Soil Biology & Biochemistry 40 (2008) 2722–2728

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Patterns of substrate utilization during long-term incubations at different temperatures

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ARTICLE INFO

Article history:
Received 21 January 2008
Received in revised form 24 June 2008
Accepted 2 July 2008
Available online 3 August 2008

Keywords:
Carbon utilization efficiency
Long-term incubation
Soil carbon
Temperature

ABSTRACT

Microorganisms play key roles in biogeochemical cycling by facilitating the release of nutrients from organic compounds. In doing so, microbial communities use different organic substrates that yield different amounts of energy for maintenance and growth of the community. Carbon utilization efficiency (CUE) is a measure of the efficiency with which substrate carbon is metabolized versus mineralized by the microbial biomass. In the face of global change, we wanted to know how temperature affected the efficiency by which the soil microbial community utilized an added labile substrate, and to determine the effect of labile soil carbon depletion (through increasing duration of incubation) on the community's ability to respond to an added substrate. Cellobiose was added to soil samples as a model compound at several times over the course of a long-term incubation experiment to measure the amount of carbon assimilated or lost as CO_2 respiration. Results indicated that in all cases, the time required for the microbial community to take up the added substrate increased as incubation time prior to substrate addition increased. However, the CUE was not affected by incubation time. Increased temperature generally decreased CUE, thus the microbial community was more efficient at 15 °C than at 25 °C. These results indicate that at warmer temperatures microbial communities may release more CO_2 per unit of assimilated carbon. Current climate–carbon models have a fixed CUE to predict how much CO_2 will be released as soil organic matter is decomposed. Based on our findings, this assumption may be incorrect due to variation of CUE with changing temperature.

1. Introduction

Uptake, storage, growth, and respiration are all functions of a microorganism that vary depending on substrate availability and environmental conditions. Carbon utilization efficiency (CUE), defined as the efficiency with which carbon taken up by the microbial community is converted into microbial biomass (Winzler and Baumberger, 1938; Clifton, 1946), is an important parameter in many carbon–climate models. In the Century model, for example, the growth yield efficiency is assumed to be 55%, meaning that 45% of the carbon taken up by microorganisms is lost as respiration (Parton et al., 1987). Models that incorporate CUE are sensitive to changes in this parameter (Thiet et al., 2006), but little is known about how CUE varies with temperature.

There is broad evidence that biological processes such as respiration and growth are affected by temperature (Alvarez et al., 1995; Gillooly et al., 2001). Previous research suggests that CUE is relatively insensitive to temperature in aquatic environments (Seto and Misawa, 1982; del Giorgio and Cole, 1998), but that it may be temperature sensitive in soil systems (Devevre and Horwath, 2000). Microbial communities use different compounds (Grayston et al., 2001) that have varying efficiency factors (Paul and Clark, 1996) and a change in temperature that leads to shifts in microbial community composition or decomposition of different substrates may result in community-level changes in CUE.

All of the approaches used to test the effects of temperature on decomposition (e.g., *in situ* warming, short-term and long-term laboratory incubations) have limitations that make investigating the impact of temperature on CUE challenging. *In situ* warming experiments maintain ongoing carbon inputs and are not subject to the artifacts created by laboratory incubations such as changes in microbial community structure due to carbon depletion or alteration of the microenvironment. However, with increases in CO_2 and temperature, plants may change their tissue chemistry, altering the

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doi:10.1016/j.soilbio.2008.07.002

C:N ratios of their litter, thus altering the microbial community belowground (Canadell et al., 1996; Phillips et al., 2002). It is also difficult to precisely manipulate temperatures or to make direct CUE measurements in the field. Short-term laboratory incubations measure only the initial response of microbial communities to temperature, and those observations may not reflect long-term responses under sustained global change. Conversely, long-term laboratory incubations often show declining soil carbon contents, decreased quality of the remaining carbon (Mikola, 1955 cited in Swift et al., 1976), and a decrease in microbial activity and biomass over time (Joergensen et al., 1990; Follett et al., 2007). The rate of depletion of labile soil carbon is directly related to incubation temperature (Bunnell et al., 1977a,b): for a given period of time, carbon becomes more depleted as temperature increases because substrates are utilized more quickly (Joergensen et al., 1990). The reliability of predictions based on long-term laboratory incubations could be questioned because of the artificiality created by the incubation of soils for an extended period of time outside of the natural environment. In particular, some may question the viability of the soil microbial population during long-term incubation without periodic inputs of carbon. Incubations are, however, a good way to investigate how a microbial community's ability to utilize substrates is affected by changes in availability of labile compounds.

