

Controls on microbial CO₂ production: a comparison of surface and subsurface soil horizons

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Abstract

Although a significant amount of the organic C stored in soil resides in subsurface horizons, the dynamics of subsurface C stores are not well understood. The objective of this study was to determine if changes in soil moisture, temperature, and nutrient levels have similar effects on the mineralization of surface (0–25 cm) and subsurface (below 25 cm) C stores. Samples were collected from a 2 m deep unsaturated mollisol profile located near Santa Barbara, CA, USA. In a series of experiments, we measured the influence of nutrient additions (N and P), soil temperature (10–35 °C), and soil water potential (–0.5 to –10 MPa) on the microbial mineralization of native soil organic C. Surface and subsurface soils were slightly different with respect to the effects of water potential on microbial CO₂ production; C mineralization rates in surface soils were more affected by conditions of moderate drought than rates in subsurface soils. With respect to the effects of soil temperature and nutrient levels on C mineralization rates, subsurface horizons were significantly more sensitive to increases in temperature or nutrient availability than surface horizons. The mean Q_{10} value for C mineralization rates was 3.0 in surface horizons and 3.9 in subsurface horizons. The addition of either N or P had negligible effects on microbial CO₂ production in surface soil layers; in the subsurface horizons, the addition of either N or P increased CO₂ production by up to 450% relative to the control. The results of these experiments suggest that alterations of the soil environment may have different effects on CO₂ production through the profile and that the mineralization of subsurface C stores may be particularly susceptible to increases in temperature or nutrient inputs to soil.

Keywords: carbon storage, CO₂, microbial respiration, Q_{10} , soil carbon, soil profile

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Introduction

Soils are often many meters deep and contain significant quantities of organic carbon in both the surface (arbitrarily defined here as 0–25 cm) and the subsurface (below 25 cm) soil horizons. Although organic C concentrations in subsurface horizons are generally much lower than in surface horizons, the total volume of soil contained in subsurface horizons can be very large. On a global basis, more than 50% of the organic C contained within 1 m deep soil profiles is found in subsurface soil horizons (Batjes, 1996).

On a per unit carbon basis, the rate of microbial mineralization of the organic C residing in subsurface

horizons is generally low and subsurface organic C pools have long mean residence times in the soil (Desjardins *et al.*, 1994; Paul *et al.*, 1997; Trumbore, 2000). Although subsurface organic C pools are not mineralized at high rates, the amount of CO₂ produced in deeper soil horizons by microbial mineralization can be substantial and, in some soils, may account for a significant portion of total soil CO₂ production (Wood *et al.*, 1993; Ajwa *et al.*, 1998; Hendry *et al.*, 1999; Gaudinski *et al.*, 2000).

Human activity can significantly affect the rates of microbial CO₂ production in soils by directly or indirectly altering the soil environment (Anderson, 1991; Rustad *et al.*, 2000; Tate, 2000). However, we do not know if subsurface C stores are particularly responsive to modifications of the soil environment.

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Since deeper soil horizons contain large quantities of sequestered organic C (Batjes, 1996; Jobbagy & Jackson, 2000), even small changes in the rates of subsurface C mineralization could significantly alter soil C dynamics over time. In order to understand whole profile C dynamics and the effects of global change on soil C storage, we need to identify the environmental controls on organic C mineralization in both surface and subsurface soil horizons.

In this study, we chose to examine how three different environmental factors soil moisture, soil temperature, and soil nutrient levels (N and P) affect the rates of soil organic C mineralization through the profile. We chose these factors because they are the most important controls on the rate of soil organic C mineralization in many ecosystems (Schimel *et al.*, 1994; Davidson *et al.*, 1998; Leiros *et al.*, 1999; Tate, 2000) and because many soils are expected to experience changes in these environmental conditions in the future (Anderson, 1991; Rustad *et al.*, 2000). Climate change is likely to have a significant impact on soil moisture and temperature conditions (IPCC, 1996; Loaiciga *et al.*, 1996), with the direct or indirect fertilization of soils by human activity (Vitousek *et al.*, 1997; Smil, 2000) potentially increasing the inputs of N and P to surface and subsurface soil horizons.

We expect the nature of the controls on microbial CO₂ production to change with depth through the soil profile. There are two reasons for this hypothesis. (i) The organic C residing at depth is generally of lower 'quality', since subsurface C stores are often protected physically and/or chemically from microbial mineralization (Desjardins *et al.*, 1994; Ajwa *et al.*, 1998; Trumbore, 2000). Herein, we define C 'quality' as the degree to which the soil organic C is resistant to microbial mineralization (Bosatta & Agren, 1999). (ii) The microbial communities residing in subsurface horizons are distinct in composition from those found in surface horizons (Fierer *et al.*, 2003; LaMontagne *et al.*, in press). We suspect that soil C dynamics and the specific controls on microbial CO₂ production will be affected by any changes in organic C 'quality' (French, 1988; Bosatta & Agren, 1999; Vance & Chapin, 2001; Mikan *et al.*, 2002) and microbial community composition (Schimel, 2001; Balsler *et al.*, 2002).

We studied a 2 m deep soil profile located in Santa Ynez, CA. We collected soil samples from throughout the profile and incubated the samples in the laboratory to measure the effects of nutrient additions (N and P), soil temperature (10–35 °C), and soil water potentials (–0.5 to –10 MPa) on microbial CO₂ production rates in surface and subsurface soil horizons.

Methods

Profile characterization

The studied profile is located on the University of California Sedgwick Reserve (34°42'N, 120°03'W), 30 miles north of Santa Barbara, CA, in the Santa Ynez Valley. The profile was excavated on a valley floor, a depositional zone underlain with the Paso Robles formation, a weakly consolidated alluvium composed largely of shale deposited in the Pleistocene era (Dibblee, 1966). The soil is classified as a Thermic Pachic Haploxeroll. The site is dominated by the annual grasses Mediterranean barley (*Hordeum murinum* L.) and brome grass (*Bromus* spp.). The climate of the region is Mediterranean, the average annual rainfall is 50 cm, and almost all of the rainfall occurs between the months of December and April.

