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Temperature sensitivity of soil organic matter decomposition and the influence of soil carbon pools and attributes

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Temperature sensitivity of soil organic matter decomposition and the influence of soil carbon pools and attributes

by

Jin Zhang

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

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THE TEMPERATURE SENSITIVITY OF SOIL ORGANIC MATTER (SOM) DECOMPOSITION HAS GAINED INCREASING INTEREST BECAUSE OF ITS POTENTIAL IMPORTANCE TO SOIL CARBON (C) CYCLING IN RESPONSE TO FUTURE GLOBAL WARMING. SOM INCLUDES A COMPLEX CONTINUUM OF ORGANIC COMPONENTS WITH VARYING CHEMICAL STRUCTURES, DIVERSE ASSOCIATIONS WITH MINERALS, AND A WIDE RANGE OF TURNOVER TIMES. RELATIVELY RECALCITRANT C MAKES UP THE BULK OF SOM AND HAS A LONGER TURNOVER TIME RELATIVE TO LABILE C, SO ITS RESPONSE TO TEMPERATURE MAY BE PARTICULARLY INFLUENTIAL ON PREDICTIONS OF FUTURE C CONCENTRATIONS IN SOILS AND IN THE ATMOSPHERE. TWO TEMPERATE GRASSLAND SOILS WERE INCUBATED AT FOUR TEMPERATURES, AND TWO MATHEMATICAL MODELS WERE FIT TO OBSERVED SOM DECOMPOSITION RATES. BOTH MODELS SHOWED THAT TEMPERATURE SENSITIVITIES OF THE RELATIVELY LABILE C WERE SIGNIFICANTLY LARGER THAN THOSE OF RELATIVELY RECALCITRANT C.

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TO EXPERIMENTALLY ASSESS HOW SOIL CHARACTERISTICS AFFECT THE TEMPERATURE SENSITIVITY OF SOM DECOMPOSITION, WE DEVELOPED AN ARTIFICIAL SOIL WITH CONTROLLED COMPOSITION. IN THIS ARTIFICIAL SOIL, TWO SOIL PARAMETERS, SOM CHEMICAL RECALCITRANCE (CELLULOSE VS. LIGNIN) AND CLAY-MINERAL COMPOSITION (MONTMORILLONITE VS. KAOLINITE), WERE VARIED, WHILE OTHER ESSENTIAL SOIL CHARACTERISTICS WERE CONTROLLED. THE INCUBATION RESULTS SHOWED THAT THE PRESENCE OF CELLULOSE ENHANCED THE DECOMPOSITION RATE OF LIGNIN. DECOMPOSITION OF HIGH-CELLULOSE ORGANIC MATTER WAS SENSITIVE TO TEMPERATURE ONLY AT 2-12°C, WHILE DECOMPOSITION OF HIGH-LIGNIN ORGANIC MATTER HAD SIMILAR TEMPERATURE SENSITIVITIES OVER THE ENTIRE TEMPERATURE RANGE OF 2-32°C. SOM DECOMPOSITION RATE WAS GREATER IN TREATMENTS CONTAINING BOTH KAOLINITE AND MONTMORILLONITE THAN IN THE TREATMENT CONTAINING PURE KAOLINITE AT TEMPERATURES OF 12°C AND ABOVE. DECOMPOSITION OF ORGANIC MATTER ASSOCIATED WITH HIGH-MONTMORILLONITE SOILS HAD HIGH TEMPERATURE SENSITIVITIES AT 2-12°C, WHEREAS DECOMPOSITION IN PURE-KAOLINITE SOIL WAS SENSITIVE TO TEMPERATURE AT 12-22°C. THE
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THE INTERACTIVE EFFECT OF SUBSTRATE AND CLAY-MINERAL COMPOSITIONS ON THE TEMPERATURE SENSITIVITY OF SOM DECOMPOSITION WAS THEN TESTED IN AN ARTIFICIAL SOIL DESIGNED TO VARY BOTH LIGNIN/CELLULOSE CONTENT AND MONTMORILLONITE/KAOLINITE CONTENT. THE RESULTS SHOWED A SIGNIFICANT INTERACTIVE EFFECT AT 2-12°C: THE TEMPERATURE SENSITIVITIES OF MONTMORILLONITE TREATMENTS WERE HIGHER IN THE PURE-CELLULOSE TREATMENT THAN IN THE PURE-LIGNIN TREATMENT; IN CONTRAST, THE TEMPERATURE SENSITIVITIES OF KAOLINITE TREATMENTS DID NOT VARY WITH SUBSTRATE COMPOSITION.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

The steady increase in the concentration of atmospheric CO$_2$ and other heat-trapping gasses has likely caused the 0.56-0.92°C increase in global mean surface temperatures over the last 100 years (IPCC 2007). Current predictions estimate that the global mean surface temperature will increase by about 0.2°C per decade, if the rate of carbon (C) emissions remains unchanged (IPCC 2007). An analysis of C fluxes into and out of the atmosphere indicates that soil CO$_2$ efflux is second only to photosynthesis in its contribution to C flux in the terrestrial ecosystem (Raich & Schlesinger, 1992). Soil CO$_2$ emissions are about eight times larger than the amount of CO$_2$ associated with fossil fuel combustion (Canadell et al., 2007). Not only is this flux significant but also the size of the C pool in terrestrial soils is large (approximately 1600 Pg C), which is more than twice the amount of C present in the atmosphere (750 Pg C) or in the terrestrial biosphere (560 Pg C) (Schlesinger, 1991). Because of its large mass, even a small change in soil organic matter (SOM) decomposition could equal or surpass the CO$_2$ emitted to the atmosphere as a result of land use change and/or fossil fuel combustion.

Despite the importance of understanding the processes that control SOM decomposition, the intrinsically heterogeneous composition of SOM greatly complicates the investigation of how SOM decomposition responds to temperature changes. SOM consists of undecomposed litter, living soil microorganisms, partially decomposed C, more recalcitrant humus, and mineral-organic complexes of various types. As a result of this heterogeneity, SOM consists of a continuum of different low- to high-molecular weight components that vary dramatically in their resistance to decomposition, with turnover times ranging from days to thousands of
years (Schlesinger, 1977; McLauchlan & Hobbie, 2004). Therefore, multiple SOM pools have been incorporated into some ecosystem models, such as CENTURY and Rothamsted, to better assess ecosystem C dynamics (Pattern et al., 1987; Jenkinson et al., 1991; Cox et al., 2000; Jones et al., 2005). By separating SOM into different compartments having different turnover times, these multi-pool models were able to better simulate soil organic C dynamics and predict SOM decomposition using site-specific parameters.

