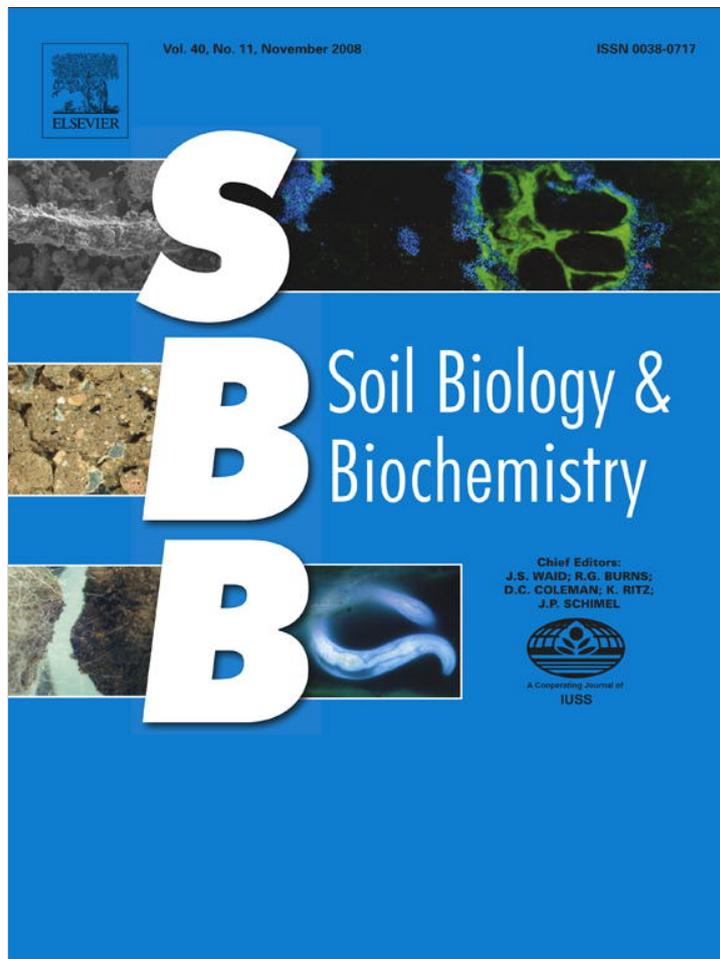


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Short communication

## Microbial biomass, functional capacity, and community structure after 12 years of soil warming

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## ABSTRACT

We examined the effect of chronic soil warming on microbial biomass, functional capacity, and community structure in soil samples collected from the Soil Warming Study located at the Harvard Forest Long-term Ecological Research (LTER) site. Twelve years of chronic soil warming at 5 °C above the ambient temperature resulted in a significant reduction in microbial biomass and the utilization of a suite of C substrates which included amino acids, carbohydrates, and carboxylic acids. Heating significantly reduced the abundance of fungal biomarkers. There was also a shift in the mineral soil microbial community towards gram positive bacteria and actinomycetes.

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Long-term changes in soil temperature regimes resulting from climate warming are expected to alter soil properties and processes. Numerous warming studies have been initiated over the past decade to examine the ecosystem-scale effects of rising temperature (Rustad et al., 2001). Most of these studies, from a soil perspective, have focused on soil respiration. While soil CO<sub>2</sub> efflux is initially stimulated by warming, an increasingly common observation is that this effect diminishes over time. That is, acclimation of soil respiration occurs in response to warming (Oechel et al., 2000; Luo et al., 2001; Bradford et al., 2008). For example, in a soil warming experiment at Harvard Forest located in central Massachusetts, warming accelerated CO<sub>2</sub> fluxes to the atmosphere in the first few years of the study (Melillo et al., 2002, 2004). However, this stimulatory effect significantly decreased after 5–6 years of warming and there was no response to heating between years 7 and 12. Similar results have been observed by others (Oechel et al., 2000; Luo et al., 2001). Proposed mechanisms underlying this observation include reduced plant production leading to lower root respiration rates, reduced microbial activity induced by soil drying, and substrate limitation due to losses of labile soil C (Oechel et al., 2000; Luo et al., 2001; Melillo et al.,

2002). Changes in microbial community structure may be another important, but understudied, mechanism influencing soil C cycling.

