

## ORIGINAL PAPER

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## Microbial response of an acid forest soil to experimental soil warming

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**Abstract** Effects of increased soil temperature on soil microbial biomass and dehydrogenase activity were examined on organic (O) horizon material in a low-elevation spruce-fir ecosystem. Soil temperature was maintained at 5 °C above ambient during the growing season in the experimental plots, and soil temperature, moisture, microbial biomass, and dehydrogenase activity were measured during the experiment. An incubation study was also conducted under three temperature regimes, 5, 15, and 25 °C, and under four moisture regimes of 20, 120, 220, and 320% to further evaluate these environmental factors on dehydrogenase activity and microbial biomass. Soil moisture content and microbial biomass controls were significantly lower (30% and 2 µg g<sup>-1</sup> soil, respectively) in the heated plots during the treatment period, suggesting that moisture content was important in controlling microbial biomass. In the incubation study, temperature appeared more important than moisture in controlling microbial biomass and dehydrogenase activity. Increasing temperature between 5 °C and 25 °C resulted in significant decreases in microbial biomass and dehydrogenase activity.

**Key words** Forest soil activity · Microbial biomass · Temperature · Moisture · Dehydrogenase

### Introduction

Atmospheric concentrations of the trace gases carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) have been increasing over the past century as a result of human activities including fossil fuel combustion, destruction of tropical rainforests, and other land use changes (Post et al. 1990). Some predict a warming climate as a direct result of these increases in greenhouse gases (Taylor and MacCracken 1990; Tirpak 1990). In light of the potential implications of climate warming on forests, the Temperature Manipulation Project (TeMP) was designed to investigate the effects of atmospheric warming on soil processes in a spruce-fir ecosystem of northern New England (Rustad and Fernandez 1998).

Microbial biomass and activities vary with seasonal patterns of soil temperature, moisture and substrate availability (Clarholm and Rosswall 1980; Sarathchandra et al. 1989). Soil microbial biomass values tend to be highest in spring and fall and lowest in summer and winter (Bååth and Söderström 1982; Sarathchandra et al. 1989; Luizao et al. 1992; Diaz-Raviña et al. 1993). However, Holmes and Zak (1994) reported that soil microbial population did not vary in size over time in a northern hardwood forest.

Microbial biomass and activities are important ecosystem characteristics to be used for predicting rates of nutrient cycling. Both microbial biomass and activities can be affected by changing soil temperature and moisture regimes (Nadelhoffer et al. 1991; Ellert and Bettany 1992; Pilbeam et al. 1993). Reports in the literature indicate that soil microbial biomass and activity may increase in response to soil warming (Alexander 1977; Sprent 1987; Lloyd and Taylor 1994). However, indirect physical effects of warmer soil temperatures, such as a decrease in soil moisture, may also have negative effects on the microbial community (Van Gestel et al. 1993). An increase in microbial population can lead to nutrient immobilization (Nadelhoffer et al. 1991;

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Holmes and Zak 1994) and therefore decrease the availability of nutrients to higher plants. However, an increase in microbial activities can lead to an increase in net mineralization and therefore an increase in nutrient availability to plants. Understanding seasonal patterns and environmental influences on both microbial biomass and activity are key to predicting changes in nutrient cycling. This study was designed to provide insight on the role of the soil microbial community in determining ecosystem response to soil warming as part of the TeMP project.

## Materials and methods

### Site description

Field research was conducted adjacent to the Howland Integrated Forest Study (HIFS) site, at Howland, Maine. The HIFS site is located in eastern central Maine (45°10'N, 68°40'W) approximately 100 km north of the Atlantic Ocean. Table 1 shows selected soil properties found at the HIFS site (Fernandez et al. 1993). A more detailed description of the site and soil characteristics are presented in Fernandez et al. (1993).

### Experimental design

This experiment utilized four 15 m × 15 m plots; two were heated plots and two were control plots. Experimentally increased soil temperature was achieved by installing high-resistance electrical heating cables in the O horizon 2 cm below the soil surface. Soil temperature was elevated by 5°C in the O horizon of the heated plots from June to November in both 1993 and 1994 (Table 1). A detailed explanation of the experimental design is provided in Rustad and Fernandez (1998).

