicate differences among column means.

	Incubation temperature (°C)				
	mol%			$\delta^{13}C$	
	35	20	5	35	5
Saturated					
16:0	13.3[a]	11.4[b]	10.9[b]	−22.30[a]	−28.58[a]
18:0	7.0[a]	5.3[b]	4.6[b]	−23.32[a]	−26.07[b]
Gram +					
i15:0	7.1[a]	8.1[a,b]	8.4[b]	−21.91[a]	−25.69[a]
a15:0	3.3[a]	3.3[a]	3.8[b]	−28.44[a]	−26.81[a]
i16:0	6.1[a]	6.6[b]	6.7[b]	−23.01[a]	−26.32[b]
i17:0i	1.3[a]	2.7[a]	2.0[a]	−23.72[a]	−26.12[a]
i17:0	11.8[a]	8.7[b]	8.3[b]	−22.52[a]	−26.32[a]
a17:0	2.6[a]	2.5[a]	2.4[b]	−25.52[a]	−26.59[a]
16:0 10me	5.1[a]	7.0[b]	7.5[b]	−24.29[a]	−27.89[b]
17:0 10me	1.1[a]	1.3[b]	1.3[b]		
i18:0	1.4[a]	1.1[b]	0.9[c]		
Gram −					
16:1ω5c	1.4[a]	1.7[b]	1.6[b]	−27.17[a]	−26.94[a]
16:1ω7c	1.5[a]	2.4[b]	4.3[c]	−28.70[a]	−27.32[a]
17:0cy	1.9[a]	2.6[b]	2.7[b]	−26.59[a]	−27.26[a]
16:1 2OH	9.6[a]	8.4[b]	6.9[c]	−25.52[a]	−26.20[a]
18:1ω7c	1.5[a]	2.1[b]	2.3[b]	−24.01[a]	−27.23[a]
19:0cy ω8c	9.9[a,b]	10.9[a]	9.2[b]	−24.49[a]	−29.24[a]
18:1ω9c	3.4[a]	2.9[b]	3.1[a,b]		
Fungi					
18:2ω6	0.3[a]	0.7[a,b]	1.1[b]	−24.00[a]	−32.37[b]
Actinomycete					
10ME 18:0	2.6[a]	2.8[a,b]	3.4[b]		

$\delta^{13}C$-CO_2 data, using cumulative respiration as the covariate, displayed a significant treatment effect ($F = 9.28$, $P = 0.0002$; Table 1). The carbon isotope ratio of microbial respiration ($\delta^{13}C$-CO_2) in the +N treatment was depleted relative to the control treatment, indicating that microbial communities were utilizing older SOM following N addition (Table 1). Peroxidase activity increased, phenol oxidase activity decreased, and hydrolytic enzyme activities were unaffected by the +N treatment (Table 1). Dissolved organic carbon concentration also tended to increase in the +N treatment, relative to control (Table 1).

Microbial community composition, as revealed by PLFA and ITS analysis, was unchanged due to N addition (Figure 3; ITS data not shown). The isotope ratio of PLFA biomarker a17:0 was depleted in ^{13}C relative to control (control = -23.4 ± 1.0‰; nitrogen treatment = -29.2 ± 1.6‰). There was

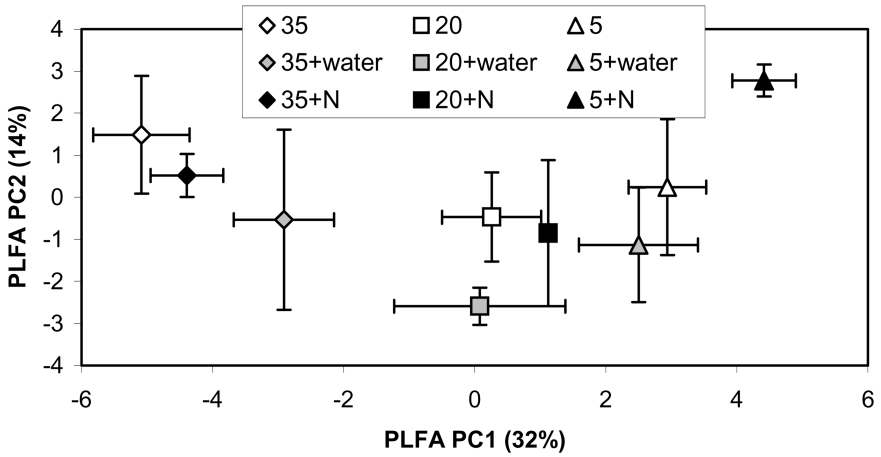

Figure 3. PLFA profiles of microbial communities in the three temperature treatments in control, + water, and + N treatments. PLFA PC1 represents the first principal component and PLFA PC2 represents the second principal component with amount of variability explained by the principal component in parentheses. Communities at different temperatures were significantly different from one another ($p < 0.05$, $n = 9$), and there were no significant treatment or temperature × treatment effects.