Soil warming studies that have examined microbial community dynamics can be divided into field or mesocosm experiments of variable duration (1–15 years) with a modest increase in soil temperature (1–3 °C; Kandeler et al., 1998; Bardgett et al., 1999; Deslippe et al., 2005; Zhang et al., 2005; Sowerby et al., 2005; Rinnan et al., 2007) and lab incubations of short duration (6–16 weeks) with soils incubated over a wide range of temperatures (5–40 °C; Zogg et al., 1997; Andrews et al., 2000; Waldrop and Firestone, 2004). Of the field studies, only two examined microbial community structure. Zhang et al. (2005) observed a 20–60% increase in the fungal:bacterial ratio at a tallgrass prairie site exposed to a ~2 °C increase in temperature over a three year period. Warming did not alter microbial biomass or respiration. After 15 years of soil warming (1–2 °C) in northern Sweden, the only response was a significant reduction in the relative abundance of fungi (Rinnan et al., 2007), the opposite effect as the one observed by Zhang et al. (2005). We still lack a clear understanding of how warming will affect the soil microbial community and in turn, how changes in the community will feedback to influence nutrient cycling dynamics.

Our objective was to examine how microbial biomass, functional capacity, and community structure have responded to long-term soil warming in a northeastern forest. Samples were collected 12 years after establishment of the Soil Warming Study located at the Harvard Experimental Forest in Petersham, MA (42.5 N,

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72.18 W). The experiment is located in an even aged, mixed deciduous forest dominated by black oak (*Quercus velutina*), red maple (*Acer rubrum*), paper birch (*Betula papyrifera*), and striped maple (*Acer pensylvanicum*) (Melillo et al., 2004). The soil is a fine loamy, mixed, mesic, Typic Dystrochrept (Gloucester series; Peterjohn et al., 1993). Mean annual temperature and precipitation are 7.6 °C and 1100 mm, respectively (Boose et al., 2002). The experimental design consists of 18 6 × 6 m plots grouped into six blocks, with the three plots within each block randomly assigned to one of three treatments: (i) a heated plot in which the average soil temperature is elevated 5 °C above ambient by the use of buried heating cables, (ii) a disturbance control plot that is identical to the heated plot except it receives no electrical power, and (iii) an undisturbed control plot that has been left in its natural state (Melillo et al., 2002).

A soil core (2.5 cm diameter) was collected from each of four 1 × 1 m subplots within each of the 18 treatment plots. Samples from each subplot were separated into two depth increments: O-horizon material and 10 cm of mineral soil. The four samples from each plot and depth increment were bulked for a total of 36 samples. The samples were sieved (2 mm), and root and litter fragments were removed. Samples were stored at 4 °C and analyzed within two weeks of collection.

Microbial functional capacity was assessed by measuring the short-term respiratory responses to 25 substrates following the method developed by Degens and Harris (1997). Each substrate (2 mL) was added to subsamples of O-horizon material (0.5 g) or mineral soil (1 g) in 20-mL vials. The substrates consisted of two carbohydrates (D-glucose, D-mannose), two amines (D-glucosamine, L-glutamine), six amino acids (L-arginine, L-asparagine, L-glutamic acid, L-histidine, L-lysine, L-serine), and 15 carboxylic acids (L-ascorbic acid, citric acid, fumaric acid, gluconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-malic acid, malonic acid, pantothenic acid, quinic acid, succinic acid, tartaric acid, uric acid, urocanic acid). Substrate solution concentrations were 75 mM for carbohydrates, 10 mM for amines and amino acids, and 100 mM for carboxylic acids. Samples were incubated for 4 h at 25 °C after substrate addition and vortexed twice to ensure even dispersion. Following the incubation period, headspace CO<sub>2</sub> concentrations were measured using an infrared gas analyzer. Active microbial biomass was estimated using the respiratory response to carbohydrate addition (glucose and mannose), which is comparable to the substrate-induced respiration (SIR) method. Total microbial biomass was determined as extractable lipid P on duplicate 1 g samples using perchloric acid digestion and measuring the released phosphate by the method of Bartlett (Kates, 1986).