A better understanding of the interaction of temperature, carbon quality, carbon utilization, and respiration will enable us to predict how microbial metabolism will respond to temperature changes and increasing periods of starvation which can be produced during long-term experiments and climate change. We used long-term incubations to study the assimilation and respiration responses to added carbon. The objectives of our research were to determine (1) if depletion of labile soil carbon, as induced by increasing duration of incubation, affected the soil microbial community's ability to respond to an added substrate, and (2) how temperature affected the efficiency by which the soil microbial community utilized an added labile substrate. Substrate additions were performed and monitored at different points during long-term incubations performed at two different temperatures.

2. Materials and methods

Our first objective was addressed by performing a series of substrate additions after different durations of prior long-term incubation to deplete labile soil carbon, while our second objective was addressed by performing these long-term incubations and substrate additions at two different temperatures.

2.1. Site description and sampling

The soils used for this experiment were obtained from the long-term field experimental plots at the Northern Great Plains Research Laboratory located near Mandan, North Dakota (46°46′ N, 100°55′ W; Frank et al., 2004). Annual precipitation at the site is 402 mm and the mean annual temperature is 5 °C (Black and Tanaka, 1997). The average growing-season length for the area is 136 d. The soils on the site are in the Temvik–Wilton silt-loam association, and are classified as Pachic or Typic Haplustolls in U.S. Soil Taxonomy (Black and Tanaka, 1997). The long-term cropping system was continuous no-till spring wheat. Surface litter and aboveground vegetation were cleared away prior to sampling and field replicates were collected from small pits to a depth of 20 cm. Replicates were sealed in separate plastic bags, returned to the laboratory, air-dried and passed through a 2 mm mesh sieve. Visible root material was removed by hand picking during sieving. Field replicates were homogenized and stored air-dry until incubations began.

2.2. Laboratory incubations

Soils were incubated at either 15 or 25 °C for four time periods: 0, 90, 360, and 600 d. For each temperature-incubation duration combination, 15 laboratory replicate samples (80 g each) were weighed and placed in plastic specimen cups (100 ml, 6.5 cm diameter). Samples were moistened to 25% water content by weight (~55% water-filled pore space) and the cups were placed in 946 ml (one-quart) canning jars. Jar lids contained rubber septa for collection of gas samples. Moisture inside the jars was maintained with a small (20 ml, 2 cm diameter) glass vial of water, and soil moisture was checked and corrected after the jars were aerated four times. Jars were aerated once per week to ensure that CO_2 concentrations did not exceed 50,000 ppm.

2.3. Substrate additions

Substrate additions were performed at the end of incubations for different durations (90, 360, and 600 d), and on samples that did not undergo any long-term incubation (i.e. 0 d). Incubated samples were split into four 20 g samples, and placed into separate jars 2 d before the addition of labile substrate. Subdivision of samples increased the number of samples available for the frequent destructive sampling required to monitor substrate remaining. Cellobiose (a glucose disaccharide derived from the partial hydrolysis of cellulose) was used as our substrate because it is a natural carbon compound that is relatively easily broken down by many microorganisms, but must be broken down extracellularly prior to incorporation into the cell. The degradation process facilitates measurements of respiration due to cellobiose addition and of remaining cellobiose concentration over a longer period of time compared to simpler substrates such as glucose or amino acids.

One milliliter of cellobiose solution (20 mg ml^{-1}) was added to the surface of each sample, resulting in an addition rate of 420 μg C g^{-1} soil. This addition represents approximately 0.021% of initial soil carbon. The addition rate was intended to add sufficient cellobiose to induce microbial uptake and respiration of the substrate without inducing a significant increase in population size. Fifty-six of the 60 samples available for destructive sampling received cellobiose, while the remaining four control samples received 1 ml of deionized (DI) water to maintain soils moisture content equivalent to that in amended soils.

2.4. Soil respiration

Soil respiration and cellobiose remaining were measured immediately after addition, then approximately every 2 h beginning 6 h after addition. Measurements continued for up to 38 h after the addition of cellobiose for a total of 14 measurement times for each temperature and duration of prior incubation combination (i.e. 14 times × 4 replicates = 56 samples). Preliminary trials indicated that this sampling frequency provided sufficient temporal resolution to observe differences in cellobiose availability, presumably due to microbial uptake, and enough time for cellobiose concentrations to reach zero in most cases. Soil respiration was monitored by analyzing CO_2 concentrations in gas samples taken from the headspace of the jars to be destructively sampled using a LI-6252 CO_2 Analyzer (LI-COR Biosciences, Lincoln, NE). Subtraction of the non-amended samples from each amended sample permitted the quantification of cellobiose-derived CO_2, though we acknowledge that any contribution of native SOM to respiration in the amended samples due to the priming effect cannot be accounted for without the use of labeled substrate.