Coupled carbon-climate models are effective tools to evaluate carbon-climate feedbacks. The temperature-decomposition relationship in these models is usually described by an exponential or Arrhenius function and most models have used a temperature sensitivity equaling 2 for decomposition of all soil C pools (Luo, 2007). That is primarily why global models that have sought to couple climate dynamics to terrestrial C dynamics have predicted a positive feedback between C cycling and climate warming (Friedlingstein et al., 2006). However, even though the temperature sensitivities of labile soil C pools have been documented, no consensus has been reached on the temperature sensitivities of more recalcitrant C pools, despite the large number of studies (Davidson & Janssen, 2006; von Lützow & Kögel-Knabner, 2009). There are two opposing views: first, that the temperature sensitivity of recalcitrant C is similar to or even larger than that of labile C, so projected global warming will increase atmospheric CO$_2$ concentration and potentially accelerate global warming (Mikan et al., 2002; Fang et al., 2005; Fierer et al., 2005; Knorr et al., 2005; Leifeld & Fuhrer, 2005; Vanhala et al., 2007; Conant et al., 2008; Hartley & Ineson, 2008); and second, that recalcitrant C is less sensitive or insensitive to temperature change, so any increases in soil CO$_2$ emissions due to global warming will be short-term (Giardina & Ryan,
The temperature sensitivity of soil respiration may be expressed as van’t Hoff’s temperature coefficient $Q_{10}$, the factor by which respiration rate is multiplied when temperature increases by $10^\circ C$ (Davidson & Janssen, 2006; von Lützow & Kögel-Knabner, 2009):

$$R_{exp} = \alpha e^{\beta T}$$  \hspace{1cm} (1)

$$Q_{10} = e^{\beta \times 10}$$  \hspace{1cm} (2)

where $R_{exp}$ is respiration rate, $T$ is measured temperature, and $\alpha$ and $\beta$ are fitted parameters.

Alternatively, the Arrhenius equation Eq. 3 describes changes in relative reaction rates (like decomposition rates) as a function of temperature:

$$k = Ae^{(-E_a/(RT))}$$  \hspace{1cm} (3)

where $k$ is the reaction rate constant, $A$ is the frequency factor, $E_a$ is the required activation energy, $R$ is gas constant, and $T$ is the temperature.

The Arrhenius equation implies that SOM with complex molecular attributes, which has a high activation energy $E_a$, is characterized by a low decomposition rate, and therefore an “inherently” high temperature sensitivity (Bosatta & Agren, 1999). This implication has been confirmed by some incubation studies using fresh litters (Fierer et al., 2005; Wetterstedt et al., 2010). When the same SOM (constant activation energy $E_a$) is decomposed at higher temperatures, there is a slower relative increase in the fraction of molecules with sufficient energy to react, and therefore the $Q_{10}$ value decreases (Kirschbaum, 1995; Wetterstedt et al.,
This implies that the highest temperature sensitivity of SOM decomposition occurs in cold regions, where large stocks of soil C are found.

However, many studies have generated results that contradict with Arrhenius kinetics (e.g. Giardina & Ryan, 2000; Reichstein et al., 2000; Luo et al., 2001; Melillo et al., 2002; Eliasson et al., 2005; Fang et al., 2006; Bradford et al., 2008). The Michaelis-Menten kinetics, in contrast, describe reaction rates that are modified by substrate concentrations and affinities of the enzymes for the substrates (Davidson et al., 2006; von Lützow & Kögel-Knabner, 2009):

\[ k = \frac{V_{\text{max}} \times C}{(K_m + C)} \]  

(4)

where \( k \) is the reaction rate, \( V_{\text{max}} \) is the maximal rate of enzymatic activity at a given temperature, \( K_m \) is the Michaelis-Menten constant, representing the affinity of enzymes for the substrates expressed as the substrate concentration at which the reaction rate equals \( V_{\text{max}}/2 \) and \( C \) is the substrate availability (substrate concentration at active site of the enzyme). Substrate availability can be directly affected by stabilization of organic compounds (e.g., by interaction with mineral surfaces and by spatial inaccessibility due to aggregation, Sollins et al., 1996) or indirectly affected by external control factors (e.g., water, oxygen and nutrient supply) (Davidson & Janssens, 2006). Both \( V_{\text{max}} \) and \( K_m \) are temperature dependent. When \( C \gg K_m \), \( K_m \) and its temperature sensitivity are unimportant, and the response of respiration to temperature reflects primarily the \( Q_{10} \) of \( V_{\text{max}}. \) \( K_m \) is an important parameter only when the substrate concentration \( (C) \) is in the range of the \( K_m \) value \( (C \approx K_m) \), and the temperature sensitivities of \( V_{\text{max}} \) and \( K_m \) can neutralize each other, called cancelling effect (Davidson et al., 2006). Gershenson et al. (2009) found that the addition of readily
available substrates significantly increased $Q_{10}$ values because substrate saturation eliminated the cancelling effect of $K_m$ on the measured $Q_{10}$ values.

The variety of $Q_{10}$ values obtained in numerous studies may result either from different intrinsic temperature sensitivities at high substrate availability or from the real temperature sensitivities influenced by different physiochemical environments. Experimental identification of which kinetics, Arrhenius or Michaelis-Menten, may contribute to the temperature response, or which physiochemical parameter may affect Michaelis-Menten kinetics, would be useful.

The stable C isotope composition of soil-respired CO$_2$ (the flux out of soil) is an important parameter that has been used to evaluate soil C dynamics (Ehleringer et al., 2000; Hanson et al., 2000; Søe et al., 2004). $^{13}$C signatures of SOM have allowed for identification of the proportion of SOM derived from C3 and C4 vegetation (e.g. Pessenda, 2001), because natural $^{13}$C labeling exploits the difference in $^{13}$C contents between C3 and C4 plants. In fields where C3 (C4) vegetation has replaced C4 (C3) vegetation, C stable isotope measurements have been reported to be an effective tool to distinguish between new and old SOM, which often are regarded as more-labile and more-recalcitrant C pools. Studies using C stable isotopes have been based on the assumption that no significant isotopic fractionation occurs during SOM decomposition and, thus, that the $^{13}$C signature of respired CO$_2$ could precisely reflect its substrate C source. However, it is controversial whether isotopic fractionation is negligible in during SOM decomposition (Lin & Ehleringer, 1997; Mary et al., 1992), and whether isotopic fractionation is affected by temperature has not been tested.

The goals of this study were to assess the temperature sensitivities of both labile and relatively recalcitrant C pools, to test the isotopic fractionation during SOM decomposition
and the temperature effect on it, and to examine how different SOM attributes (chemical recalcitrance and clay mineral interaction) would affect temperature sensitivities of SOM decomposition in vitro.