Microbial community composition was based on the extraction of total fatty acid methyl esters (FAMES) by mild alkaline hydrolysis (Grigera et al., 2006). Identification of FAMES was by comparison of retention time of equivalent chain length with known standards (Bacterial Acid Methyl Esters CP Mix, Supelco USA) and confirmed by gas chromatograph mass spectrometry (Drijber et al., 2000). Concentrations of FAMES were calculated from peak areas and reported as nmol g<sup>-1</sup> soil. A total of 36 FAMES were retained for analysis. FAME nomenclature followed that of the IUPAC-IUB Commission on Biochemical Nomenclature (IUPAC-IUB, 1978). Specific fatty acids were used to represent fungal and bacterial groups. Fungal biomass was represented by C18:2cis9,12 (Stahl and Klug, 1996). Ten FAMES were summed to represent bacterial biomass: iC15:0, aC15:0, C15:0, iC16:0, C16:1cis7, iC17:0, aC17:0, C17:0, cyC17(9), and cyC19(11) (Grigera et al., 2006). The fatty acid C16:1cis11 was used as a biomarker for arbuscular mycorrhizal (AM) fungi (Olsson, 1999). Actinomycetes were quantified by 10Me fatty acids: 10MeC17:0, 10MeC18:0 and i10MeC18:0 (Kroppenstedt, 1985).

**Table 1**

Respiration (μg CO<sub>2</sub>-C g<sup>-1</sup> soil) in response to addition of a range of simple organic substrates. There were no significant differences between control and disturbance control plots, so only data from the control and heated plots are shown. Values are means ± one standard error

Biomarker	O-horizon		Mineral soil	
	Control	Heated	Control	Heated
<i>Carbohydrates</i>				
Glucose	30.1 (5.8)	18.2 (5.4)	4.31 (0.72)	2.42 (0.32)
Mannose	25.3 (5.4)	12.4 (3.0)	3.45 (0.55)	1.65 (0.31)
<i>Amines and amino acids</i>				
Arginine	17.6 (3.0)	11.7 (3.2)	1.74 (0.29)	1.45 (0.28)
Asparagine	22.8 (3.6)	11.3 (3.3)	2.88 (0.48)	1.70 (0.37)
Glucosamine	3.6 (1.4)	1.9 (0.7)	0.63 (0.27)	0.51 (0.25)
Glutamic acid	29.7 (5.0)	15.5 (2.6)	3.17 (0.38)	2.00 (0.53)
Glutamine	26.7 (3.3)	18.0 (3.0)	2.54 (0.38)	2.20 (0.46)
Histidine	20.2 (3.6)	9.2 (2.8)	1.35 (0.35)	0.85 (0.33)
Lysine	20.5 (4.6)	13.9 (3.4)	2.73 (0.34)	2.81 (0.94)
Serine	17.6 (2.9)	10.2 (2.5)	3.11 (1.38)	0.93 (0.28)
<i>Carboxylic acids</i>				
Ascorbic acid	46.2 (7.4)	42.6 (6.6)	8.58 (1.35)	7.46 (1.02)
Citric acid	61.4 (15.2)	42.3 (11.2)	11.20 (3.17)	7.08 (1.75)
Fumaric acid	59.3 (11.2)	35.5 (5.3)	9.68 (1.12)	5.28 (0.75)
Gluconic acid	33.5 (5.7)	17.9 (3.8)	5.19 (0.44)	2.99 (0.48)
Ketobutyric acid	52.8 (9.1)	35.6 (6.9)	9.11 (0.58)	6.05 (0.57)
Ketoglutaric acid	34.4 (8.4)	20.1 (3.7)	4.20 (0.72)	2.19 (0.48)
Ketovaleric acid	49.8 (10.5)	40.0 (10.2)	9.78 (2.18)	8.65 (1.52)
Malic acid	46.8 (8.4)	28.6 (5.3)	7.90 (1.19)	4.96 (0.61)
Malonic acid	48.3 (11.7)	23.7 (6.9)	5.13 (1.52)	2.37 (1.09)
Pantothenic acid	11.0 (2.4)	6.9 (2.5)	1.87 (0.27)	0.99 (0.27)
Quinic acid	32.4 (5.6)	17.9 (4.8)	5.03 (0.59)	3.07 (0.55)
Succinic acid	37.6 (7.6)	20.9 (4.7)	6.44 (0.96)	3.32 (0.37)
Tartaric acid	19.3 (4.5)	8.7 (2.0)	2.83 (0.34)	2.02 (0.48)
Uric acid	28.7 (5.2)	20.4 (3.1)	4.55 (0.41)	3.15 (0.43)
Urocanic acid	20.3 (5.1)	16.1 (2.5)	2.89 (0.54)	2.45 (0.63)