2.5. Analysis of cellobiose remaining

Cellobiose remaining was determined using the sulfuric acid–anthrone method for water-soluble carbohydrates (Brink et al., 1960), in which soil extracts are hydrolyzed by addition of sulfuric acid, reacted with anthrone, and measured photometrically to determine total carbohydrate concentration. Preliminary measurements by this method in non-amended soils showed very low levels of total carbohydrates, and conversely, spike recovery determinations were close to 100% (data not shown). Both suggest that using the sulfuric acid–anthrone method to measure cellobiose remaining was not significantly affected by extant soil carbohydrates. At each of the 14 measurement times, four replicate samples were analyzed for cellobiose. Aliquots (5 g) of soil were extracted with 50 ml of DI water in a 125 ml Erlenmeyer flask and shaken for 30 min. The suspension was poured into 50 ml round-bottom centrifuge tubes, balanced, and centrifuged for 10 min at 10,000 rpm. A 25 ml of extract supernatant was transferred to a 100 ml volumetric flask and diluted to 100 ml with DI water. The extract solutions were stored at 4 °C for 3–4 d before analysis.

Five milliliters of standard, blank (DI water), or soil extract were transferred to a glass test tube. Ten milliliters of anthrone–sulfuric acid reagent (0.2% anthrone in 95% (v/v) sulfuric acid) was rapidly added to the glass test tubes. The solution was mixed then allowed to cool for 10 min. After cooling, 10 ml of the solution were transferred to glass cuvettes. The absorbance was read at 625 nm in a spectrophotometer and converted to cellobiose concentration using a standard calibration curve. Soil cellobiose concentrations were determined using the following equation:

$$\mu\text{g cellobiose g}^{-1}\text{ moist soil} = A \times s \times \frac{100\text{ mL}}{25\text{ mL extract}} \frac{50\text{ mL extract}}{5\text{ g moist soil}} \quad (1)$$

where A is the solution absorbance at 625 μm and s is the slope of the standard curve.

2.6. Total lipid analysis

After the CUE experiment was complete, total lipid weight was measured as an indicator of microbial biomass on samples incubated in parallel to those of the main experiment using the same methods. Soil samples (80 g) were incubated at 15 or 25 °C for 60, 150, 450, or 600 d. Soils were only incubated at 25 °C for day-0. After incubation the samples were frozen at −20 °C until total lipid analysis could be performed. On the first day of analysis, 4 g of each soil sample was weighed and put it into a 50-mL glass test tube. The phosphate buffer was made with 1.4626 g of monobasic sodium phosphate and 5.579 g of dibasic sodium phosphate mixed in 1000 mL of DI H_2O, pH 7.4. An addition of 11.5 mL of extractant (130 mL phosphate buffer, 313 mL MeOH, 157 mL $CHCl_3$) was made to each 4 g sample, mixed by vortex for 15 s, and allowed to stand for 4 h at room temperature. The sample was then centrifuged for 10 min at 2500 rpm, and the supernatant poured off into a separatory funnel. The addition of buffer, mixing, and centrifugation steps were repeated, allowing the tube to stand for 30 min following centrifugation. The supernatants were combined, followed by an addition of 6 mL of chloroform and 6 mL of phosphate buffer to the separatory funnel, swirled, and allowed to separate overnight. The next day the chloroform layer (bottom layer) was decanted into a 50-mL round-bottom flask and dried with a rotary evaporator set to 37–40 °C (Bossio and Scow, 1998). After drying, the sample was weighed to obtain lipid weight.

2.7. Calculations and statistical analyses

Here we define CUE as the proportion of added cellobiose that is presumed to be metabolized and released, or assimilated into the microbial biomass without any assumption about microbial growth during cellobiose assimilation. This definition differs from the definition frequently used in the literature as the proportion of substrate that is assimilated into the biomass alone and from the definition of "growth yield efficiency" (Thiet et al., 2006), which requires that no microbial growth occurs. We therefore calculated carbon utilization efficiency using the following equation:

$$\text{CUE} = 1 - \left(\frac{\text{cellobiose} - \text{derived } CO_2 \text{ at } t_x}{(\text{cellobios e remaining at } t_0 \times 0.421) - (\text{cellobios e remaining at } t_x \times 0.421)} \right) \times 100$$

where t_x is the time when no cellobiose is remaining determined using curve fitting approach, and thus the denominator becomes equal to the amount of cellobiose initially added. Cellobiose remaining was multiplied by 0.421 to convert cellobiose to carbon. Our estimates of CUE are likely larger than those determined using more strict definitions, but our concern was the response of the estimate to temperature and incubation time.