**Dissertation Organization**

In chapter 2, a traditional laboratory incubation experiment was conducted to assess the temperature sensitivities of both labile and relatively recalcitrant C pools. We incubated soils collected from Rhodes Research and Demonstration Farm of Iowa State University at four temperatures, and a first-order two-component model was applied for the obtained SOM decomposition rates to calculate the $Q_{10}$ values of labile and relatively recalcitrant C. These model-fitted $Q_{10}$ values were then compared to the $Q_{10}$ values of SOM in the same fresh soils (more labile) and soils after pre-incubation (more recalcitrant). This chapter was formatted following the submission guidelines for Plant and Soil.

In chapter 3, the same soil as in chapter 2 was incubated at four different temperatures, and $^{13}$C of both bulk SOM and respired CO$_2$ were measured. The obtained C stable isotopic data were fitted with a Rayleigh equation to test the hypothesis that soil microbial respiration was associated with a fractionation effect and that temperature could affect the isotopic fractionation. This chapter was formatted following the submission guidelines for Oecologia.

In chapter 4, we developed a novel artificial soil with controlled compositions that mimicked natural soil and was appropriate for soil microbial growth. We were able to control soil characteristics such as texture, SOM content, water potential and pH and to vary the soil properties that we were most interested in, including SOM chemical recalcitrance and soil clay-mineral compositions. A traditional laboratory soil incubation experiment was then conducted using this artificial soil, which allowed us to assess the temperature sensitivities of
SOM decompositions and how it was affected by SOM and clay compositions individually. This chapter was formatted following the submission guidelines for European Journal of Soil Science.

In chapter 5, the artificial soil described in chapter 4 was used to test for an interactive effect of SOM chemical recalcitrance and soil clay-mineral compositions on the temperature sensitivity of SOM decomposition. This chapter was formatted following the submission guidelines for European Journal of Soil Science.

In chapter 6, general conclusions are made from the results of all experiments, and an overview on the subject of temperature and SOM decomposition is provided. Needed future research is also suggested by our current knowledge of this subject.

References


CHAPTER 2. DECOMPOSITION OF RELATIVELY RECALCITRANT CARBON IS LESS SENSITIVE TO TEMPERATURE THAN LABILE CARBON IN A TEMPERATE MOLLIC SOIL

A paper submitted to *Plant and Soil*

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**Abstract** The response of labile carbon (C) and recalcitrant C to elevated soil temperature is still not well understood. To address this gap in knowledge, two incubation experiments were conducted using a Mollic soil from Iowa, USA. Two samples were collected from under different vegetations: *Phalaris arundinacea* (PHAR) (a C3 species) and *Tripsacum dactyloides* (TRDA) (a C4 species). The first experiment incubated intact soils and the second incubated soils that had already lost some C during a pre-incubation period. A first-order two-component parallel model and a simple exponential model were employed to characterize the temperature sensitivities ($Q_{10}$) of relatively labile and recalcitrant C pools. Results from the first-order two-component parallel model indicated that obtained $Q_{10}$ values of the relatively labile C (PHAR, 2.71; TRDA, 2.48) were significantly larger ($P<0.001$) than...
those of relatively recalcitrant C (PHAR, 1.73; TRDA, 1.38). Results from the exponential model produced Q_{10} values of relatively labile C (PHAR, 2.22; TRDA, 2.16) that also were significant larger (P<0.001) than those of relatively recalcitrant C (PHAR, 1.73; TRDA, 1.86). These results suggest that different C pools may respond differently to temperature changes, which questions the assumption of uniform temperature sensitivities used in some soil C dynamic models.

**Keywords** Temperature sensitivity • Relatively labile carbon pool • Relatively recalcitrant carbon pool • Global warming • Soil incubation • Mean turnover time

**Introduction**

The steady increase in the concentration of atmospheric CO\textsubscript{2} and other heat-trapping gasses has likely caused the 0.56-0.92°C increase in global mean surface temperatures over the last 100 years (IPCC 2007). Current predictions estimate that the global mean surface temperature will increase by about 0.2°C per decade, if the rate of C emissions remains unchanged (IPCC 2007). An analysis of C fluxed in and out of the atmosphere indicates that soil CO\textsubscript{2} efflux is second only to photosynthesis (100-120 Pg C y\textsuperscript{-1}) in its contribution to C flux in the terrestrial ecosystem (Raich and Schlesinger 1992). This flux is ten times larger than the amount of CO\textsubscript{2} associated with fossil fuel combustion (Canadell et al. 2007). Not only is this flux significant but also the size of the C pool in terrestrial soils is large (approximately 1600 Pg C), which is more than twice the amount of C present in the atmosphere (750 Pg C) or in the terrestrial biosphere (560 Pg C) (Schlesinger 1991). Because
of its large mass, even a small change in soil organic matter (SOM) decomposition could equal or surpass the CO$_2$ emitted to the atmosphere as a result of land use change and/or fossil fuel combustion.

Despite the importance of understanding the processes that control SOM decomposition, the intrinsically heterogeneous composition of SOM greatly complicates the investigation of how SOM decomposition responds to temperature change. SOM consists of undecomposed litter, living soil microorganisms, partially decomposed C, more recalcitrant humus, and mineral-organic complexes of various types. As a result of this heterogeneity, SOM consists of a continuum of different low- to high-molecular weight components that vary dramatically in their resistance to decomposition, with turnover times ranging from days to thousands of years (Schlesinger 1977; McLauchlan and Hobbie 2004).

Comparison of temperature sensitivities of soil C decomposition and net primary productivity (Kirschbaum 1995, 2000; Rustad et al. 2001; Raich et al. 2006) as well as model simulations (Parton et al. 1987; Jenkinson et al. 1991; Lloyd and Taylor 1994) predict a decrease of global soil organic C inventories with increasing surface air temperatures, thereby providing a positive feedback to the global C cycle. Carbon in SOM pools may indeed have different sensitivities to temperature increases, which would impact predictive models. For example, the labile C pool may respond differently from the recalcitrant soil C (Reichstein et al. 2005; Davidson and Janssens 2006). Moreover, the recalcitrant C pool affects the long-term soil C dynamics due to its large contribution to SOM inventories (Schimel et al. 1994). The potential of a varying response to warming within the SOM pool, as well as the dominance of relatively recalcitrant soil C, makes it important to consider
comprehensively both relatively labile and recalcitrant C pools to predict how SOM
decomposition will respond to future global warming.

The objective of this study was to evaluate whether C quality influences the temperature
sensitivity of SOM decomposition; that is, whether relatively labile and recalcitrant organic
materials have similar temperature sensitivities ($Q_{10}$). Temperature sensitivities can be
expressed as $Q_{10}$, which is the increase of metabolic activity as temperature increases $10^\circ$C.
To evaluate this hypothesis, we compared two sets of $Q_{10}$ values that were obtained from two
incubation experiments conducted at 4, 14, 24, and $34^\circ$C. The first experiment was a more
traditional approach and derived $Q_{10}$ values from fitting SOM decomposition rates in a short-
term incubation to a first-order two-component parallel model, which theoretically describes
the amount of relatively labile and recalcitrant C pools. The second experiment was a novel
approach where $Q_{10}$ values were obtained by fitting the SOM decomposition rates (both at
the beginning on day 1 and after 36-day pre-incubation) into a simple exponential model.
Whereas $Q_{10}$ values obtained at the beginning of incubation indicate the temperature
sensitivities of relatively labile C pools, $Q_{10}$ values obtained after pre-incubation reflect the
temperature sensitivities of more stable C pools.