Selected physical and chemical characteristics of the soil profile are given in Table 1; additional information can be found in Fierer *et al.* (2003). The total carbon and nitrogen content was determined using a Fisons NA1500 C/N analyzer. Soil inorganic C concentrations, as determined by standard methods (Allison & Moodie, 1965), are very low in the profile samples so total carbon is equivalent to total organic C and respiration measurements should not be affected by carbonate dissolution. Soil pH was measured with a pH meter (Corning Model 320) after equilibrating 15 g of dry soil with 15 mL of deionized water for 30 min. Particle size and Olsen-P analyses were conducted at the Division of Agriculture and Natural Resources Analytical Laboratory, University of California Cooperative Extension (Davis, CA, USA) using standard methods. Horizon designations were assigned using field and laboratory data in accordance with the USDA soils classification scheme (Soil Survey Staff, 1996). Soil temperatures were measured with two Type K thermocouples permanently installed in the trench walls at each sampling depth. Temperature averages were automatically logged every 2 h with a Campbell CR10x (Logan, UT, USA) data logger. We used the chloroform fumigation–extraction method described by Fierer & Schimel (2002) to measure the microbial biomass levels of samples collected in October 2000.

Sample collection and processing

We excavated the profile in April 2000; the trench measured 10 m × 2 m with a depth of 4 m. Immediately after excavation, the trench walls were sealed with an opaque plastic vapor barrier, roofed, and insulated. The trench structure was designed to permit repeated sampling of the soil profile in a relatively undisturbed state. On three different dates, we collected soil samples

Table 1 General characteristics of the soil profile sampled

Sampling depth (cm)	pH	% C	% N	Olsen P (ppm)		Texture	Horizon	CHCl ₃ extractable-microbial biomass C (µg C g soil ⁻¹)	Average daily temperature (°C)	Range in daily temperatures (°C)	Field %H ₂ O		
				% N	% C						Experiment 1 (3/2001)	Experiment 2 (2/2002)	Experiment 3 (6/2001)
0–5	6.1	3.2 (0.26)	0.30 (0.024)	33	Loam	A	676 (30.0)	24	3.5–53	33 (1.0)	29 (0.05)	5.9 (0.55)	
5–15	6.2	2.0 (0.068)	0.21 (0.011)	25	Loam	A	209 (19.1)	25	8.9–40	29 (0.40)	19 (0.21)	9.1 (0.66)	
15–25	6.2	1.7 (0.020)	0.18 (0.0016)	19	Loam	A	73.9 (10.2)	25	12–32	29 (0.41)	20 (0.05)	13 (0.72)	
50	6.7	0.83 (0.019)	0.10 (0.0022)	13	Loam	AB	57.7 (23.0)	22	13–26	30 (0.16)	18 (0.10)	19 (1.0)	
100	6.9	0.84 (0.022)	0.10 (0.0031)	16	Clay loam	B	79.0 (22.4)	20	14–23	30 (0.68)	19 (0.05)	22 (0.16)	
200	7.5	0.16 (0.024)	0.050 (0.0022)	10	Clay loam	B	24.7 (20.2)	18	14–22	31 (1.1)	28 (0.14)	27 (0.68)	

Average daily temperatures and temperature ranges were measured from March 2001 to September 2001. Moisture contents were determined at the time of sample collection by mass difference before and after drying at 120°C for 48 h. One standard error reported in parentheses. *N* = 3 for each sampling depth.

for the three separate experiments (March 2001, February 2002, and June 2001 for Experiments 1–3, respectively). Gravimetric soil moisture contents at the three sample collection times are shown in Table 1.

The depth increments for soil sampling are shown in Table 1. Three replicate soil samples were collected from each profile at the surface depth increments (0–5, 5–15, and 15–25 cm) by digging vertically from the surface at three randomly chosen locations within 5 m of the trenches. We collected subsurface soil samples by horizontally coring from the inner walls of the trenches; we discarded the outer 25 cm of each core (the 25 cm closest to the trench wall) to ensure that the collected soil was not directly affected by the presence of the excavated trench. Three replicate samples were taken at each depth from randomly chosen locations within the trench. The samples were immediately transported back to the laboratory, sieved to 4 mm, homogenized, and stored at 4 °C for no more than 2 weeks before the start of the individual experiments. Prior to sieving, we removed all visible root and fresh litter material from the samples. Care was taken to prevent cross-contamination of the soil samples during and after collection.

Experiment 1: water potential and soil respiration

With Experiment 1 we determined the influence of soil water potential on C mineralization rates through the soil profile. We chose to adjust the samples to a series of soil water potentials, not water contents, because water potential is a more accurate index of water availability to microorganisms (Harris, 1981). Three replicate soil samples from each depth were adjusted to each of four target soil water potentials (–0.5, –1.5, –5, and –10 MPa) and, after an equilibration period, respiration rates were measured simultaneously on all samples.

Soils were equilibrated to the appropriate water potentials using an isopiestic equilibration technique modified from Harris *et al.* (1970). Isopiestic equilibration allows for more accurate and consistent control of soil water potential than gravimetric water content adjustment, particularly at water potentials lower than –1.5 MPa (Harris *et al.*, 1970). Soil samples were adjusted to water contents close to the target water potentials (as determined by preliminary experiments) by either drying at 20 °C or wetting with deionized H₂O. Individual soil samples (approximately 8 g dry weight) were placed inside 120 mL gas-tight glass Mason jars fitted with rubber septa that had been lined on all interior surfaces with approximately 30 mL of 3% agar. The agar was amended with varying concentrations of NaCl to achieve the desired water potentials along with CuSO₄ (20 g L⁻¹) and 10% H₂SO₄ (5 mL L⁻¹) to limit microbial growth on the agar. All

samples were equilibrated simultaneously at 20 °C for 11 days inside the agar-lined jars. After this equilibration period, samples were maintained at 20 °C in the agar-lined jars and we measured basal respiration rates using a series of static incubations over a 5 day period. We used an infrared gas analyzer (Licor Model LI-6252) to measure headspace CO₂ concentrations. The jars were vented if CO₂ concentrations exceeded 2%.