To compare results between temperatures and duration of prior long-term incubation, we determined CUE only at the point when cellobiose remaining reached zero. To know this time precisely, best fit model parameters for the cellobiose remaining data were determined using Curve Expert v1.38 (D. Hyams, Starkville, MS, http://curveexpert.webshop.biz). The software fits data to a large library of models and ranks the best-fitting models based on the lowest standard error. The last four cellobiose concentration data points from the 90-day long-term incubation at 25 °C, and the last two points from the 600-day long-term incubation at 25 °C were omitted from the curve fitting because cellobiose concentrations had already reached constant near-zero values. Cumulative CO_2 respiration data were similarly fit to determine the total amount of CO_2 respired up to the point when cellobiose remaining reached zero.

Carbon utilization efficiency values were calculated for each replicate, and differences between means for each temperature and duration of long-term incubation were compared by PROC MEANS and ANOVA using PROC GLM (SAS Institute, Cary, NC). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Cellobiose decomposition

The general pattern of cellobiose decomposition consisted of rapid initial uptake, followed in most cases by a brief plateau between 6–16 h at 25 °C and 12–24 h at 15 °C. This pattern of cellobiose decomposition was similar for all combinations of temperature and duration of prior incubation, except for samples at 25 °C with no prior incubation and those incubated for 600 d at 25 °C (Fig. 1). Following the plateau, cellobiose decomposition returned to rates similar to those observed initially. In most cases, the cellobiose concentration data were best fit by a third-order polynomial (cellobiose remaining $= a + bt + ct^2 + dt^3$), with r^2

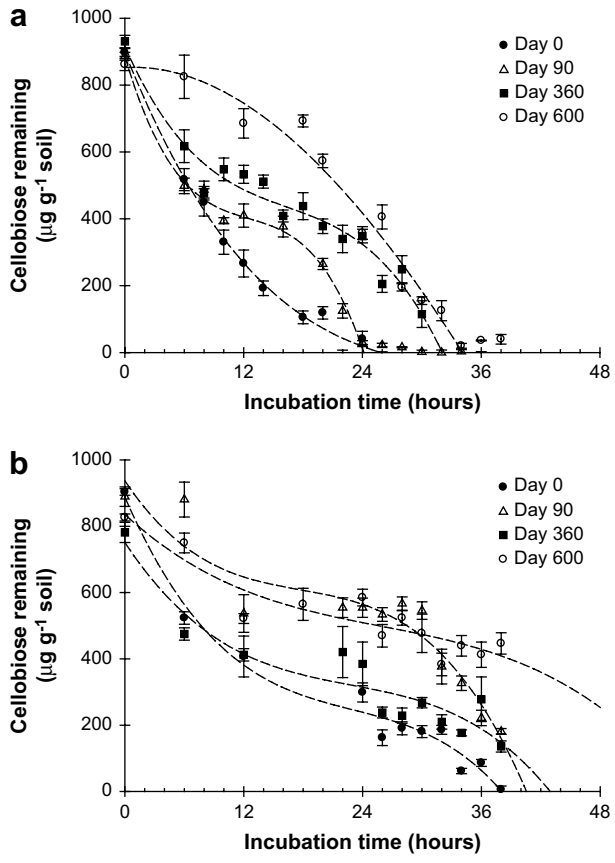

Fig. 1. Third-order polynomial curve fits of cellobiose remaining data at 25 °C (a) and 15 °C (b) for all incubation times. The last five data points of the day-90 at 25 °C incubation and the last two points of the day-600 at 25 °C incubation were excluded from the curve fits because of constant, near-zero cellobiose concentrations. Plotted values are means; error bars represent one standard error of the mean.

generally >0.9 for each combination of temperature and duration of prior incubation. For consistency, the times required for cellobiose concentrations to reach zero were determined using the model parameters from the third-order polynomial fit, even when this was not the best-fitting model.

Times to cellobiose depletion showed a strong interaction between duration of prior incubation and incubation temperature (Table 1). Cellobiose decomposition was more rapid at warmer temperatures (Fig. 1), such that cellobiose was depleted approximately 10 h sooner at 25 °C than at 15 °C for each point in the long-term incubation. Longer periods of prior incubation also led to longer times for cellobiose to be depleted.