Materials and methods

Soil characteristics

The studied soils were collected from planted grass monocultures at Iowa State University’s
Rhodes Research and Demonstration Farm ($42.00^\circ$N, $93.25^\circ$W) in Marshall County Iowa.
The climate is strongly continental with a mean annual temperature of 8.7°C. The monthly mean air temperatures range from -8.1°C in January to 23.2°C in June. The mean annual precipitation is 880 mm, 14% of which occurs from November-February. Several species of C3 and C4 grasses were planted in 2001. The soils that were selected for this study included those covered under *Phalaris arundinacea* (PHAR), a C3 species, and *Tripsacum dactyloides* (TRDA), a C4 species. The soils are in the Nodaway series, which are fine-silty, mesic, Mollic Udifluvents (Oelmann 1981). They are fertile, moist, highly productive soils that were formerly planted to continuous corn without crop rotation.

Experiment 1: Determination of relatively labile C $Q_{10}$ values

Characteristics of the soils collected from PHAR and TRDA plots are shown in Table 1. In each plot, 10 soil cores were collected from the 0-15 cm depth using soil augers. Extracted cores were mixed together to generate a single well-mixed sample from each plot. All visible roots and fresh litter materials were removed from the collected soil, and the samples were passed through a 4-mm sieve to remove large litter fragments and particles. The samples were then thoroughly mixed by hand and air-dried. Ten g of soil (dry weight equivalent) were incubated in individual 75-ml vials at a water potential of -0.03 MPa, which is thought to be in the optimum moisture range for decomposition of SOM (Harris 1981). Separate sets of three replicate samples were used to measure respiration rates. Vials were placed in four incubators (Fisher Scientific, Pittsburgh, PA) at 4, 14, 24, and 34°C. The vials were tightly capped with lids to keep the same water potential throughout the experiment, but they were opened occasionally to allow air exchange. To ensure that moisture remained constant,
samples were weighed periodically and, if needed, deionized water was added during the incubation period.

The rate of CO$_2$ production of the soil samples was measured on days 1, 3, 5, 11, 22, and 36 of the incubation experiment. The unequal interval was designed to capture the asymptotic decrease commonly observed in incubation experiments (e.g. Schimel et al. 1994; Townsend et al. 1997). During each measurement, vials containing the soil samples were sealed with lids and flushed with CO$_2$-free air. The sealed vials were placed back in the incubator for a period of time that was sufficient to accumulate quantifiable amounts of CO$_2$ in the headspace, which ranged from 30 min (on the initial days of the experiment) to several hours (during the later days of the experiment). The vials were then taken out of the incubator and flushed with CO$_2$-free air through a LI-820 IRGA (Infrared Gas Analyzer, LI-COR, Lincoln, NE) to measure the CO$_2$ flux. The IRGA data were calibrated with CO$_2$ standards of known concentration to evaluate the amount of CO$_2$ respired in a certain interval and these were used to calculate SOM mineralization rates.

A first-order two-component parallel model was used to fit the obtained time-series of SOM decomposition rates to investigate Q$_{10}$ values of both relatively labile and recalcitrant C pools. This model was found to adequately describe SOM dynamics during soil incubation experiments (Kätterer et al. 1998):

$$RR = \alpha C_0 K_e^{-K_l t} + (1 - \alpha)C_0 K_e^{-K_r t} \quad 0 \leq \alpha \leq 1; \quad (1)$$

where $C_0$ is the initial amount of total C in the substrate, $\alpha C_0$ and $(1-\alpha)C_0$ are the initial amounts of C in the relatively labile and recalcitrant pools, respectively, and $K_l$ and $K_r$ are
the corresponding rate constants of relatively labile and recalcitrant C pools (Kätterer et al. 1998).

The model was fitted to the time-series for the highest incubation temperature ($T_{34}$) by simultaneously optimizing values for $\alpha$, $K_l$, and $K_r$, by using an algorithm for non-linear least squares (Ralston and Jennrich 1978). For all temperatures below 34°C, the value for $\alpha$ was the same as that estimated for $T_{34}$; i.e., the proportion of relatively labile and recalcitrant C was assumed to be constant in spite of temperature change. Thereafter, the model was fitted to the time-series for the remaining incubation temperatures by optimizing $K_l$ and $K_r$. Mean turnover time for each component was calculated as the reciprocal of the decomposition rate constant ($K_l$ and $K_r$).

The next step was to describe the dependence of $K_l$ and $K_r$ to temperature using the exponential $Q_{10}$ function (modified from Kätterer et al. 1998).

$$K_{34} / K(T) = Q_{10}^{(T_{34} - T) / 10}$$  \hspace{1cm} (2)

By fitting $K_l$ and $K_r$ at different temperatures, we obtained the $Q_{10}$ value of relatively labile and recalcitrant C pools. The coefficient of determination ($R^2$) calculated by linear regression (modeled vs. measured values) was used as a measure for goodness of fit.

Because the labile C pool is responsible for most of the initial CO$_2$ efflux (Schimel et al. 1994), the initial SOM decomposition rates (2 hours after rewetting of dry soils) were used to calculate the $Q_{10}$ values of relatively labile C by fitting these measured decomposition rates into the exponential equation (e.g., Fierer et al. 2005):

$$RR_T = B \cdot e^{kT}$$  \hspace{1cm} (3)
\[ Q_{10} = e^{10k} \]  \hspace{1cm} (4)

where \( RR_T \) is the decomposition rate (in mg CO\(_2\)-C g\(^{-1}\) soil-C d\(^{-1}\)) at temperature \( T \) (in °C), and \( B \) and \( k \) are the exponential fit parameters. Whereas the \( Q_{10} \) fitted by the first-order two-component parallel model is theoretical (under the assumption that CO\(_2\) evolution is from two C pools); the one obtained from the exponential equation (Eq. 4) is empirical.

Because the 36-day pre-incubation presumably decomposed much of the relatively labile C, the remaining C was considered relatively recalcitrant (these terms are considered relative because on a continuum, there likely is no clear delineation between labile verses recalcitrant C). Therefore, by comparing the \( Q_{10} \) values of SOM decomposition at different incubation periods (i.e. at the beginning on day 1 vs. after the 36-day pre-incubation), it is then possible to evaluate whether relatively labile C is more temperature sensitive than relatively recalcitrant C.