At each depth and water potential, a fourth subsample that had been isopiesticly equilibrated in an identical manner to the samples used for respiration measurements was used for soil water potential determination. Water potential in these subsamples was measured after 13 days of equilibration (the third day of respiration measurements) using a thermocouple psychrometer equipped with a Richards thermocouple (Decagon Devices, Inc, Pullman, WA, USA, Model SC-10a) that was calibrated using a KCl standard curve ranging from 0 to -10 MPa.

Experiment 2: soil temperature and soil respiration

In Experiment 2, we tested the influence of soil temperature on short-term C mineralization rates through the soil profile. Soil samples (5 g dry weight equivalent) were incubated at field moistures (Table 1) in individual 50 mL plastic tubes sealed with gas-tight lids fitted with rubber septa. We used separate sets of replicate samples to measure respiration rates at each of six temperatures (10–35 °C, 5° increments). All samples were maintained at 5 °C until being warmed to the target temperatures in a controlled temperature incubator (Lab-Line Instruments, Dubuque, IA, USA). After a 6 h equilibration period at the target temperature, CO₂ accumulation in the headspace of each tube was measured over a 24 h period using the technique described for Experiment 1. Soil temperatures were measured continuously using Type K thermocouples and a Campbell CR10x (Logan, UT, USA) data logger to assure that the actual soil temperature did not deviate significantly from the target temperature. Since soil temperature treatments were applied consecutively using a single incubator and starting with the lowest temperature, the 35 °C samples spent 10 more days at 5 °C than the 10 °C samples.

Experiment 3: N and P limitations to CO₂ production

With Experiment 3, we assessed how N and P amendments to soil profile samples affect C mineralization rates. For each of the four treatments (+N, +P, +N+P, and control) four replicate soil samples (10 g dry weight equivalent each) were weighed into individual 50 mL plastic tubes. After the moisture contents of all samples were adjusted to approximately

-0.5 MPa by the addition of deionized H₂O, all samples were incubated at 20 °C for 2 weeks. After this preliminary incubation period, 1 mL of the appropriate nutrient solution was added to each sample. The +N solution was 0.5 M NH₄NO₃ (an addition of 100 µmol N g soil⁻¹), the +P solution was 0.06 M K₂HPO₄ (an addition of 6 µmol P g soil⁻¹), and the +N+P solution was 0.5 M NH₄NO₃ and 0.06 M K₂HPO₄. For the control treatments, we added 1 mL of deionized H₂O to the soil samples. All solutions were adjusted to pH 7 using 1 M NaOH and HCl. Immediately after the addition of the nutrient solutions, all tubes were sealed with gas-tight lids and headspace CO₂ accumulations were measured over the course of a 5 day period, using the technique described for Experiment 1. Tubes were vented if headspace CO₂ concentrations exceeded 2%.

Data analysis

For all three experiments, we calculated the specific respiration rates (µg C g soil C⁻¹ h⁻¹) over the incubation period (5 days for Experiments 1 and 3, 24 h for Experiment 2). The actual respiration rates vary considerably between samples collected from different profile depths. In order to compare the relative responses of soils from different depths to the various treatments and to make variances independent of means, we normalized all respiration data using the procedures described in the captions for Figs 1–3. Both the normalized, or relative, respiration rates and the actual respiration rates are reported.

We used Eqn (1), a linear function with log-transformed data, to summarize the response of respiration rates to the range of water potentials:

$$\log y_w = a(\log \Psi) + b, \quad (1)$$

where y_w is the relative respiration rate at a given water potential, Ψ is the water potential (absolute value in bars), and a and b are rate constants. We conducted one-way ANOVA procedures with Bonferroni adjustments as *post hoc* tests (SYSTAT, 2000) to determine the effect of soil depth on α values. We also used two sample *t*-tests (SYSTAT, 2000) to compare the average relative respiration rates for surface (0–25 cm) and subsurface (25–200 cm) soil samples at specific soil water potentials.

In Experiment 2, we used the empirically derived van't Hoff function (van't Hoff, 1898) to describe the dependence of C mineralization on soil temperature:

$$y_T = de^{kT}, \quad (2)$$

where y_T is the actual respiration rate at a given temperature (in µg C g soil C⁻¹ h⁻¹), T is the temperature in °C, and d and k are constants. From Eqn (2), we calculated the Q_{10} value, the average increase in

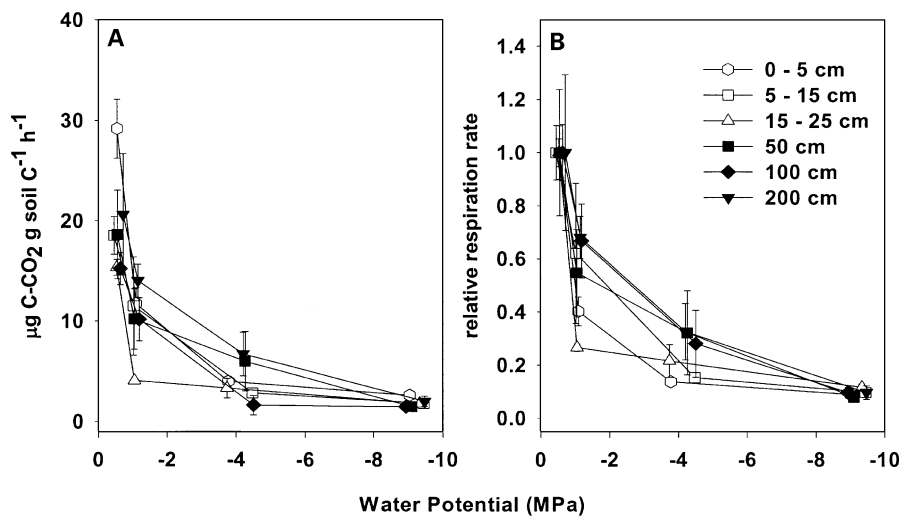


Fig. 1 Relationship between actual (a) and relative (b) respiration rates with soil water potential. Relative rates were calculated as the ratio between the average respiration rate at the highest water potential (-0.5 MPa) for the specific soil depth and the actual respiration rate for a given field replicate. $N = 3$ for each sampling depth. Bars indicate ± 1 standard error.