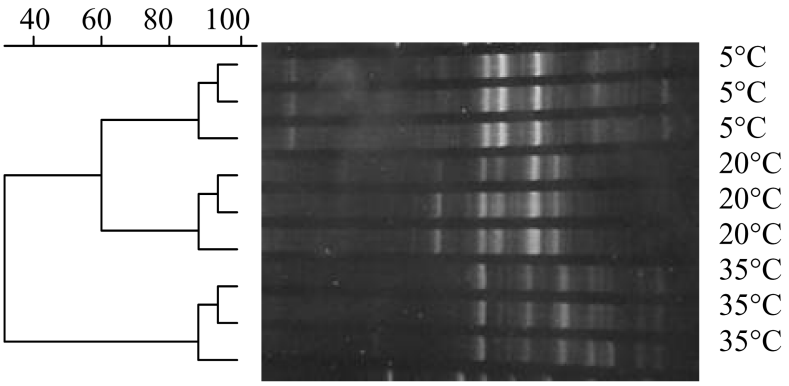

Figure 4. Similarity analysis of amplified bacterial ITS regions of the microbial DNA in soil incubated at different temperatures. The x-axis represents the Pearson's correlation coefficient among treatments. Samples that cluster together have greater similarity. The ITS banding pattern from the original polyacrylamide gel is shown in the digital image. Each lane in the image corresponds to a sample in the similarity analysis.

some indication that fungal populations were also using older SOM (18:2ω6; control = -25.4 ± 1.1‰; nitrogen treatment = -29.5 ± 6.5‰), but this was not significant.

Discussion

Temperature

The changes in PLFA and ITS patterns, enzyme activities, kinetic parameters of microbial respiration, and isotope ratios of respired CO_2 provide strong evidence for a shift in microbial community composition and function related to elevated soil temperatures. Elevated incubation temperatures led to an increase in the size of the substrate pool utilized by soil microbes, a result that has been observed previously (Macdonald et al. 1995; Zogg et al. 1997). Furthermore, we found that the rate constant (k) for microbial respiration was highest at 20 °C, near the mean monthly air temperature of the site (range = 24–27 °C over the course of the year), which is consistent with research describing different temperature optima for microbial respiration in soils from different climatic regions (Dalias et al. 2001). Therefore it is possible that the microbial community in this study was adapted to a temperature range closer to 20 °C than either 35 or 5 °C.

In addition to increasing the pool size of available C and altering the rate constant, increasing temperatures led to increases in the utilization of old C, independent of the total quantity of C consumed. Increased utilization of older, recalcitrant SOM with increases in temperature has been previously measured. For example, Loiseau and Soussana (1999) measured increased utilization of old C with an increased temperature of only 3 °C over a 2-year period at elevated CO_2 but not ambient CO_2. Andrews et al. (2000) using the label from FACE sites, concluded that microbes at lower temperatures were not able to utilize old SOM that microbes at 20 and 40 °C could utilize. They concluded that at low temperatures there is either a change in the kinetics of carbon mineralization or a shift in the C pool being mineralized. Our data indicate that both the kinetics of carbon mineralization and the size and type of soil C mineralized is changing.

Importantly, the greater respiration of more recalcitrant C-pools at higher temperatures may be explained, in part, by changes in the composition and metabolic capacity of the microbial community that mediates C cycling processes (Macdonald et al. 1995; Zogg et al. 1997; Loiseau and Soussana 1999). Microbial communities differ in their genetic capacity to produce oxidative enzymes (Kirk and Farrell 1987), and it is evident from our ITS data that changes in the composition of the microbial community occurred in response to changes in temperature. Although we expected to see increased respiration of larger and older C pools accompanied by increased enzyme activity, we instead observed a decrease in cellulytic and hemicellulytic enzyme activities and no change in oxidative enzyme activities. Since enzyme activity was determined at the end of the 103 day experiment, and since soils at higher temperatures effectively respired a larger pool of C, enzyme activity may have been lower at higher soil temperatures because of a depletion of the available substrate pool. This idea is supported by the lower DOC concentration observed in

the higher temperature soils. While the activities of the hydrolytic enzymes decreased by nearly 50% in the 35 °C treatment compared to the 5 °C treatment, oxidative enzyme activities remained constant or even tended to increase. Thus the ratio of oxidative enzyme activity to hydrolytic enzyme activity increased with increasing temperature. This pattern suggests that the oxidative enzymes may have been a mechanism for the enhanced degradation of the older, more recalcitrant SOM at higher temperatures.

Increases in soil temperature may also have affected the physical–chemical processes controlling carbon stabilization and destabilization. For example, since the recalcitrance of a molecule is defined by the activation energy required to break the chemical bonds (Thornley and Cannell 2001) and since the Arrhenius equation shows that the higher the activation energy (E_a) for a reaction, the higher its temperature dependence, elevated temperature will theoretically accelerate the breakdown of recalcitrant compounds to a greater degree than the breakdown of labile compounds. This might then alter the biochemical forms of carbon substrates available to soil organisms, thereby shifting the composition of the microbial community to organisms that have a competitive advantage. However, elevated temperature may also theoretically increase carbon *stabilization* through the same mechanisms (Thornley and Cannell 2001), resulting in no net increase in C availability to soil microorganisms via abiotic processes. The relative importance of biotic and abiotic mechanisms of carbon stabilization and destabilization is an important area of further study.