Table 1
Estimated time required for cellobiose remaining to reach zero as determined by best fit models of cellobiose concentrations after addition, and total CO_2 respiration and carbon utilization efficiency at this point

Incubation temperature (°C)	Incubation time before addition (d)	Time to cellobiose remaining = 0 (h)	Total CO_2 respiration ($\mu g\ g^{-1}$ soil)	CUE (%)
15	0	38.2 ± 0.4	131.3 ± 4.3	65.4 ± 1.5^{a*}
	90	40.7 ± 0.2	80.1 ± 2.1	78.5 ± 1.1^{b*}
	360	43.6 ± 1.1	94.3 ± 8.9	71.4 ± 2.1^{c*}
	600	54.5 ± 2.7	67.2 ± 4.6	80.5 ± 1.2^{b*}
25	0	26.7 ± 0.6	151.0 ± 2.8	60.2 ± 0.8^{A}
	90	26.7 ± 0.3	136.3 ± 2.2	63.4 ± 1.0^{B}
	360	32.3 ± 0.8	107.6 ± 4.8	72.5 ± 1.0^{C}
	600	35.1 ± 1.0	124.1 ± 3.8	65.7 ± 1.6^{B}

Small letters indicate significant differences between incubation duration within 15 °C. Large letters indicate significant differences between incubation duration within 25 °C. The asterisks represent significant differences between temperatures at each incubation period. All values are mean ± one standard error of the mean.

3.2. Cellobiose-derived CO_2 respiration

Addition of cellobiose to soils caused a significant increase in respiration compared to non-amended samples for all incubation periods and temperatures. The greatest cumulative basal respiration for non-amended soils was 13.4 $\mu g\ CO_2$-C g soil^{-1} after 38 h at 15 °C and 17.6 $\mu g\ CO_2$-C g soil^{-1} after 38 h at 25 °C, both without prior incubation, while cellobiose-derived respiration was 155 $\mu g\ CO_2$-C g soil^{-1} and 176 $\mu g\ CO_2$-C g soil^{-1} for 15 °C and 25 °C, respectively. Increasing duration of long-term incubation typically decreased the cumulative respiration for non-amended soils during the experiment. After 600 d of incubation, samples respired 3.15 $\mu g\ CO_2$-C g soil^{-1} after 38 h at 15 °C and 2.24 $\mu g\ CO_2$-C g soil^{-1} after 38 h at 25 °C. The same respiration response to duration of prior incubation was observed for cellobiose-amended samples. Cumulative cellobiose-derived respiration during the 38 h after cellobiose addition decreased from 155 $\mu g\ CO_2$-C g soil^{-1} with no prior incubation at 15 °C to 47.2 $\mu g\ CO_2$-C g soil^{-1} after 600 d of incubation at 15 °C, and from 176 to 129 $\mu g\ CO_2$-C g soil^{-1} after 600 d of incubation at 25 °C. Overall, CO_2 respiration during the 38 h incubations was greater at 25 °C than at 15 °C for all prior incubation periods.

Patterns of cumulative cellobiose-derived CO_2 respiration showed an initial lag in respiration, followed by a high rate of respiration, leading to a maintenance phase where respiration leveled off, particularly at 25 °C (Fig. 2). This suggested a logistic pattern (CO_2 respired $= a/(1 + b \times e^{(-ct)})$), which was indeed one of the best-fitting models across all combinations of duration of prior incubation and temperature ($r^2 > 0.87$ for all cases). Cellobiose-derived respiration curves at 25 °C began to plateau when cellobiose concentrations approached depletion, indicating the microbial community was returning to a maintenance phase (Fig. 3). For samples incubated at 15 °C, respiration rates did not reach a plateau. This is consistent with the longer than observed times required for cellobiose concentrations to reach zero (Table 1). The total amount of cellobiose-derived CO_2 (i.e. up to when estimated cellobiose concentrations reached zero) was estimated for each incubation using logistic model fits. Total cellobiose-derived respiration at 15 °C without prior incubation was estimated to be 131 $\mu g\ CO_2$-C g soil^{-1}. While total cellobiose-derived respiration estimates were lower after long-term incubation (67.2 $\mu g\ CO_2$-C g soil^{-1} after 600 d of incubation), we expected to see a consistent decline in total respiration with increasing prior incubation. The decline, however, was not monotonic. Respiration was generally greater for incubations at 25 °C compared to 15 °C, ranging from 107.6 to 152 $\mu g\ CO_2$-C g soil^{-1}. The amount of cellobiose-derived CO_2 was also lower after long-term incubation at 25 °C compared to no prior incubation, but there was no consistent relationship between decline in respiration and prior incubation duration.

3.3. Carbon utilization efficiency

Carbon utilization efficiency (CUE) estimated at the point when cellobiose was depleted was lowest without prior incubation for both 15 °C and 25 °C (65.4% and 59.5%, respectively), compared to samples previously incubated for 90, 360, and 600 d (Table 1). Estimated CUE values were also significantly lower at 25 °C compared to 15 °C for each prior incubation period except for samples incubated for 360 d, which were not significantly different (Table 1).