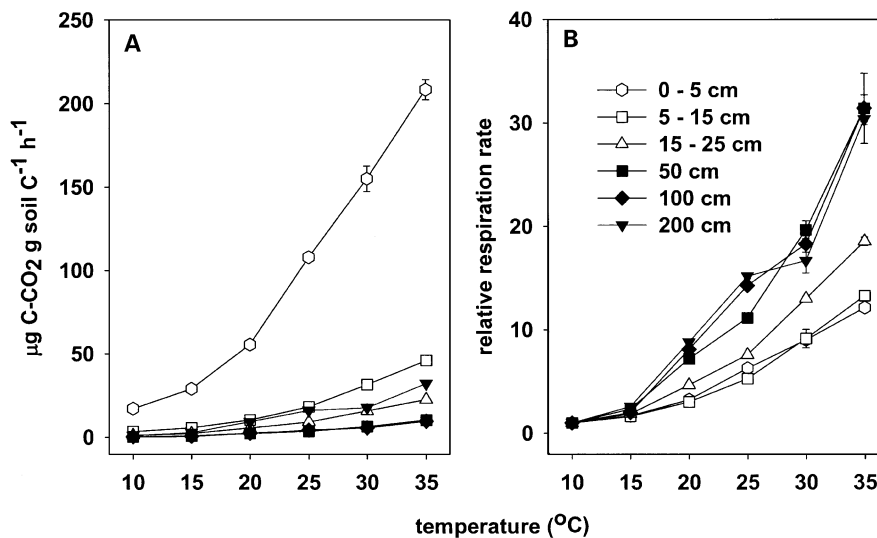


Fig. 2 Relationship between actual (a) and relative (b) respiration rates with soil temperature. The relative rate was calculated as the ratio between the actual respiration rate for a given field replicate and the average respiration rate at 10°C for a given soil depth. $N = 3$ for each sampling depth. Bars indicate ± 1 standard error.

respiration rates for a 10°C increase in temperature:

$$Q_{10} = e^{10k}. \quad (3)$$

Values for Q_{10} and the rate constant d in Eqn (2) were compared using one-way ANOVAS (SYSTAT, 2000) in order to assess the significance of soil depth on specific respiration rates (d) and the temperature responses of respiration rates (Q_{10}).

For Experiment 3, we conducted two separate sets of one-way ANOVAS. Within each sampling depth, we compared actual respiration rates with the various treatments to determine if nutrient additions had a significant effect on respiration rates relative to the control treatment. We also compared the influence of soil depth on relative respiration responses with each of the three nutrient amendments (+N, +P, +N + P).

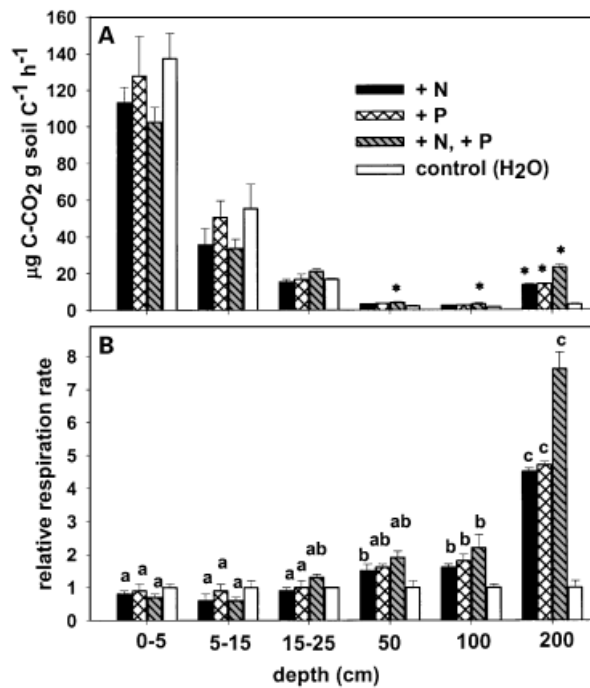


Fig. 3 Average actual (a) and relative (b) respiration rates for the 5 day period following nutrient amendments. Relative rates were calculated as the ratio between the actual respiration rate for a given field replicate and the average respiration rate of the control treatment (+ H₂O) at a given soil depth. In (a), statistical analyses were conducted separately within each sampling depth; * = actual respiration rates are significantly different ($P < 0.05$) from the control. In (b), we compare the influence of soil depth on relative respiration rates for each nutrient treatment. Statistical analyses were performed separately for each of the three nutrient treatments; means with the same letter were not significantly different ($P < 0.05$) from the other sampling depths. $N = 4$ for each sampling depth. Bars indicate one standard error.

Results

Water potential and C mineralization rates

C mineralization rates were highly dependent on soil water potential (Fig. 1). The linear function used to describe the relationship between respiration rates and water potentials (Eqn (1)) fit the individual data sets well and the r^2 values were > 0.85 for each field replicate sample ($N = 3$ per depth). The relative respiration rates at the intermediate water potentials (-1 and -4 MPa) were slightly lower for surface soils than for subsurface soils. However, the slope values (a in Eqn (1)) were not significantly related to soil depth ($P = 0.45$), so we can conclude that, across the full range of tested water potentials, the fundamental relationship between soil C mineralization rates and soil water potential does not change appreciably with soil depth.