Experiment 2: Determination of relatively recalcitrant C \( Q_{10} \) values

The second goal of this study was to evaluate the temperature sensitivity of relatively recalcitrant SOM. To achieve this goal, most readily available C must be removed from the soil samples without interruption. A subset of the samples employed in the previous soil incubation experiment was randomly selected under the assumption that most readily available C was mineralized after 36 days of incubation at 24°C. We selected both PHAR- and TRDA-derived SOM samples that were incubated in sets of 12 replicates in individual 75-ml vials at 24°C for 36 days. The temperature of 24°C was chosen to represent room...
temperature, and 36 days were assumed to permit the mineralization of readily-available C as indicated by the previous experiment (Fig. 1). The vials were tightly capped with lids to keep the moisture at -0.03 MPa, and they were opened occasionally to allow air exchange. After the initial 36 days of incubation, 12 soil samples of both PHAR- and TRDA-derived SOM were randomly separated into four groups of three vials. The soil samples were subsequently incubated in four incubators set at four different temperatures (4, 14, 24, and 34°C). After an hour of pre-incubation time, the vials were sealed with lids and flushed with CO$_2$-free air. The sealed vials were placed back in the incubator to let CO$_2$ accumulate in the headspace for 5 hours. The vials were then flushed with CO$_2$-free air through a LI-820 IRGA to measure the CO$_2$ flux. The IRGA data were calibrated with CO$_2$ standards of known concentrations to evaluate the amount of CO$_2$ respired in a certain interval, which were used to calculate SOM decomposition rates. Assuming that most readily available C was decomposed after the initial 36 days of incubation, the obtained decomposition rates were then used to calculate the $Q_{10}$ values of relatively recalcitrant C by fitting the obtained data to the exponential equation explained in the previous experiment.

Model fitting and statistic analysis

The experimental data were fitted to Eqs. 1, 2, and 3 to determine the values of $K_f$, $K_r$, and $\alpha$ by the standard least square method (JMP 7.0, SAS Institute, Cary, NC). All statistical analyses were conducted using JMP 7.0. Effects of temperature and incubation time on the $Q_{10}$ values were tested using two-way analysis of variance (ANOVA). In addition, Student’s
t-tests were used to compare the $Q_{10}$ values of relatively labile and recalcitrant SOM. Data were considered significantly different at $\alpha=0.05$ level.

Results

Experiment 1: Determination of relatively labile C $Q_{10}$ values

The C mineralization rates (Fig. 1) of the studied soils during the 36 days of incubation ranged from 1.3-13.3 mg CO$_2$-C g$^{-1}$ soil-C d$^{-1}$ at the initial stage of incubation, to 0.6-1.8 mg CO$_2$-C g$^{-1}$ soil-C d$^{-1}$ at day 36. As expected, both soils showed significantly different C mineralization rates (Fig. 1) among the four different temperatures (4, 14, 24, and 34$^\circ$C) at the beginning of soil incubation (from days 1 to 5) for both soils ($P<0.001$), with higher temperatures inducing higher C mineralization rates. Later in the incubation, respiration rates exhibited no significant differences on days 11 ($P=0.561$) and 36 ($P=0.183$). However, on day 22, the C mineralization rate at 4$^\circ$C was significant lower ($P=0.014$) than at higher temperatures in the TRDA soil.

The C mineralization rates were fitted by the first-order two-component parallel model (Eq. 1 and 2). The model inputs and outputs are summarized in Tables 2 and 3. Percentage of relatively labile C was obtained by fitting the respiration data at 34$^\circ$C to the first-order two-component model and this was assumed to be constant at all temperatures. Most treatments showed a good fit to the first-order two-component parallel model ($R^2 > 0.6$, with the exception of soils that were incubated at 4$^\circ$C, Table 2). PHAR soils consisted of 4.2 ± 0.4% relatively labile C (as determined under these conditions), and TRDA soils consisted of 5.2 ±
0.7% relatively labile C. Rate constants of relatively labile and recalcitrant C differed by two orders of magnitude. Results from the model indicate that most relatively labile C (PHAR: 99.9%; TRDA: 85.3%) but only a small fraction of relatively recalcitrant C (PHAR: 4.4%; TRDA: 5.5%) was respired at the highest temperature (34°C) after 36 days of pre-incubation. The total amount of respired C obtained from this first-order two-component parallel model was compared to the cumulative respired CO₂ (Table 2), and no significant difference was found (P>0.05). Whereas mean turnover times of relatively labile C varied from days to months, those of relatively recalcitrant C varied in years (Table 2). Q₁₀ values of relatively labile C pools in both soils were significantly higher than those of relatively recalcitrant C pools (t=6.1, P<0.001 for PHAR soils; t=25.9, P<0.001 for TRDA soils) (Table 3).

Experiment 2: Determination of relatively recalcitrant C Q₁₀ values

Carbon mineralization rates of soil samples at four different temperatures after a 36-day pre-incubation period at 24°C are shown in Fig. 2. As expected, these rates were significantly smaller than the C mineralization rates observed at the initial stage of experiment 1 (Fig. 1). Table 4 shows the parameters obtained by fitting the data collected both at the initial stage (experiment 1) and after 36-days of pre-incubation (experiment 2) to Eqs. 1 and 2, which adequately described the increase in C mineralization rates 4 to 34°C (R²>0.80). For both PHAR and TRDA soils, Q₁₀ values at the beginning of the incubation experiment were significantly larger than those after 36 days of incubation (PHAR: t=7.1, P<0.001; TRDA: t=5.6, P<0.001). Soils samples after incubation under PHAR had greater respiration than the soils collected under TRDA (Fig. 2).
Discussion

Decomposition rates of SOM were higher in our study than those reported by Fierer et al. (2003), Conen et al. (2006), and Yang et al. (2007) but were lower than those reported by Vance and Chapin (2001). Underlying causes for these differences are difficult to determine because the studies relied on soils collected from various ecosystems and in different seasons. For instance, collecting soils during a cool season (e.g., Fierer et al. 2003; Conen et al. 2006) may cause low soil C mineralization rates. At lower temperatures, relatively low production of root exudates promotes lower soil respiration rates (Uselman et al. 2000). Furthermore, the soils analyzed in our study corresponded to monocultures of grasses, and it is known that the belowground allocation of C in prairie grasses is higher than that in crops and temperate forests (Al-Kaisi and Grote 2007). As a result, these prairie soils tend to exhibit greater SOM content and root activities. Although the studied grass monocultures have been established for four years and it is likely that they have not yet enhanced SOM content significantly, the monocultures could have promoted active belowground C cycling, which is reflected by the high rates of SOM decomposition. Because of this process, it might then be expected that our respiration rate data are higher than those of temperate forest ecosystems that have similar C contents (e.g. Yang et al. 2007). Moreover, the measured SOM decomposition rates differ from those of other studies potentially as a result of the different C contents. For example, taiga forest ecosystems have higher SOM contents and as a result exhibit higher soil C decomposition rates (Vance and Chapin 2001).
Results from the first-order two-component parallel model suggested that the relatively labile C pool comprised a small fraction of the total SOM (4.2% for PHAR and 5.2% for TRDA, Table 2), which is comparable to the amount of the most active C pool usually used in SOM dynamic models (e.g., Parton et al. 1987; Townsend et al. 1997) and to the amount found in some experimental studies that relied on different tools to quantify the labile C pool (e.g. McLauchlan and Hobbie 2004; Zou et al. 2005). In our study, the relatively recalcitrant C pools could be interpreted as the sum of the intermediate and passive C pools in those studies where three C pools are used (Townsend et al. 1997; Knorr et al. 2005; Yang et al. 2007).