The data were analyzed by two way analysis of variance (ANOVA) to determine the effect of treatment (control, disturbance control, heated) and horizon (O-horizon, mineral soil) on microbial biomass, substrate utilization and biomarker abundance. A non-parametric procedure (PROC RANK, PROC GLM, SAS Institute) was selected because the data violated the assumptions of normality

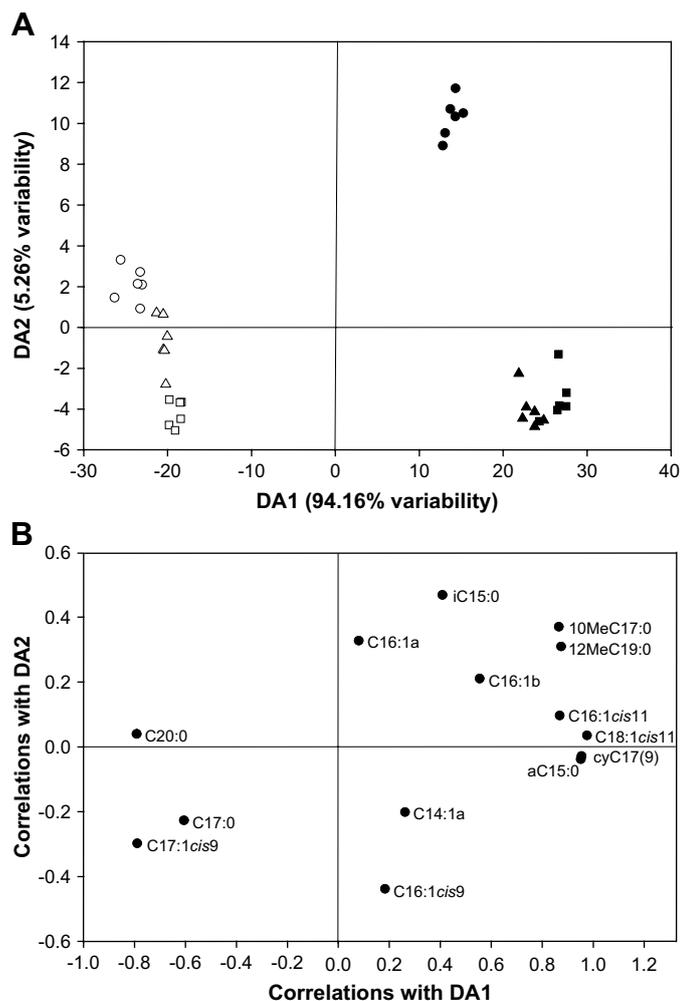
**Table 2**

Extractable lipid P and the quantity of FAME biomarkers representing bacterial and fungal groups (nmol g<sup>-1</sup> soil). There were no significant differences between control and disturbance control plots, so only data from the control and heated plots are shown. Values are means ± one standard error