When relative respiration rates for samples from all depths are analyzed together using Eqn(1), the dependence of mineralization rates on soil water potential is summarized by ($r^2 = 0.85$)

$$\log y_w = -0.82(\log \Psi) + 0.6. \quad (4)$$

Although the slope of C mineralization rates vs. water potential does not change significantly across the full range of tested water potentials, the surface soils tended to have lower relative respiration rates at -1.5 and -5 MPa (Fig. 1). If we compare the average relative respiration rates of surface (0–25 cm) and subsurface (25–200 cm) soil samples at these two water potentials, we find that surface soils have, on average, lower rates at both -1.5 and -5 MPa ($P = 0.04$ and 0.01 , respectively).

Soil temperature and C mineralization rates

Respiration rates increased exponentially between 10°C and 35°C (Fig. 2) in all samples. The van't Hoff function (Eqn (2)) adequately describes the relationship between soil respiration and soil temperature, and the r^2 values for individual field replicates were always > 0.90 . The mean values for the rate constant (Eqn (2)) declined exponentially with soil depth (Table 2), a trend that is also evident in Fig. 2a. The calculated Q_{10} values (Table 2) changed significantly with soil depth ($P = 0.002$), with Q_{10} values increasing consistently with depth through the soil profile. In each case, the Q_{10} values calculated for the surface samples (0–25 cm depth) were significantly lower ($P < 0.05$ in all cases) than the Q_{10} values measured for the subsurface samples (50–200 cm). The increase in Q_{10} values with depth is evidence that C mineralization rates are more sensitive

Table 2 Temperature dependence on respiration rates through the soil profile

Sampling depth (cm)	D	Q_{10}
0–5	7.1 ^a (0.14)	2.8 ^a (0.01)
5–15	1.3 ^b (0.13)	3.0 ^a (0.10)
15–25	0.50 ^c (0.04)	3.2 ^a (0.14)
50	0.13 ^d (0.01)	3.7 ^b (0.14)
100	0.12 ^d (0.01)	3.8 ^b (0.09)
200	0.40 ^{cd} (0.02)	4.1 ^b (0.10)

Q_{10} and The rate constant d and Q_{10} are parameters calculated from Eqns (2) and (3), respectively. Values were calculated for the temperature range of 10 – 35°C . Mean d and Q_{10} values that are not marked with the same letter differ significantly ($P < 0.05$) between depths. One standard error reported in parentheses. $N = 3$ for each sampling depth.

to temperature in subsurface soil horizons than in surface horizons.

Nutrient limitations to C mineralization

When the results from each sampling depth are analyzed independently, we find that the effects of the nutrient treatments on actual respiration rates (Fig. 3a) were only significantly different from the control treatment at depths of 50 cm and below ($P = 0.04$, 0.03 , and 0.001 for 50, 100, and 200 cm, respectively). In the surface soils, the addition of N caused a slight (but not significant) decrease in respiration rates relative to the control. At the deeper soil depths, the addition of either N or P separately tended to increase mineralization rates compared to the control, and the addition of N and P together (+ N + P) caused the greatest increase (Fig. 3a). The effects of the three nutrient treatments on relative respiration rates (Fig. 3b) were significantly related to soil depth ($P < 0.001$ in all cases).

Discussion

These results should only be considered a qualitative prediction of how CO_2 production in undisturbed soil profiles would be affected by changes in soil moisture, temperature, or N and P additions. Field respiration rates are likely to be different from the *in vitro* rates measured in these experiments since the soils were sieved and only the more 'active' or labile SOM would be mineralized over the relatively short incubation periods (Townsend *et al.*, 1995). However, the majority of the microbial CO_2 production in intact profiles is likely to come from the mineralization of the more labile SOM pools (Townsend *et al.*, 1997; Trumbore, 2000), so our experimental results should qualitatively represent the effects that changes in soil moisture, temperature, and nutrient conditions would have on microbial CO_2 production *in situ*. Since we did not measure the effects of these environmental variables on the size of the microbial biomass pools, our results can only be used to predict the short-term effects that changes in soil moisture, temperature, and nutrient conditions would have on microbial CO_2 production.

Across the full range of tested soil water potentials, microbial respiration rates in surface and subsurface horizons responded similarly to changes in soil water potential (Fig. 1). However, if we independently examine respiration rates at the mid-range of tested water potentials (-1.5 and -5 MPa), we find that surface soils have lower relative respiration rates than the subsurface soils (Fig. 1b). These results suggest that conditions of moderate drought may have a larger

relative impact on the rates of C mineralization from surface soil horizons.

Comparing our results describing the relationship between soil water potential and microbial respiration with published studies is difficult because the techniques used to adjust and measure soil water potentials are not consistent among studies (Rodrigo *et al.*, 1997). Nevertheless, our overall results (summarized by Eqn (4)) are similar to the results obtained in other studies where C mineralization rates have been measured at water potentials below the level of saturation (Wilson & Griffin, 1975; Sommers *et al.*, 1981; Orchard & Cook, 1983).

Respiration rates in subsurface soils are more sensitive to changes in soil temperature than the rates in surface soils. The average Q_{10} values measured for the surface soils are relatively high, but within the range of Q_{10} values (generally 1.8–3) reported in similar studies (Howard & Howard, 1993; Kirschbaum, 1995; Katterer *et al.*, 1998). In contrast, the Q_{10} values calculated for the subsurface soil samples are higher than most of the Q_{10} values reported in the literature, with the notable exception of soils incubated at cold temperatures (Mikan *et al.*, 2002). Differences in methodologies may partially account for the high Q_{10} values calculated in this experiment. In many published studies, Q_{10} values are calculated after incubating soil samples in the laboratory and measuring CO_2 evolution over time periods of weeks to months (Kirschbaum, 1995; Katterer *et al.*, 1998). Without additional C inputs, the respiration rates in incubated soils tend to decrease rapidly at first, with rates continuing to decrease slowly over time as the supplies of readily decomposable C are exhausted. At higher temperatures, the progression of the decomposition process will occur more rapidly, resulting in lower Q_{10} values when respiration rates are integrated over prolonged incubation periods (Kirschbaum, 1995; Dalias *et al.*, 2001). In this study, respiration rates would not have time to decrease appreciably over the relatively short incubation period (24 h); therefore, the calculated Q_{10} values pertain to a transient loss of readily metabolizable C and are likely to be higher than those reported from similar studies with longer incubation times.