Finally we attempted to determine the microbial groups that may be responsible for greater access to older SOM by analysis of the isotope ratios of microbial PLFAs. Isotope ratios of microbial PLFAs differed among fatty acids by as much as 10‰, suggesting that microbial PLFAs can be used to distinguish microbial utilization of different carbon sources under some circumstances. Temperature related fractionation, however, seemed to affect the isotope ratios of microbial PLFA's, where PLFAs at 5 °C were depleted in ^{13}C compared to 35 °C, which would indicate that they were made up of older C. Since temperature-related fractionation, as well as community shifts can cause large (up to 7‰) changes in the $\delta^{13}C$ of fatty acids (Abraham et al. 1998), simple interpretations of this type are equivocal. The biophysical processes causing fractionation of microbial lipids are an important topic for further study.

Water addition

Increasing soil water content did not significantly reduce microbial degradation of older soil carbon or microbial respiration in general, but we did observe a decrease in phenol oxidase enzyme activity. We also did not observe a change in microbial community composition in the +water treatment relative to control. The soil moisture in the control soil (0.43 g g^{-1}) was already very wet

and so the increase in moisture content from the control soil to the +water treatment was not a severe treatment.

Nitrogen addition

Phospholipid biomarker and ITS patterns were unaffected by nitrogen addition after 103 days, indicating that microbial community composition and biomass was unresponsive to the +N treatment. Different studies have shown varying effects of nitrogen addition on microbial biomass, including decreases (McAndrew and Malhi 1992; Arnebrandt 1994; Arnebrandt et al. 1996; Ajwa et al. 1999), increases (Salinas-Garcia et al. 2002) and no effect (Schmidt et al. 2000), even after many years of N addition. Although there was no change in community composition or microbial biomass, the depleted isotope ratio of respired CO_2 indicates that microbial communities were stimulated by N to decompose older SOM. The increase in peroxidase activity in the +N treatment points to a possible mechanism for greater access to older SOM. Oxidative enzymes can degrade the phenolic compounds within soil organic matter, making relatively more recalcitrant organic material available for microbial metabolism.

The controls on soil oxidative enzyme activity and the degradation of older SOM are extremely important and not well understood. Loiseau and Soussana (1999) found that nitrogen addition decreased the decomposition rate of old SOM in a low CO_2 atmosphere but stimulated old SOM decomposition in a high CO_2 environment. They reasoned that nitrogen inhibited phenol oxidase activity at low CO_2, and under high CO_2 nitrogen stimulated the 'priming effect'. However, the priming effect may also be caused by an induction of oxidative enzyme activity (Kuzyakov et al. 2000). Thus, their data seem to suggest that N addition can either stimulate or inhibit oxidative enzyme activity, depending upon relative resource availability. When C availability is low, high N may inhibit oxidative enzyme activity, and when C availability is high N may stimulate oxidative enzyme activity.

In our experiment, N addition stimulated peroxidase enzyme activity, potentially a central mechanism leading to greater microbial utilization of old soil C. It is thought that N addition should decrease oxidative enzyme activity and therefore the decomposition rates of phenolic, potentially more recalcitrant, compounds. This idea comes primarily from experiments on basidiomycete fungi degrading lignin and litter. Many results with other types of fungi, for example, soft rot fungi, have found either stimulatory or no effect (Fog 1988). As Fog (1988) notes, the effect of adding N on decomposition may depend on the composition of the microbial community, especially fungi, because, for example, white rot, brown rot, and soft rot fungi respond differently to N additions. The ultimate effect of N on decomposition and SOM degradation may depend on a complex interaction between substrate availability, microbial community composition and the enzymatic capacity of the microbial community.

We attempted to identify microbial biomarkers that were responsible for the enhanced utilization of SOM in the +N treatment by analysis of the isotope ratios of microbial PLFAs. The composition of the microbial community did not measurably change in the +N treatment, thus, isotope ratios of PLFAs could be compared among treatments without considering microbial fractionation due to differences in community composition. The depleted isotope ratios of the Gram + PLFA biomarker a17:0, and the fungal biomarker 18:2ω6 indicate that fungal and some Gram + organisms preferentially utilized older SOM following N addition. This may provide a link between functionally important microbial biomarkers (fungi and Gram + in this case), changes in soil oxidative enzyme activities, and the utilization of distinct pools of soil C.

This study provides evidence for changes in the microbial community composition, function, and C pool utilization due to changes in soil temperature and N addition. Changes in soil temperature and N availability can alter the forms of C utilized by microbial communities through changes in the production of oxidative enzymes, rather than just affecting their respiration rate constants. This result points toward a need to further understand the effects of changes in soil temperature and N availability on the composition and function of the soil community, and the resulting feedback to the decomposition processes.

Acknowledgements

We would like to acknowledge the help of Don Herman, Paul Brooks, Mark Conrad, Tamas Torok, Egbert Schwartz, and Eoin Brodie for laboratory assistance and advice on the manuscript. We would also like to acknowledge the Kearney Foundation for Soil Science Research and CA AES Project 6117-H for support.

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