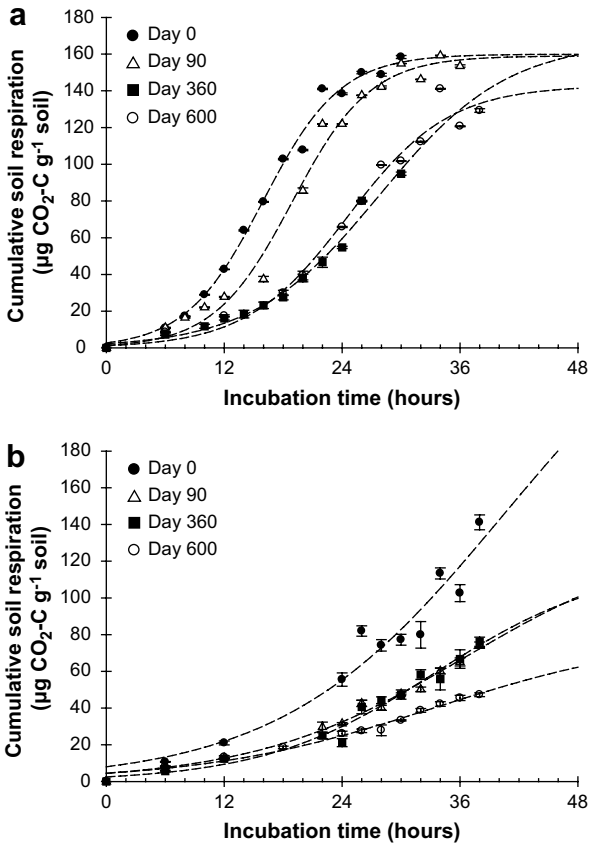

Fig. 2. Logistic model curve fits of cumulative cellobiose-derived CO_2 respiration at 25 °C (a) and 15 °C (b) for all incubation times. Plotted data points are control-subtracted means; error bars represent one standard error of the mean.

3.4. Total lipid analysis

Total lipid concentrations for each duration of prior incubation were generally greater at 15 °C than at 25 °C (except for 450 d), but the differences were not significant (Table 2). There were also no significant differences in total lipid content between incubation periods within each incubation temperature. While a decline in microbial biomass might be anticipated as incubation times increase, the evidence suggests that the microbial biomass was relatively constant throughout the incubation, but this may be due to high variability in the measurements.

4. Discussion

In this study we found that CUE was temperature dependent, but remained relatively constant over long-term incubation period. The change in CUE due to temperature could be attributed to how temperature affected either cellobiose uptake or respiration. The shape of the respiration and cellobiose uptake response by microbial communities to carbon additions was similar regardless of incubation duration or temperature. Lags in cellobiose uptake and respiration increased during longer incubations. These increased lags could be attributed to a reduced microbial biomass as seen in previous studies (Anderson and Domsch, 1985; Follett et al., 2007), but we did not observe decreases in microbial biomass using total lipid analyses.

To evaluate CUE at the point when added cellobiose concentrations reached zero, we fitted cellobiose concentration data to a third-order polynomial and CO_2 respiration data to a logistic model. Model fitting must always balance the desire for the best correlation with the selection of a model that is biologically meaningful. It is reasonable to believe that the logistic model used to fit the cumulative CO_2 respiration data has biological significance, and better fitting models for some replicates resulted in only marginally better correlations but had minimal biological significance. The respiration responses (at 25 °C) for all incubation durations followed a similar logistic pattern, whereas at 15 °C the pattern was not as distinct. Model fits, however, were very good for both temperatures (mean $r^2 = 0.982$ at 25 °C and $r^2 = 0.958$ at 15 °C). While a third-order polynomial model has little apparent biological significance, it was the most frequent best-fitting model, and it was also best able to simulate the vertically oriented logistic-type of behavior frequently observed in the data. Better fitting models for some replicates resulted in only marginally better correlations and were not better justified for biological relevance. Generally, data from 25 °C incubations resulted in better fits than incubations at 15 °C (mean $r^2 = 0.938$ versus 0.891).

Initial lag times in respiration and cellobiose uptake increased after longer incubation times. This response may have been caused by a decrease in the size of the microbial community during incubation (Anderson and Domsch, 1985; Follett et al., 2007), but we did not observe this with our total lipid analyses. Anderson and Domsch (1978) demonstrated a relationship between substrate-induced maximum initial respiration rates and microbial biomass in the soil. Using this relationship, we estimated microbial biomass in this study to be 30–35 mg biomass C 100 g^{-1} soil at 25 °C at day-0. The initial lag in respiration at both temperatures without prior incubation suggests the microbial community may be in an enzyme-induction phase, or may be taking up and storing the cellobiose without using it for repairing structures prior or growth. This rapid uptake response has been shown previously when microorganisms are carbon limited (Nguyen and Guckert, 2001; Blagodatsky et al., 2002; Jones and Murphy, 2007).