Regardless of the specific Q_{10} values, it is clear that organic matter mineralization becomes more temperature dependent with depth through the soil profile. Studies focused on surface organic horizons have also observed an increase in Q_{10} values with depth (Bunnell *et al.*, 1977; Leiros *et al.*, 1999). Few other studies have looked at the temperature dependence of mineralization rates at soil depths greater than 25 cm. Data presented in Lomander *et al.* (1998) suggest that

between 15 °C and 25 °C, CO₂ production was more strongly influenced by temperature in subsoil (30–55 cm) than in topsoil (0–25 cm) horizons. However, Winkler *et al.* (1996) observed the opposite trend, with the respiration rates in the surface A horizon having a greater temperature sensitivity than rates in the underlying B horizon.

There are three possible explanations as to why, in our study, respiration rates are more sensitive to temperature in the soil subsurface than at the soil surface: differences in microbial community composition, a decrease in C quality with soil depth, or an interaction between CO₂ production and nutrient availability. Of course, these proposed explanations, discussed in detail below, are merely speculative and more research is needed in order to pinpoint accurately the specific mechanisms responsible for the change in Q_{10} values with soil depth.

We know that, in the studied profile, subsurface microbial communities are distinct from surface communities (Fierer *et al.*, 2003; LaMontagne *et al.*, in press) and that microbial communities may have different thermal optima due to physiological adaptations to specific temperature regimes (Latter & Heal, 1971; Stark & Firestone, 1996; Balser, 2000). In this case, where average annual temperatures through the profile differ by up to 7 °C (Table 1), differences in the thermal optima of the microbial communities may partly account for the significant increase in Q_{10} values with soil depth. However, since surface communities experience higher average annual temperatures, we would expect subsurface communities to have lower, not higher, thermal optima than the surface communities.

Although the distinct nature of surface and subsurface microbial communities may partly explain the distinct temperature responses, a decrease in C quality with soil depth is a better explanation for the observed increase in Q_{10} values. As mentioned previously, we are defining C 'quality' as the degree to which soil organic C is resistant to microbial mineralization if we assume that there is an inverse relationship between soil organic C quality and respiration rates (per unit C), the deeper soils have a larger proportion of recalcitrant, lower quality, and soil organic C. The decrease in the quality of soil organic C with depth is reflected in the statistically significant decrease in the rate constant d (Eqn (2)) with soil depth (Mikan *et al.*, 2002). There are two possible explanations for the decrease in organic C quality with depth: an increase in physical protection due to the stabilization of organic C by soil minerals (Hassink & Whitmore, 1997) or a decrease in chemical lability of the organic C for microbial metabolism (Bosatta & Agren, 1999). The degree of physical protection, which we would expect to increase at

elevated temperatures (Thornley & Cannell, 2001), should not contribute to the observed increase in Q_{10} values with depth through the soil profile. However, the enzymatic reactions required to metabolize organic C of lower lability have higher activation energies and temperature dependence than reactions metabolizing higher quality C (Bosatta & Agren, 1999; Mikan *et al.*, 2002). Therefore, as the soil C in deeper horizons is of lower quality than the C contained in surface horizons, we would predict that the mineralization of subsurface C should be inherently more temperature dependent than the mineralization of the higher quality C found in surface horizons.

Another possible explanation for the observed increase in Q_{10} values with soil depth is that there is a positive feedback between CO₂ production and the mineralization of nutrients other than C. Besides increasing the rate of C mineralization by soil microorganisms, higher soil temperatures may also increase the rates of mineralization of key nutrients such as N and P. An increased availability of N and P would enable soil microorganisms, particularly those in nutrient-limited environments, to produce more of the enzymes required for C mineralization (Tate, 2000). If enzyme production is rapid enough to increase the enzyme pool sizes significantly over the relatively short incubation periods used in this study, C mineralization rates may respond accordingly. In other words, higher soil temperatures may induce a positive feedback response by increasing both the activity of individual enzymes and the number of active enzymes found in soil. If this scenario is accurate, we would expect Q_{10} values to be higher in soils where low nutrient levels limit the production of the enzymes required for C mineralization. Our data suggest that the subsurface microorganisms are more nutrient limited than the microorganisms residing in the surface horizons (see below), so this interaction between C mineralization and nutrient availability may be another possible explanation for the observed increase in Q_{10} values with soil depth.

Figure 3 shows that the relative impact of nutrient additions on microbial CO₂ production increases with soil depth. If the magnitude of the increase in relative respiration rates is indicative of the degree of nutrient limitation to C mineralization, we can surmise that the degree of N and P limitation to respiration increases with depth through the soil profile. At the surface depths, C mineralization is not strongly limited by either N or P since the addition of N and P tends to lower respiration rates relative to the control (Fig. 3b). At greater depths, microbial CO₂ production is strongly N and P limited; at the 2 m depth the addition of either N or P resulted in a four-fold increase in SOM mineralization rates relative to the control (Fig. 3b).

Without nutrient amendments, subsurface microorganisms may have insufficient N and P to synthesize the metabolic machinery (primarily RNA and enzymes) required to support higher rates of organic C mineralization. Other studies have also found that rates of C metabolism in subsurface samples are often limited by N and P availability (Thornton-Manning *et al.*, 1987; Swindoll *et al.*, 1988; Ajwa *et al.*, 1998). The total soil nutrient concentrations do not explain the high degree of nutrient limitation for C mineralization in the subsurface horizons of the studied profile; both C:N and C:P ratios decrease with depth through the profile (Table 1). However, total nutrient concentrations are often a poor indicator of nutrient availability since N and P soils are often found in forms that are physically and/or chemically inaccessible to microorganisms (Schlesinger, 1991; Vitousek & Howarth, 1991).