Sampling was conducted monthly in the fall of 1993 and summer of 1994. On each sampling date, soil thermocouples were used to measure soil temperature. Soil samples were removed from the O horizon by inserting steel pipe (4.1 cm inner diameter) into the soil. The samples were carefully extruded from the pipe and placed into individual Ziplock bags and stored on ice until taken to the laboratory for storage at 4°C. Samples were analyzed individually and later averaged by plot. Before analyses, samples were sieved through a 19-mm mesh screen to reduce variability and interference from plant roots (Ross 1988). All analyses were completed within 14 days of sampling except for the samples for microbial biomass collected in October and November which were analyzed in January of 1994. Ross (1991) found a 61% reduction in microbial biomass after 14 months of storage, but did not speculate on the chronology of this reduction. We recognize the possible influence of the time factor on the October and November samples; however, as our storage time was much shorter than that of Ross (1991), it is unlikely they were as large as those reported by Ross (1991) and this factor is considered in our interpretations.

### Laboratory analyses

All results are reported on an oven-dry soil basis. Dehydrogenase activity (DHA) was measured as described by Tabatabai (1982), but this protocol was modified to account for the volume and large adsorptive capacity of the O soil material. Therefore, 6 g of O soil sample was mixed with 0.06 g of CaCO<sub>3</sub>, and 2 g of this mixture was placed in each of three 10-ml test tubes.

Microbial biomass was determined by substrate induced respiration (SIR) as described by Anderson and Domsch (1978) and Parkinson and Paul (1982). This method was chosen because of limited sample sizes available in this study. It should be recognized that SIR is not a direct measure of microbial biomass, but a commonly accepted estimate of this soil parameter. Ten grams of field moist soil was weighed into a pint-sized Mason jar, covered, and allowed to acclimate to room temperature for 24 h. Then, 0.2 g of glucose with 0.5 g of talc was added to the soil. After mixing with a stirring rod for 1 min, the jar was covered. A 0.5-ml sample of gas was immediately removed from the head space through a septum in the lid of the jar. Subsequent samples were removed every hour for 3 h. Each sample was analyzed immediately for CO<sub>2</sub> concentration on a Gow-Mac Series 580 gas chromatograph. The difference between the amount of CO<sub>2</sub> respired each consecutive hour was calculated and the largest amount respired in a 1-h period was used as the maximum initial respiratory response. To convert maximum initial respiration rate to microbial biomass C, a conversion factor was calculated by comparing the results of SIR to chloroform fumigation-extraction (FE; Voroney et al. 1993) on 20 sub-samples. This method was chosen because of its rapid nature and applicability to soils of low pH (Voroney et al. 1993). The conversion equation was:

$$x = 89.14y \quad (1)$$

where  $x$  is micrograms microbial C g<sup>-1</sup> dry soil and  $y$  is the maximum initial rate of soil respiration, expressed in milliliters CO<sub>2</sub> g<sup>-1</sup> dry soil h<sup>-1</sup>.

### Incubation study

A laboratory incubation study was conducted to examine O horizon soil material responses to a range of temperatures and moistures under controlled conditions. In the fall of 1993, O horizon material was collected at the HIFS site and stored in a large plastic bag at 4°C. In February 1994, the soil was sieved through a 19-mm screen and air-dried for 5 days. After drying, soil samples were homogenized and remoistened with deionized water to moisture levels of 20, 120, 220, and 320%, henceforth referred to as M1, M2, M3, and M4, respectively. There were 6 replicates for each moisture level for a total of 24 samples. Samples were allowed to equilibrate for 24 h before initial DHA and microbial biomass were determined. Total mass of the samples was measured, and samples were placed into individual plastic jars and covered. Two sub-samples of each moisture level were then incubated at either 5, 15, or 25°C (T1, T2, and T3, respectively) for 4 weeks. At the end of each week, the samples were weighed and any mass loss was assumed to be due to moisture loss and the appropriate amount of deionized water was added. Dehydrogenase activity was determined at the end of each week and after 4 weeks final DHA and SIR were determined.

**Table 1** Selected organic horizon soil properties calculated from quantitative soil pit data, Howland, Me

pH (H <sub>2</sub> O)	pH (CaCl <sub>2</sub> )	Total C	Total N	C/N	Total S (%)	Ex Ca	Ex Mg	Ex K	Ex Na	Ex Al	CEC
						(cmolc kg <sup>-1</sup> )					
3.6	2.8	47.1	1.3	38	0.16	9.5	2.8	1.7	0.3	5.4	30.1