By fitting a three-pool first-order model to their four forest soils respiration data, Yang et al. (2007) found that the average mean residence times of labile, intermediate, and passive C were 219 days, 78 years, and thousands of years, respectively. The relatively short turnover times in our study may be due to high quantities of recent exudates in the rhizosphere of these grasses, high microbial biomass, or to the strong disturbance occurring in the soil preparation process, for instance sieving and drying, that might have broken down some aggregates, and released some relatively labile C that had once been well protected (Franzluebbers et al. 2000).

Our results show a dependency of C mineralization rates to temperature change, which has been documented in other studies (e.g., Townsend et al. 1995; Liski et al. 1999; Fierer et al. 2005). The sensitivity of soil respiration to temperature change is frequently evaluated through $Q_{10}$ values (Parton et al. 1987; Kätterer et al. 1998; Davidson and Janssens 2006), and our overall $Q_{10}$ values are comparable to those reported in other studies. For instance, Lenton and Huntingford (2003) reviewed a series of laboratory studies to show that the $Q_{10}$
values of soil respiration had a mean value of 2.51 (n=167, SD=1.37). Further, Kätterer et al. (1998) compiled results of soil C mineralization experiments at temperatures ranging from 5 to 35°C, finding that estimated \( Q_{10} \) values varied between 1.35 and 2.88.

The availability of soil C presumably is on a continuum from liable to recalcitrant. We have chosen to label values determined in our study as relatively liable C and relatively recalcitrant C. Absolute availability of C would be a function of stability of the remaining C. Thus, the longer the incubation period, the greater the stability that would be expected of the remaining C. In experiment 2, soils were incubated (36 days) under common conditions to a uniform starting point and then the remaining C was incubated at different temperatures.

A comparison of \( Q_{10} \) values between soils at different incubation periods shows that the initial soil respiration rates were more sensitive to temperature change than those taking place in soils that had been incubated for 36 days (Table 4). Because most of the relatively labile C (PHAR: 98.1%, TRDA: 83.8%) was consumed within the first 36 days of pre-incubation (Table 2), soils employed to measure respiration rates after this pre-incubation period had reduced amounts of relatively labile C. Consequently, the C quality of these soils likely was significantly lower relative to the soils that were not pre-incubated. Franzluebbers et al. (2000) reported that C mineralized during the first three days of incubation after air drying primarily originates from biologically active pools of SOM. Further, Conant et al. (2008) concluded that, once about 8% of total soil C has been respired during a much longer pre-incubation process, the remaining C was relatively recalcitrant. The two obtained sets of \( Q_{10} \) values in this study have slightly different means. The first set of \( Q_{10} \) values was derived from a mathematical model reported to adequately describe the soil microbial respiration processes (Kätterer et al. 1998). To compare \( Q_{10} \) values of different C pools, this first-order
two-component model assumes fixed pool sizes for the relatively labile and recalcitrant C pools at different temperatures. The second set of $Q_{10}$ values was obtained by directly fitting a simple exponential equation using soil C mineralization data at two different incubation periods (at the beginning on day 1 vs. after 36-day pre-incubation period), therefore providing a more realistic assessment of temperature sensitivities of relatively labile and recalcitrant C pools. Despite different ways in which these two sets of $Q_{10}$ values were obtained, a consistent trend shows that relatively labile C is more temperature sensitive than relatively recalcitrant C.

To explain why different studies report different findings and why our finding that SOM quality affects the sensitivity of respiration rates to temperature, it is necessary to analyze the factors that control SOM recalcitrance. SOM quality depends on chemical recalcitrance, physical protection, and biological availability (Sollins 1996; McLauchlan and Hobbie 2004). Chemical recalcitrance is considered dependant on molecular weight, C/N ratio, and the presence of aromatic or other complex structures (Krull et al. 2003). Whereas physical protection is usually regarded as the chemical stabilization of SOM to clay mineral surfaces (Sollins et al. 1996), biological availability reflects the accessibility of SOM in soil aggregates by soil microbes and enzymes (Oades and Waters 1991). All of these factors (chemical recalcitrance, physical protection, and biological availability) are likely to affect independently the temperature sensitivity of SOM. As a result, relatively recalcitrant soil C may differ in its response to temperature change, depending on the main source of SOM recalcitrance.

Bosatta and Agren (1999) hypothesized the presence of an inverse relationship between litter C quality and temperature sensitivity. C quality was defined as the number of enzymatic
steps required to release CO$_2$ from an organic compound, only assuming intrinsic chemical recalcitrance. As a result, microbial enzymes and free energy act as the determining factor of temperature sensitivity. The inverse relationship between litter C quality and temperature sensitivity was supported by results from incubation experiments where fresh litter and roots were added to soils (Fierer et al. 2005) and by results from incubation experiments of four tundra organic soils with different C qualities (Mikan et al. 2002). In both cases, it was likely that the organic C had little association with soil physical particles because organic C is abundant in soils amended with fresh litter or in tundra organic soils.

In contrast to the hypothesized inverse relationship between C quality and temperature sensitivity, a number of incubation experiments involving mineral soils have revealed that relatively recalcitrant SOM might have lower or similar temperature sensitivity than relatively labile SOM (e.g., Townsend et al. 1995, 1997; Liski et al. 1999; Giardina and Ryan 2000; Fang 2005). Results from our study also suggest a lower temperature sensitivity associated with relatively recalcitrant C pools. The low temperature sensitivity found in our study may be explained by the low C content of these soils (PHAR: 1.95%, TRDA: 1.34%). Although SOM is stabilized by many processes: physical protection in soil aggregates, chemical bonding onto minerals, and intrinsic chemical recalcitrance; chemical recalcitrance may be the only important factor in litter or soil organic layers where organic C is not readily associated with soil aggregates (Thornley and Cannell 2001). In most mineral soils, however, active physico-chemical processes between organic C and soil particles make physical stabilization (i.e. physical protection and chemical bonding) an important factor that could affect the temperature sensitivity of SOM decomposition (Thornley and Cannell 2001). In a study to assess the impact of land-use change on soil C sequestration in agricultural soils,
Galdo et al. (2003) found that most of the relatively recalcitrant C was associated with micro-aggregates in the silt and clay fraction, indicating more permanent C sequestration. In mineral soils, increasing temperatures could induce both higher SOM association with mineral particles and higher rate constants of soil microbial respiration (see Eq. 1), which would ultimately cause competition for available organic matter substrates (Thornley and Cannell 2001). Under these conditions, some SOM is possibly stabilized at higher temperatures rather than decomposed by microorganisms. Consequently, the response of soil C decomposition to temperature could depend on the combined effect of these mechanisms: chemical C quality and physical mineral association. More work needs to be done to elucidate how these mechanisms could, specifically and interactively, affect the temperature dependency of soil C mineralization.