Within a given incubation temperature, decreasing amounts of labile, native SOM available prior to cellobiose addition due to increasing lengths of prior incubation did not generally affect CUE. Long-term laboratory incubations are frequently used to biologically fractionate labile from non-labile soil organic matter (Paul et al., 2006; Follett et al., 2007). A separate long-term incubation of the same soil under the same conditions resulted in a significant decline in respiration rates: down from 3.14 ± 0.53 to 1.45 ± 0.14 μg CO_2-C g^{-1} soil d^{-1} after 532 d of incubation at 15 °C, and from 7.91 ± 1.13 to 2.57 ± 0.99 μg CO_2-C g^{-1} soil d^{-1} after incubation at 25 °C. Overall, long-term incubation at 15 °C resulted in the mineralization of 4.2% of initial soil C and 7.6% of initial soil C at 25 °C (data not shown). Significantly decreased microbial activity (as measured by CO_2 respiration rates) with increasing incubation time is usually attributed to the consumption of labile material and decreased ability of the microbial population to mineralize the remaining, more recalcitrant material. Some studies have also observed significantly decreased microbial biomass after long-term incubation because of the depleted substrate supply (Follett et al., 2007). One might hypothesize that these phenomena may alter the microbial community composition and its ability to respond to new substrate additions. Consistent CUE values for long- and short-term incubations suggest that microorganisms respond predictably to added substrate regardless of whether their current carbon substrate is labile or resistant. The components comprising CUE (carbon uptake and respiration) were, however, impacted by carbon depletion. Changes in microbial community structure or co-metabolism in response to depletion of soil carbon substrates seemed to impact the components of CUE more significantly than the value of CUE itself. Our observations of the capacity of microorganisms to respond to labile substrate additions even after long-term incubation suggests that at least one concern over the artificiality created by long-term incubations can be eliminated.