Biomarker	O-horizon		Mineral soil	
	Control	Heated	Control	Heated
Extractable lipid P	1013 (53)	751 (93)	106.1 (4.6)	77.0 (5.0)
<i>Bacteria</i>				
iC15:0	129 (11)	121 (10)	14.0 (0.6)	12.3 (1.6)
aC15:0	49 (3)	39 (2)	11.0 (0.6)	6.9 (0.7)
C15:0	31 (2)	25 (1)	2.5 (0.1)	2.3 (0.4)
iC16:0	113 (6)	118 (8)	8.5 (0.4)	9.1 (1.6)
C16:1cis7	18 (1)	17 (0.9)	3.7 (0.2)	2.7 (0.3)
iC17:0	24 (1)	24 (1)	4.6 (0.2)	4.2 (0.4)
aC17:0	30 (2)	24 (1)	4.9 (0.2)	3.9 (0.3)
cyC17(9)	35 (2)	28 (3)	5.9 (0.3)	4.1 (0.4)
C17:0	35 (2)	29 (2)	2.7 (0.1)	2.2 (0.3)
cyC19(11)	258 (21)	224 (17)	33.9 (1.7)	26.4 (2.7)
<i>Actinomycetes</i>				
10MeC17:0	25 (2)	22 (2)	4.6 (0.3)	4.3 (0.4)
i10MeC18:0	20 (2)	25 (2)	2.9 (0.1)	2.8 (0.3)
10MeC18:0	30 (2)	31 (3)	3.0 (0.1)	2.9 (0.5)
<i>Fungi</i>				
C18:2cis9,12	321 (18)	258 (26)	12.7 (1.4)	9.1 (1.4)
<i>AM Fungi</i>				
C16:1cis11	48 (7)	42 (3)	7.6 (0.4)	5.6 (0.6)

and homogeneity of variance for standard analysis of variance. The Ryan–Einot–Gabriel–Welsch multiple range test was used to determine significant differences among means at  $P < 0.05$ . There were no significant differences between the two control treatments (control versus disturbance control), thus we focus our discussion below on how heating altered microbial dynamics. Additionally, there were no significant interactions between treatment and horizon for any of the variables analyzed, nor a significant block effect. Discriminate analysis with stepwise selection was also used to analyze the FAME data (Proc Stepdisc, SAS Institute) to determine if heating altered microbial community composition. The discriminate model was then evaluated using canonical discriminant analysis (PROC CANDISC; SAS Institute).

Heating significantly reduced total microbial biomass (determined by extractable lipid P) by 26% and active microbial biomass (determined by substrate-induced respiration following carbohydrate addition) by 45% ( $P < 0.0001$ ; Tables 1 and 2). Lower microbial biomass values are concomitant with reduced labile C contents in the heated plots (Bradford et al., 2008). Amounts of total and active microbial biomass were significantly higher in the O-horizon compared to mineral soil ( $P < 0.0001$ ). Soil moisture content was 33% lower in heated plots for the organic horizon, but there were no significant differences in moisture content between treatments for the mineral soil.



**Fig. 1.** Results of canonical discriminant analysis of the fatty acid methyl ester (FAME) data: (A) canonical discriminant scores for control (squares), disturbance control (diamonds), and heated (circles) samples collected from the O-horizon (open symbols) and mineral soil (closed symbols). (B) Correlations of individual FAMES with the first and second canonical discriminant functions.

Substrate utilization was significantly lower in the heated compared to control plots ( $P < 0.0001$ ; Table 1). On average, utilization of carbohydrates, amino acids, and carboxylic acids was significantly reduced by 45%, 41%, and 35%, respectively, in the heated plots ( $P < 0.0001$  for all three substrate classes). However, no heating effect was observed when the data were normalized to account for differences between treatments in microbial biomass ( $P = 0.4667$ ), indicating that the observed substrate utilization patterns were due to the lower levels of microbial biomass in the heated plots. Utilization of all three substrate types was higher in the O-horizon compared to mineral soil, but this difference was also due simply to higher microbial biomass levels in the organic layer.

A multivariate analysis was used to identify FAMES responsive to the heat treatment and to assess overall differences in microbial community composition. Stepwise discriminant analysis retained 14 significant FAMES for canonical correlation analysis. This analysis showed significant separation between all treatments (Fig. 1a). The discriminant functions associated with the first and second eigen values, DA1 and DA2, accounted for 94.16% and 5.26% of the variance for a total explained variance of 99.42%. Correlations of individual FAMES with the first and second canonical discriminant functions are plotted in Fig. 1b. Although, heated plots shifted negatively from the control plots ( $P < 0.001$ ) for both organic and mineral horizons along DA1, this axis largely separated mineral from organic soil horizons. In contrast, heated plots were shifted positively along DA2, especially for the mineral soil. The relative increase in iso-branched and 10-methyl fatty acids suggests a shift in the microbial community towards gram positive bacteria and actinomycetes in the heated plots. Analysis of variance corroborated the increased relative abundance of actinomycetes in the heated plots ( $P = 0.0021$ ; Table 3).