Ex = exchangeable

CEC = cation exchange capacity

**Table 2** Monthly means for selected soil variables from heated (H) and control (C) plots (TRT treatment)

Variable	TRT	Month							
		Sept	Oct	Nov	Dec <sup>a</sup>	May <sup>a</sup>	June	July	Aug
Temp <sup>b</sup>	H	18.7 <b>b</b> * <sup>c</sup>	9.0 <b>d</b> *	7.5 <b>d</b> *	1.3 <b>e</b>	5.6 <b>f</b>	12.9 <b>c</b> *	19.1 <b>b</b> *	21.5 <b>a</b> *
	C	14.3 <b>b</b>	4.3 <b>e</b>	3.2 <b>e</b>	1.2 <b>f</b>	6.5 <b>d</b>	10.6 <b>c</b>	14.5 <b>b</b>	18.1 <b>a</b>
Mois	H	75 <b>d</b>	99 <b>bcd</b> *	150 <b>b</b>	110 <b>c</b> *	193 <b>a</b>	166 <b>a</b> *	124 <b>bc</b> *	93 <b>d</b> *
	C	99 <b>c</b>	154 <b>abc</b>	144 <b>abc</b>	145 <b>b</b>	191 <b>a</b>	195 <b>a</b>	159 <b>ab</b>	134 <b>bc</b>
DHA	H	6.76 <b>d</b>	4.00 <b>d</b>	7.31 <b>cd</b>	11.48 <b>bc</b> *	20.29 <b>a</b>	14.51 <b>a</b> *	15.89 <b>ab</b> *	8.34 <b>c</b> *
	C	4.87 <b>c</b>	4.00 <b>c</b>	10.04 <b>c</b>	24.40 <b>a</b>	24.18 <b>a</b>	23.23 <b>ab</b>	22.71 <b>a</b>	16.94 <b>b</b>
SIR	H	4.65 <b>bd</b> *	4.10 <b>b</b> *	6.76 <b>ad</b>	5.11 <b>bcd</b> *	7.16 <b>a</b>	7.98 <b>a</b>	6.88 <b>ad</b> *	7.46 <b>ac</b> *
	C	6.89 <b>bc</b>	7.00 <b>bc</b>	7.89 <b>ab</b>	6.74 <b>b</b>	7.73 <b>ab</b>	7.98 <b>ab</b>	10.72 <b>a</b>	9.11 <b>ac</b>

<sup>a</sup> No treatment applied

<sup>b</sup> Dependent variables: *TEMP* Temperature (°C), *MOIS* Moisture (%), *DHA* (dehydrogenase activity), triphenylformazan ( $\mu\text{g g}^{-1}$  soil), *SIR* (substrate induced respiration), microbial biomass ( $\mu\text{g g}^{-1}$  soil)

<sup>c</sup> Within treatments, values followed by the same letter are not significantly different ( $\alpha=0.05$ ) between months; within months, values followed by '\*' indicate significant treatment effects ( $\alpha=0.05$ )

### Statistical analyses

The field study was designed as a single factor randomized complete block design with two treatments and two replications. The incubation study was designed as a  $3 \times 4$  factorial with one replication. Multivariate Analysis of Variance (MANOVA) with repeated measures and Tukey's mean separation was conducted on ranked data using SAS (SAS Institute 1990) with an alpha level of 0.05.

## Results and discussion

### Seasonal patterns

To assess potential seasonal patterns in microbial biomass and DHA, only control plot data were used to avoid any possible interactions with treatment effects. Table 2 shows the O horizon soil monthly means for selected variables.

DHA was lowest in the fall of 1993 following a relatively dry period at the HIFS site and was positively correlated with soil moisture (Table 3), but not with soil temperature. As fall progressed, soil moisture content increased and DHA increased, despite lower soil temperatures. This may explain the lack of correlation between soil temperature and DHA. Increases in soil organic matter (SOM) from litterfall may have also

played a role in increasing DHA because this procedure is a measurement reflecting rates of primary decomposition (Tabatabai 1982). Slow decomposition of SOM during winter likely increases the availability of oxidizable substrates to microbial populations in the spring. As temperatures become more favorable, there would be a flush of microbial activity associated with the oxidation of this material (Ivarson and Sowden 1970; Ross 1972). Evidence for this flush at HIFS was demonstrated in the O horizon in May, with DHA values similar to values from December (Table 2).

Organic soil microbial biomass was highest in July, a warm, relatively moist month. When both temperature and moisture were combined in a multiple regression to predict soil microbial biomass, a significant but small amount of the variability was accounted for by the equation ( $P < 0.0001$ ;  $R^2 = 0.30$ ). Christ et al. (1997) described a similar significant relationship between soil temperature, moisture, and  $\text{CHCl}_3$ -labile C, N, and P for O horizon soils from reference plots at HIFS. In their study on a forested sandy soil, Goncalves and Carlyle (1994) reported that microbial biomass did not respond to warmer summer soil temperatures because microbial biomass was restricted by soil moisture contents. Results reported here agree with Goncalves and Carlyle (1994), as the lowest level of microbial biomass occurred in September, when soil moisture content was lowest. As fall progressed, soil microbial biomass began to increase with increasing soil moisture content, despite lower soil temperatures. Sarathchandra et al. (1989) reported a build-up of microbial biomass during the colder winter months but did not directly attribute the increase to temperature or moisture but to a dominance of fungal biomass decomposing dead plant biomass.