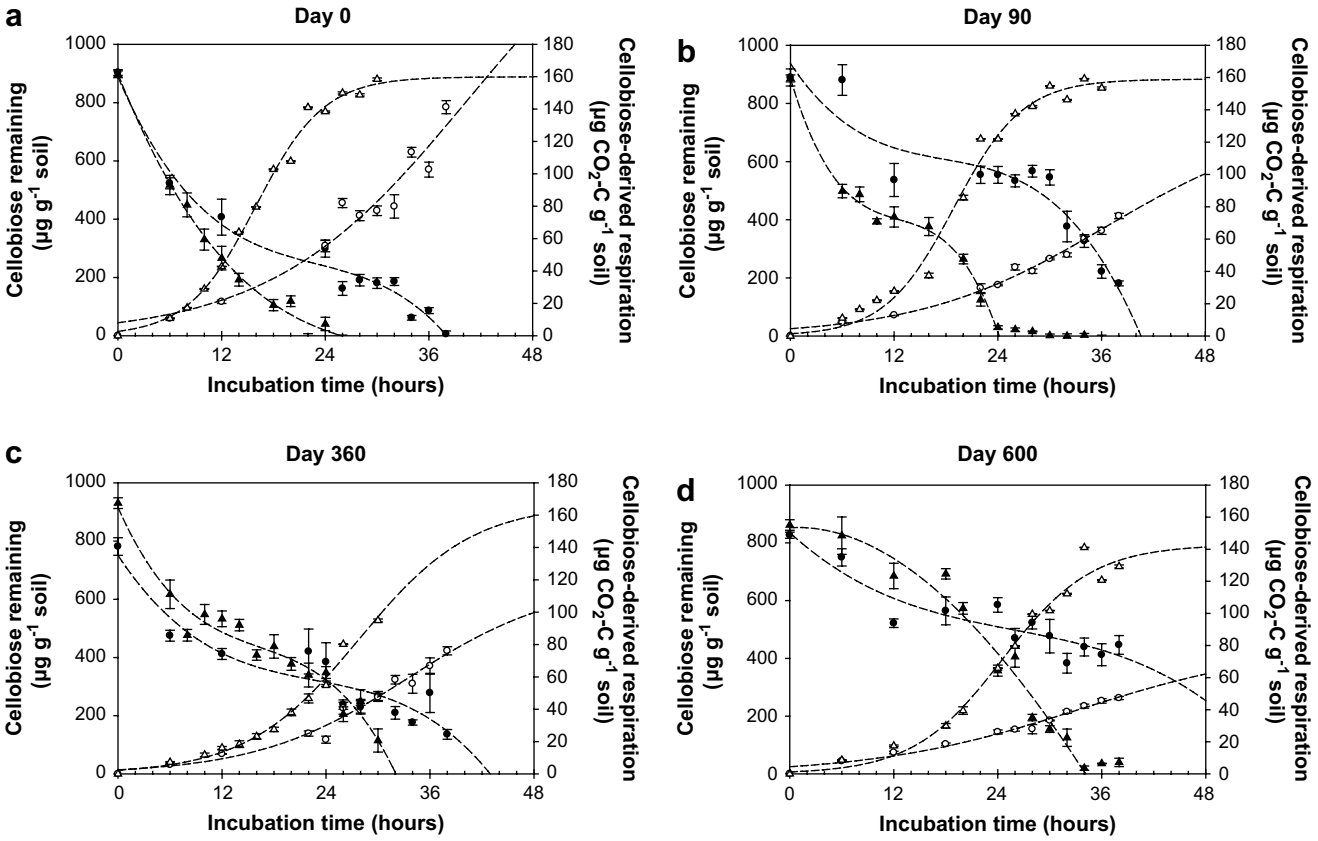

Fig. 3. Overlays of cellobiose remaining (left axis, closed symbols) and cumulative cellobiose-derived CO_2 respiration (right axis, open symbols) with curve fits at 25 °C (▲) and 15 °C (●) for day-0 (a), day-90 (b), day-360 (c), and day-600 (d) Plotted values are means; error bars represent one standard error of the mean.

The decreased respiration and slower uptake response to the addition of cellobiose at 15 °C compared to 25 °C can be attributed to several factors: diffusion of the substrate is slower at lower temperatures, cell metabolism is slower, microbial biomass is smaller at lower temperatures, or microbial community structure differed between temperatures. The impacts of temperature on the supply of carbon to the microorganisms consists of two components, the rate of diffusion and the rate at which substrate is made available to organisms (Ågren and Wetterstedt, 2007). Temperature impacts on diffusion are unlikely to contribute substantially to observed rate differences because incubation was conducted at water content that maximized substrate diffusion through the soil matrix (Skopp et al., 1990), reducing the effect of low water content on soil diffusion. Also, the concentration of cellobiose added was high enough to lead to high rates of substrate availability. Cell metabolic activity is affected by temperature, so a lower temperature could result in a lower metabolic rate and thus slower uptake (Alvarez et al., 1995; Gillooly et al., 2001). Without prior long-term incubation, the lag time differed between the two temperatures, when there should have been no difference in community size or carbon depletion, so temperature may have inhibited microbial uptake and growth at 15 °C. Anderson and Domsch (1985) observed a smaller soil microbial biomass pool at a warmer (28 °C) compared to a cooler (15 °C) temperature, possibly due to higher maintenance energy requirements at warmer temperatures so less biomass can be supported (Joergensen et al., 1990; Alvarez et al., 1995). While the differences were not statistically significant, our total lipid data suggest that microbial biomass was smaller at the warmer temperature. The differences in the respiration response curves at 25 °C for initial observations (without prior incubation and after 90 d) compared to later observations (after 360 and 600 d of incubation) may indicate a community shift as well. The capacity to break down cellobiose is common to several microbial taxa, so it is likely that organisms other than those dominant at the start of incubation could fulfill the same function even if community structure or size changed (Brookes, 1995).

Table 2
Total lipid concentrations for each incubation period at each temperature

Incubation temperature (°C)	Incubation period before substrate addition (d)	Total lipid concentration (μg g^{-1} soil)
15	60	0.610 ± 0.209
	150	0.907 ± 0.231
	450	0.69*
	600	0.757 ± 0.246
25	0	0.799 ± 0.416
	60	0.488 ± 0.209
	150	0.618 ± 0.307
	450	0.732 ± 0.149
	600	0.601 ± 0.026

Observed values are means; error estimates represent one standard error of the mean. Asterisks indicate $n = 1$.

While prior incubation duration had little effect on CUE, increasing temperature resulted in a decreased CUE. These results indicate that CUE is temperature dependent, so that if global temperatures rise there may be more CO_2 released from the decomposition of soil organic matter than is predicted. Current climate–carbon models have a fixed CUE to predict the amount of CO_2 released due to decomposition. Changes in temperature appear to affect the microbial response to added cellobiose, but future experiments need to be performed with labeled substrates of varying quality. Soil organic matter is a heterogeneous mixture of

substrates of varying quality, and complex materials have lower efficiency yields which could lead to lower CUE and smaller increases in biomass per unit of substrate decomposed than what was demonstrated in this research study (Paul and Clark, 1996). Understanding how microorganism metabolism is affected by temperature changes and substrate quality will enable accurate forecasts of how climatic changes will influence decomposition and the carbon cycle.

Acknowledgements

Support for this project was received from NSF Ecosystems Program of the Division of Environmental Biology. The authors wish to thank Rebecca Greenwood for her work in the laboratory and Robert Kolberg, USDA-ARS Mandan, ND for his assistance in site selection and field sampling.

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