The total quantity of the fungal biomarker C18:2cis9,12 ( $P = 0.0221$ ) and the AM fungal biomarker C16:1cis11 ( $P = 0.0214$ ) was lower in the heated compared to control plots (Tables 2 and 3). The relative abundance of these markers, however, was not different among treatments ( $P = 0.1032$  and  $0.8514$  for fungi and AM fungi, respectively). Neither the absolute ( $P = 0.0645$ ) nor the relative ( $P = 0.3808$ ) abundance of total bacterial markers was significantly different among treatments due to the fact that some bacterial markers increased while others decreased (Tables 2 and 3).

**Table 3**

Relative abundance of FAME biomarkers representing bacterial and fungal groups (mol%). There were no significant differences between control and disturbance control plots, so only data from the control and heated plots are shown. Values are means  $\pm$  one standard error

Biomarker	O-horizon		Mineral soil	
	Control	Heated	Control	Heated
<i>Bacteria</i>				
iC15:0	4.11 (0.23)	4.26 (0.10)	4.58 (0.06)	4.91 (0.13)
aC15:0	1.58 (0.09)	1.38 (0.06)	3.59 (0.15)	2.82 (0.16)
C15:0	0.99 (0.05)	0.90 (0.02)	0.80 (0.04)	0.90 (0.04)
iC16:0	3.62 (0.11)	4.24 (0.29)	2.78 (0.04)	3.52 (0.30)
C16:1cis7	0.57 (0.03)	0.59 (0.02)	1.21 (0.04)	1.13 (0.03)
iC17:0	0.77 (0.03)	0.86 (0.02)	1.52 (0.06)	1.75 (0.10)
aC17:0	0.96 (0.03)	0.87 (0.03)	1.59 (0.03)	1.64 (0.12)
cyC17(9)	1.13 (0.04)	1.00 (0.04)	1.94 (0.04)	1.71 (0.09)
C17:0	1.14 (0.09)	1.02 (0.02)	0.89 (0.03)	0.90 (0.02)
cyC19(11)	8.22 (0.47)	7.91 (0.22)	11.1 (0.36)	10.91 (0.41)
<i>Actinomycetes</i>				
10MeC17:0	0.80 (0.08)	0.77 (0.04)	1.51 (0.03)	1.75 (0.05)
i10MeC18:0	0.65 (0.05)	0.90 (0.09)	0.96 (0.06)	1.16 (0.05)
10MeC18:0	0.94 (0.05)	1.13 (0.12)	0.98 (0.01)	1.13 (0.07)
<i>Fungi</i>				
C18:2cis9,12	10.31 (0.61)	9.11 (0.59)	4.10 (0.28)	3.65 (0.17)
<i>AM Fungi</i>				
C16:1cis11	1.53 (0.20)	1.51 (0.07)	2.48 (0.06)	2.32 (0.10)

In summary, 12 years of continuous soil warming resulted in significantly lower levels of microbial biomass likely due to a reduced availability of labile C compounds. Lower levels of microbial biomass in the heated plots translated to reduced utilization of a range of simple organic substrates. The primary differences observed in microbial community composition were between the O-horizon and mineral soil samples; however, there was a shift towards gram positive bacteria and actinomycetes with prolonged heating of mineral soil despite a significant overall reduction in microbial biomass. The abundance of the two fungal biomarkers was also reduced by the heating treatment. This is consistent with several other studies where a decrease in fungal biomass or relative fungal abundance has been observed (Waldrop and Firestone, 2004; Rinnan et al., 2007). It is also possible that the composition of the fungal community has been altered by chronic warming; however, this cannot be assessed with fatty acid analysis.

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