Despite varying results obtained in controlled laboratory experiments, many field studies indicate that temperature responses persist for a limited time and often result in apparent acclimation over time (Giardina and Ryan 2000; Jarvis and Linder 2000; Melillo et al. 2002). These studies suggest that higher temperatures will not necessarily induce higher CO\textsubscript{2} in the long term, especially in the mineral soils. Increasing temperature, however, could affect world regions differently as a result of their geographic and climate characteristics. In high latitude regions, for example, increasing temperatures may induce soil CO\textsubscript{2} emission dramatically due to the potential thawing of permafrost soils (Dioumaeva et al. 2002); in wetland areas, higher temperatures may promote evaporation, exposing the thick organic matter to the air and therefore boosting the soil respiration (Waddington et al. 1998). However, in temperate and tropic soils, the stronger mineral-organic matter associations may counteract the increased potential of soil respiration induced by higher temperature.
Consequently, the assumption about the uniform characteristics of SOM in some models (Parton et al. 1987; Jenkinson et al. 1991; Lloyd and Taylor 1994) should be corrected.

**Conclusions**

To evaluate how SOM quality and temperature could affect SOM decomposition rates, the temperature sensitivities of relatively labile and recalcitrant C pools were evaluated in two incubation experiments using both a first-order two-component parallel model and an exponential model. Results from these experiments reveal that whereas mean turnover times for relatively labile C in the studied soils range from days (at higher temperatures) to months (at low temperatures), those for relatively recalcitrant C ranged from two (at higher temperatures) to eight years (at lower temperatures). C mineralization rates of relatively labile and recalcitrant C pools responded distinctly to temperature change, with relatively labile C being decomposed more quickly at elevated temperatures in comparison to relatively recalcitrant C. It is hypothesized that the lower temperature dependency of relatively recalcitrant C may be due to a higher degree of stabilization to soil particles compared to relatively labile C.

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Table 1 Main characteristics of the soils used for incubation experiments. PHAR is *Phalaris arundinacea*; TRDA is *Tripsacum dactyloides*. Soils belonged to the Nodaway soil series and the surface textural class was silt loam

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>N (g kg(^{-1}))</th>
<th>C (g kg(^{-1}))</th>
<th>C/N ratio</th>
<th>P(^{a}) (mg kg(^{-1}))</th>
<th>K(^{b}) (mg kg(^{-1}))</th>
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\(^{a}\) Extracted with Bray-P1

\(^{b}\) Extracted with 1 M NH\(_4\)OAc
Table 2 Parameters of the first-order two-component parallel model (Kätterer et al. 1998). PHAR is *Phalaris arundinacea*; TRDA is *Tripsacum dactyloides*. See the “Materials and methods” section for an explanation of the model. The ratio of labile to recalcitrant C was assumed to remain constant during decomposition at four temperatures. $\alpha$ is the percentage of labile C; $K_l$ and $K_r$ are the rate constants of labile and recalcitrant carbon pools, $R^2$ is the coefficient of determination, L is labile C, and R is recalcitrant C. Values are means ± sd (n=3)

<table>
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<th>$\alpha$ (%)</th>
<th>$K_l$ (10(^{-2}) d(^{-1}))</th>
<th>$K_r$ (10(^{-2}) d(^{-1}))</th>
<th>$R^2$</th>
<th>C respired (L) (%)</th>
<th>C respired (R) (%)</th>
<th>Total C respired (%)</th>
<th>Cumulative CO(_2)-C (%)</th>
<th>Turnover time (L) (d)</th>
<th>Turnover time (R) (y)</th>
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<td>16.1</td>
<td>7.3</td>
</tr>
<tr>
<td>PHAR</td>
<td>4</td>
<td>19.5</td>
<td>4.2±0.4</td>
<td>2.5±0.7</td>
<td>3.3±2.2</td>
<td>0.29</td>
<td>59.9</td>
<td>1.2</td>
<td>3.8</td>
<td>3.6±0.5</td>
<td>39.3</td>
<td>8.4</td>
</tr>
<tr>
<td>TRDA</td>
<td>34</td>
<td>13.4</td>
<td>5.2±0.7</td>
<td>28.8±4.7</td>
<td>15.9±5.0</td>
<td>0.94</td>
<td>85.3</td>
<td>5.5</td>
<td>9.2</td>
<td>10.1±1.0</td>
<td>3.5</td>
<td>1.7</td>
</tr>
<tr>
<td>TRDA</td>
<td>24</td>
<td>13.4</td>
<td>5.2±0.7</td>
<td>11.3±1.1</td>
<td>11.8±2.4</td>
<td>0.87</td>
<td>83.8</td>
<td>4.1</td>
<td>7.8</td>
<td>8.7±0.8</td>
<td>8.8</td>
<td>2.3</td>
</tr>
<tr>
<td>TRDA</td>
<td>14</td>
<td>13.4</td>
<td>5.2±0.7</td>
<td>5.1±0.8</td>
<td>7.7±2.3</td>
<td>0.62</td>
<td>71.5</td>
<td>2.6</td>
<td>5.7</td>
<td>6.5±0.4</td>
<td>19.7</td>
<td>3.8</td>
</tr>
<tr>
<td>TRDA</td>
<td>4</td>
<td>13.4</td>
<td>5.2±0.7</td>
<td>1.8±0.6</td>
<td>6.8±2.6</td>
<td>0.16</td>
<td>40.6</td>
<td>2.4</td>
<td>4.2</td>
<td>4.6±0.2</td>
<td>55.7</td>
<td>4.0</td>
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</tbody>
</table>
Table 3 Mean $Q_{10}$ values ± sd (n=3) of labile and recalcitrant carbon pools estimated from the first-order two-component parallel model (see the “Materials and methods” section for details). PHAR is *Phalaris arundinacea*; TRDA is *Tripsacum dactyloides*. $R^2$ is the coefficient of determination. L is labile C, and R is recalcitrant C

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>C Pool</th>
<th>$Q_{10}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHAR</td>
<td>L</td>
<td>2.71±0.30</td>
<td>0.99</td>
</tr>
<tr>
<td>PHAR</td>
<td>R</td>
<td>1.73±0.12</td>
<td>0.98</td>
</tr>
<tr>
<td>TRDA</td>
<td>L</td>
<td>2.48±0.06</td>
<td>0.99</td>
</tr>
<tr>
<td>TRDA</td>
<td>R</td>
<td>1.38±0.06</td>
<td>0.97</td>
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</table>
Table 4 Mean $Q_{10}$ values ± sd (n=3) obtained from respiration rates of relatively labile (measured on day 1) and relatively recalcitrant (after a 36-day pre-incubation period) SOM. PHAR is *Phalaris arundinacea*; TRDA is *Tripsacum dactyloides*.