Seasonal soil microbial biomass fluctuations are not consistent with the results reported by Holmes and Zak (1994). They reported no significant seasonal fluctuations in microbial biomass C and N for homogenized Oa, A, and E horizon material from northern hardwood forest stands. They reasoned that the microbial

**Table 3** Significant ( $\alpha=0.05$ ) correlation coefficients ( $r$ ) between measured variables for each treatment and soil type

Variable pairs <sup>a</sup>		Control	Heated
Temp	Mois	–	–0.36
Temp	DHA	–	–
Temp	SIR	+0.26	–
Mois	DHA	+0.22	+0.28
Mois	SIR	+0.19	+0.35
SIR	DHA	+0.22	+0.39

<sup>a</sup> Abbreviations for dependent variables: *TEMP* temperature (°C), *MOIS* moisture (%), *DHA* triphenylformazan ( $\mu\text{g g}^{-1}$  soil), *SIR* microbial biomass ( $\mu\text{g g}^{-1}$  soil)

population was stable throughout the year because of constant inputs of SOM, particularly root exudates. However, their homogenized soil included mineral material and therefore may not be directly comparable to softwood O material results reported here.

### Treatment effects

Table 2 shows data for all months when field measurements were made, although there was no treatment during December and May. Only data from treatment period months were used in statistical analyses. Soil temperature significantly increased with experimental warming treatment, soil moisture and microbial biomass significantly decreased and DHA decreased numerically. Soil moisture content was lower in the heated plots during the treatment period every month except for November. Soil moisture content of the heated plots may not have decreased in November because of higher precipitation rates that time of year, minimizing the warming effect on soil moisture. Unlike the control plots, soil moisture content was negatively correlated with soil temperature in the heated plots (Table 3).

The data suggest that DHA was more strongly influenced by soil moisture content than soil temperature in these moist northern coniferous forest soils (Table 3). For example, November soil temperature was significantly greater in the heated plots but there were no significant treatment effects in soil moisture or DHA. In December, there was no significant temperature treatment, but there was significantly lower soil moisture and DHA in the heated plots. In June, July and August treated plots were significantly warmer, and both soil moisture content and DHA decreased in the heated plots during this period.

Microbial biomass was significantly lower in the heated plots in all treatment months except November and June. Table 3 shows the positive correlation between soil microbial biomass and soil moisture, therefore, it is not surprising that without a significant de-

crease in soil moisture in November, there was not a significant decrease in microbial biomass. However, in June, there is a significant decrease in soil moisture content, but no difference in microbial biomass. It is possible that soil moisture content in the control plots may have been adequate for the soil microbial population, despite the treatment effect. Christ et al. (1997) reported no effect of soil warming on soil moisture, nor any effect of soil warming on microbial biomass. The results from both of the experiments at HIFS describe a positive relationship between soil moisture content and soil microbial biomass populations.

### Incubation study

The incubation study was conducted for four consecutive weeks to evaluate the interactive effects of temperature and moisture on microbial processes under controlled conditions. Microbial activity changed significantly over time, but microbial biomass did not (Table 4). Dehydrogenase activity was not significantly different between time zero and week one; however in all subsequent weeks DHA was significantly greater than at time zero and the previous week(s). For between subject effects, temperature had a significant effect on microbial biomass. Moisture had a significant effect on DHA and SIR. There were no significant temperature-moisture interactions.

Table 4 shows microbial activity and biomass data from the beginning and end of the incubation study, and the difference between the two at each temperature and moisture regime. Each temperature regime is averaged across all the moisture regimes; each moisture regime is averaged across all temperature regimes. Within T1, DHA was significantly greater at the end of the incubation study than at the beginning. Temperature regimes 2 and 3 both increased, but these increases were not significant. The change in DHA in T1 was significantly greater than the changes in T2 or T3 (Table 4). The high DHA in T1 was consistent with the inverse relationship between soil temperature and DHA

**Table 4** DHA and microbial biomass at the start and after a 4-week interim at different temperature and moisture levels