We are not able to identify specifically the reasons why surface and subsurface soils have distinct microbial responses to nutrient additions. In published studies, the observed effects of nutrient additions on the microbial mineralization of soil organic C tend to be highly variable. Depending on the specific soil type examined, nutrient additions can have a positive effect, a negative effect, or no effect on microbial respiration rates (Fog, 1988). The particular response of a soil to nutrient additions is likely to be a function of a variety of factors, including: microbial community composition, microbial physiology, nutrient concentrations, and the chemistry and availability of organic C (Fog, 1988; French, 1988; Agren *et al.*, 2001; Vance & Chapin, 2001). Because these factors and their interactions are complex and poorly understood, we cannot pinpoint a single mechanism that explains the differential responses of surface and subsurface soils to nutrient additions.

Overview

The relative responses of C mineralization rates to changes in soil moisture, temperature, and nutrient levels varied with soil depth. Most strikingly, increases in soil temperature or nutrient levels led to comparatively large increases in the relative rates of microbial CO₂ production from subsurface soil horizons. There are two main implications of our experimental results. (i) The relative magnitude of the controls on C mineralization is different for surface and subsurface soil horizons. The C dynamics of the whole soil profile cannot be adequately understood by solely studying surface soil horizons. (ii) By increasing soil temperatures and nutrient inputs to the soil subsurface, climate change and the direct or indirect fertilization of soils by human activity may substantially increase the rates of organic C mineralization in deeper soil horizons. Since

subsurface soil horizons contain large quantities of sequestered organic C, any increase in subsurface mineralization rates could, over time, have significant effects on global C dynamics.

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References

- Agren GI, Bosatta E, Magill AH (2001) Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. *Oecologia (Berlin)*, **128**, 94–98.
- Ajwa HA, Rice CW, Sotomayor D (1998) Carbon and nitrogen mineralization in tallgrass prairie and agricultural soil profiles. *Soil Science Society of America Journal*, **62**, 942–951.
- Allison LE, Moodie CD (1965) Carbonate. In: *Methods of Soil Analysis, Part 2. Agronomy*, Vol. 9 (ed. Black C), pp. 1379–1400. American Society of Agronomy, Madison, WI.
- Anderson JM (1991) The effects of climate change on decomposition processes in grassland and coniferous forests. *Ecological Applications*, **1**, 326–347.
- Balser TC (2000) *Linking microbial communities and ecosystem functioning*. PhD thesis, University of California, Berkeley, 220 pp.
- Balser TC, Kinzig AP, Firestone MK (2002) The functional consequences of biodiversity. In: *The Functional Consequences of Biodiversity* (eds Kinzig A, Pacala S, Tilman D), pp. 265–293. Princeton University Press, Princeton, NJ.
- Batjes NH (1996) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science*, **47**, 151–163.
- Bosatta E, Agren GI (1999) Soil organic matter quality interpreted thermodynamically. *Soil Biology & Biochemistry*, **31**, 1889–1891.
- Bunnell FL, Tait DE, Flanagan PW, VanCleve K (1977) Microbial respiration and substrate weight loss – I: a general model of the influences of abiotic variables. *Soil Biology & Biochemistry*, **9**, 33–40.
- Dalias P, Anderson JM, Bottner P, Couteaux M (2001) Temperature responses of carbon mineralization in conifer forest soils from different regional climates incubated under standard laboratory conditions. *Global Change Biology*, **6**, 181–192.
- Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biology*, **4**, 217–227.
- Desjardins T, Andreux F, Volkoff B, Cerri CC (1994) Organic carbon and ¹³C contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. *Geoderma*, **61**, 103–118.
- Dibblee TW (1966) *Santa Ynez Mountains: Geology of the Central Santa Ynez Mountains, Santa Barbara County, California, Bulletin*, **186**. California Division of Mines and Geology, Santa Barbara, CA, USA.

- Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biology & Biochemistry*, **35**, 167–176.
- Fierer N, Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology & Biochemistry*, **34**, 777–787.
- Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Reviews of the Cambridge Philosophical Society*, **63**, 433–462.
- French DD (1988) Some effects of changing soil chemistry on decomposition of plant litters and cellulose on a Scottish (UK) moor. *Oecologia (Berlin)*, **75**, 608–618.
- Gaudinski JB, Trumbore SE, Davidson EA, Zheng S (2000) Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry (Dordrecht)*, **51**, 33–69.
- Harris RF (1981) Effect of water potential on microbial growth and activity. In: *Water Potential Relations in Soil Microbiology* (eds Parr J, Gardner W, Elliott L), pp. 23–95. Soil Science Society of America, Madison.
- Harris RF, Gardner WR, Adebayo AA, Sommers LE (1970) Agar dish isopiestic equilibration method for controlling soil water potential of solid substrates. *Applied Microbiology*, **19**, 536–537.
- Hassink J, Whitmore AP (1997) A model of the physical protection of organic matter in soils. *Soil Science Society of America Journal*, **61**, 131–139.
- Hendry MJ, Mendoza CA, Kirkland RA, Lawrence JR (1999) Quantification of transient CO₂ production in a sandy unsaturated zone. *Water Resources Research*, **35**, 2189–2198.
- Howard DM, Howard PJA (1993) Relationships between CO₂ evolution, moisture content and temperature for a range of soil types. *Soil Biology & Biochemistry*, **25**, 1537–1546.
- IPCC (1996) *Climate Change 1995: The Science of Climate Change*, 364 pp. Cambridge University Press, Cambridge.
- Jobbagy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, **10**, 423–436.
- Katterer T, Reichstein M, Andren O, Lomander A (1998) Temperature dependence of organic matter decomposition: a critical review using literature data analyzed with different models. *Biology and Fertility of Soils*, **27**, 258–262.
- Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biology & Biochemistry*, **27**, 753–760.
- LaMontagne MG, Schimel JP, Holden PA (2003) Comparison of subsurface and surface soil bacterial communities in California grassland as assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16s rDNA. *Microbial Ecology* (in press).
- Latter PM, Heal OW (1971) A preliminary study of the growth of fungi and bacteria from temperate and Antarctic soils in relation to temperature. *Soil Biology & Biochemistry*, **3**, 365–379.
- Leiros MC, Trasar-Cepeda C, Seoane S, Gil-Sotres F (1999) Dependence of mineralization of soil organic matter on temperature and moisture. *Soil Biology & Biochemistry*, **31**, 327–335.
- Loaiciga HA, Valdes JB, Vogel R, Garvey J, Schwarz H (1996) Global warming and the hydrologic cycle. *Journal of Hydrology*, **174**, 83–127.
- Lomander A, Katterer T, Andren O (1998) Carbon dioxide evolution from top- and subsoil as affected by moisture and constant and fluctuating temperature. *Soil Biology & Biochemistry*, **30**, 2017–2022.
- Mikan CJ, Schimel JP, Doyle AP (2002) Temperature controls of microbial respiration in Arctic tundra soils above and below freezing. *Soil Biology & Biochemistry*, **34**, 1785–1795.
- Orchard VA, Cook FJ (1983) Relationship between soil respiration and soil moisture. *Soil Biology & Biochemistry*, **15**, 447–453.
- Paul EA, Follett RF, Leavitt SW, Halvorson A, Peterson GA, Lyon DJ (1997) Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. *Soil Science Society of America Journal*, **61**, 1058–1067.
- Rodrigo A, Recous S, Neel C, Mary B (1997) Modelling temperature and moisture effects on C–N transformations in soils: comparison of nine models. *Ecological Modelling*, **102**, 325–339.
- Rustad LE, Huntington TG, Boone RD (2000) Controls on soil respiration: implications for climate change. *Biogeochemistry*, **48**, 1–6.
- Schimel DS, Braswell BH, Holland EA, McKeown R, Ojima DS, Painter TH, Parton WJ, Townsend AR (1994) Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. *Global Biogeochemical Cycles*, **8**, 279–293.
- Schimel JP (2001) Biogeochemical models: implicit vs. explicit microbiology. In: *Global Biogeochemical Cycles in the Climate System* (eds Schulze E, Heimann M, Harrison S, Holland E, Lloyd J, Prentice I, Schimel D), pp. 177–183. Academic Press, New York.
- Schlesinger WH (1991) *Biogeochemistry: An Analysis of Global Change* (443 pp. Academic Press, New York).
- Smil V (2000) Phosphorous in the environment: natural flows and human interferences. *Annual Review of Energy in the Environment*, **25**, 53–88.
- Sommers L, Gilmour C, Wildung R, Beck S (1981) The effect of water potential on decomposition processes in soil. In: *Water Potential Relations in Soil Microbiology* (eds Parr J, Gardner W, Elliott L), pp. 97–117. Soil Science Society of America, Madison, WI.
- Soil Survey Staff (1996) *Keys to Soil Taxonomy*. United States Department of Agriculture, Natural Resources Conservation Service, 644 pp.
- Stark JM, Firestone MK (1996) Kinetic characteristics of ammonium-oxidizer communities in a California oak woodland-annual grassland. *Soil Biology & Biochemistry*, **28**, 1307–1317.
- Swindoll CM, Aelion CM, Pfaender FK (1988) Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation response of subsurface microbial communities. *Applied and Environmental Microbiology*, **54**, 212–217.
- Systat (2000) Systat for Windows. SPSS Inc., Evanston, Illinois.
- Tate RL (2000) *Soil Microbiology* (508 pp. John Wiley & Sons, New York).
- Thornley JHM, Cannell MGR (2001) Soil carbon storage response to temperature: an hypothesis. *Annals of Botany (London)*, **87**, 591–598.
- Thornton-Manning JR, Jones DD, Federle TW (1987) Effects of experimental manipulation of environmental factors on phenol mineralization in soil. *Environmental Toxicology and Chemistry*, **6**, 615–622.

- Townsend AR, Vitousek PM, Trumbore SE (1995) Soil organic matter dynamics along gradients in temperature and land use on the island of Hawaii. *Ecology*, **76**, 721–733.
- Townsend AR, Vitousek PM, Desmarais DJ, Tharpe A (1997) Soil carbon pool structure and temperature sensitivity inferred using CO₂ and ¹³CO₂ incubation fluxes from five Hawaiian soils. *Biogeochemistry (Dordrecht)*, **38**, 1–17.
- Trumbore SE (2000) Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications*, **10**, 399–411.
- Vance ED, Chapin FS (2001) Substrate limitations to microbial activity in taiga forest floors. *Soil Biology & Biochemistry*, **33**, 173–188.
- van't Hoff J (1898) *Lectures on Theoretical and Physical Chemistry. Part 1. Chemical Dynamics* (pp. 224–229). Edward Arnold, London.
- Vitousek PM, Howarth RH (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, **13**, 87–115.
- Vitousek PM, Aber JD, Howarth RH, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: source and consequences. *Ecological Applications*, **7**, 737–750.
- Wilson JM, Griffin DM (1975) Water potential and the respiration of microorganisms in the soil. *Soil Biology & Biochemistry*, **7**, 199–204.
- Winkler JP, Cherry RS, Schlesinger WH (1996) The Q₁₀ relationship of microbial respiration in a temperate forest soil. *Soil Biology and Biochemistry*, **28**, 1067–1072.
- Wood BD, Keller CK, Johnstone DL (1993) *In situ* measurement of microbial activity and controls on microbial CO₂ production in the unsaturated zone. *Water Resources Research*, **29**, 647–659.