Week	Variable <sup>a</sup>	Temperature regime			Moisture regime			
		T1	T2	T3	M1	M2	M3	M4
Week 0	DHA	7.72 a <sup>b</sup>	6.62 a	8.72 a	2.64 c	3.84 c	7.71 b	16.35 a
	SIR	5.78 a	5.30 a	5.59 a	0.28 c	5.79 b	7.63 a	7.87 a
Week 4	DHA	18.03 a	9.39 c	12.52 b	2.33 d	4.65 c	16.12 b	30.15 a
	SIR	6.39 a	5.31 b	4.35 c	0.27 c	6.02 b	7.58 a	7.53 a
Net change	DHA	10.30 a*	2.78 b	3.79 b	-0.31 c	0.81 c	8.42 b*	13.80 a*
	SIR	0.61 a*	0.00 b	-1.21 c*	-0.02 a	0.24 a	-0.05 a	-0.34 a

<sup>a</sup> DHA triphenylformazan ( $\mu\text{g g}^{-1}$  soil), SIR microbial biomass ( $\mu\text{g g}^{-1}$  soil)

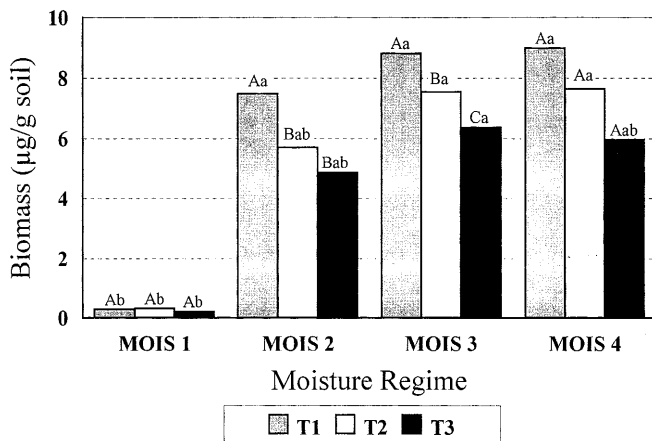
<sup>b</sup> Within rows and treatment type (T or M), values followed by the same letter are not significantly different ( $\alpha=0.05$ ); values followed by an "\*" indicate significant ( $\alpha=0.05$ ) differences between week 0 and week 4

reported in the field experiment when soil moisture was not limiting.

Microbial biomass in T1 was significantly greater at the end of the incubation study, showed no change in T2 and decreased significantly in T3 (Table 4). The high microbial biomass values at T1 are inconsistent with the field results which showed a positive correlation with soil temperature in the control plots (Table 3). One possible explanation may be that field soil temperatures were generally much cooler than the range employed in the study. The positive relationship described between soil temperature and soil microbial biomass in the field did not include any temperatures above 21.5 °C. The incubation study showed that for O horizon material over a wide soil moisture range, 25 °C may not be optimal for the indigenous microbial populations.

Dehydrogenase activity increased between the beginning and end of the 4-week incubation period for all moisture regimes except M1, where DHA decreased (Table 4), which is consistent with the field study (Table 3). Changes in microbial biomass after 4 weeks of incubation were not significantly different among moisture regimes (Table 4), which is inconsistent with field results (Table 3).

Figure 1 shows microbial biomass values at Week 4 of the incubation study. The figure suggests that M1 was too low (20%) to sustain a large viable microbial biomass. This suggests that between M1 (20%) and M2 (120%) a threshold exists above which soil moisture is no longer limiting and, above this threshold, soil temperature had a greater influence on microbial biomass. In the field study, microbial biomass was positively correlated to soil moisture, but this was for field moisture contents ranging from 75% to 195% (Table 1). In fact, close examination of the field data shows that, except for September, when soil moisture content in the heated plots was close to 120% and the control plots



**Fig. 1** Microbial biomass from week 4 of the incubation study. Upper case letters indicate significant differences among temperature regimes within moisture regimes. Lower case letters indicate significant differences among moisture regimes within temperature regimes

were above 120%, there was significantly less microbial biomass in the heated plots (Table 1). Increasing temperature clearly results in declining microbial biomass for all moisture regimes.

The field and laboratory data together suggested that cooler, moister soil conditions favor more active microbial populations and may lead to nutrient mineralization while warmer, moister soil conditions result in a shift towards increased microbial biomass that may lead to nutrient immobilization. Soil microbial response to experimental soil warming was most evident when soil moisture was not limiting. Results suggested that an optimum soil moisture content range existed between 20% and 120% for microbial biomass. Above this range, soil temperature was more important in controlling the size of microbial biomass. Future research needs to better define the complex interactions between soil moisture content and temperature, and their effects on forest soil microbial communities. The interplay described here in microbial community response to soil warming and moisture, and consequences for nutrient cycling, would be only initial stages of the ecosystem shifts expected over longer time frames.

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