Respiration rates were fitted into an exponential equation until optimal parameters $B$ and $k$ were obtained; $R^2$ is the coefficient of determination.

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Incubation stage</th>
<th>C quality</th>
<th>$B$</th>
<th>$K (10^{-2})$</th>
<th>$Q_{10}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHAR</td>
<td>Day 1</td>
<td>L</td>
<td>0.76±0.17</td>
<td>8.0±0.7</td>
<td>2.22±0.16</td>
<td>0.96</td>
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<td>TRDA</td>
<td></td>
<td></td>
<td>0.94±0.22</td>
<td>7.7±0.7</td>
<td>2.16±0.16</td>
<td>0.96</td>
</tr>
<tr>
<td>PHAR</td>
<td>After 36-day pre-incubation</td>
<td>R</td>
<td>0.54±0.17</td>
<td>5.5±1.0</td>
<td>1.73±0.18</td>
<td>0.82</td>
</tr>
<tr>
<td>TRDA</td>
<td></td>
<td></td>
<td>0.22±0.06</td>
<td>6.2±0.9</td>
<td>1.86±0.18</td>
<td>0.89</td>
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</tbody>
</table>
Fig. 1 Mean SOM decomposition rates (mg CO$_2$ - C g$^{-1}$ soil-C d$^{-1}$) ± sd (n=3) of soils collected under *Phalaris arundinacea* (PHAR) and *Tripsacum dactyloides* (TRDA) at four different temperatures during a 36-day incubation period. Decomposition rates were measured on days 1, 3, 5, 11, 22, and 36.
Fig. 2 Mean SOM decomposition rates (mg CO$_2$ -C g$^{-1}$soil-C d$^{-1}$) ± sd (n=3) of soil samples collected under *Phalaris arundinacea* (PHAR) and *Tripsacum dactyloides* (TRDA) at four different temperatures after a 36-day pre-incubation period at 24$^\circ$C
CHAPTER 3. CARBON ISOTOPE FRACTIONATION DURING SOIL ORGANIC MINERALIZATION IS TEMPERATURE DEPENDENT AND CONSISTENT WITH A RAYLEIGH FRACTIONATION MODEL

A paper submitted to *Oecologia*

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**Abstract**

The natural abundance of stable isotopes of carbon has been an important technique to investigate soil carbon dynamics, especially in ecosystems where a shift of C3 and C4 vegetation alters the isotopic signature of soil organic matter. This approach is robust if there is no or negligible carbon isotope fractionation effects associated with belowground processes, but few studies have evaluated this assumption. The goal of this study was to test if a measurable fractionation effect exists during microbial respiration in soils. For this test, we employed soils from monocultures of *Phalaris arundinacea* (a C3 grass) and *Tripsacum dactyloides* (a C4 grass) that were planted on a corn field. Soils were incubated at three different temperatures, and soil respiration rates and the carbon isotopic composition of respired CO\(_2\) were measured through time. An asymptotic decrease in respiration rates coincided with an asymptotic change in isotopic composition. Respired CO\(_2\) was strongly depleted in \(^{13}\)C at the beginning of the incubation experiments, and it progressively became
less depleted in $^{13}\text{C}$, regardless of type of planted vegetation (C3 vs. C4). A Rayleigh equation adequately described the obtained isotopic trend, indicating that a strong fractionation effect occurred during microbial respiration. Soil microbes discriminated more against $^{13}\text{C}$ at low temperatures (14°C) relative to the isotope discrimination obtained at higher temperatures (24 and 34°C). We hypothesize that the stronger fractionation effect at lower temperature is the result of (1) decreased microbial activity and the relative abundance of labile carbon substrates and/or (2) change in microbial community composition and function.

**Keywords**

Carbon isotope · Isotopic fractionation · Soil respiration · Global warming · Soil organic matter

**Introduction**

The natural abundance of $^{13}\text{C}$ has been used to address ecophysiological and biochemical questions, ranging from understanding the biochemical pathways of microorganisms (Kreuzer-Martin 2007) and vascular plants (Farquhar et al. 1989) to studying ecosystem gas exchange (Yakir and Sternberg 2000) and the biogeochemical interactions among plants, soils, and the atmosphere (Ehleringer et al. 2002). In particular, the stable carbon isotope composition of soil-respired CO$_2$ (the flux out of soil) is an important parameter that has been used to evaluate both soil carbon dynamics (Ehleringer et al. 2000; Hanson et al. 2000; Søe et al. 2004) and the role of soils in the carbon budgets of local (Broadmeadow et al.
The advantage of stable isotopes is that their natural abundances serve a dual role as both natural integrators and tracers of ecological processes. As integrators, stable isotopes permit an evaluation of the net outcome of a number of processes that vary both in space and time, without disrupting the natural activity or behavior of the component that is being investigated (e.g. Handley and Raven 1992; Högberg 1997; Robinson 2001). As tracers, stable isotopes permit the identification of biochemical processes by following the transformations and fate of a component in situ (e.g. Ehleringer et al. 2002; Buhring et al. 2006; Aboka et al. 2009). The use of natural abundances of stable isotopes as both integrators and tracers requires that different potential sources have relatively fixed isotopic signatures, and these signatures should be distinct from one another. In particular, to trace the movement of a component or an element, no significant isotopic fractionation should occur during the movement of that component within the system that is being investigated (Ehleringer et al. 2002). For example, belowground processes are difficult to evaluate and quantify with most research techniques, but the carbon isotopic composition ($\delta^{13}C$) of soil-emitted CO$_2$ has permitted the distinction between soil organic matter (SOM)-derived and root-derived CO$_2$ (e.g., Rochette et al. 1999; Ekblad and Högberg 2000). Further, $\delta^{13}C$ values of SOM have allowed an identification of the proportion of SOM derived from C3 and C4 vegetation (e.g., Pessenda 2001). The conclusions reached by these studies are based on the assumption that no significant isotopic fractionation occurs during SOM decomposition. Under this assumption, $\delta^{13}C$ values of soil-emitted CO$_2$ reflect those of the CO$_2$-produce substrates. Results from some studies provide
support to this assumption by suggesting that a negligible isotope fractionation effect occurs during autotrophic (Lin and Ehleringer 1997) and rhizosphere (Cheng 1996) respiration. However, results from other studies suggest the presence of an isotopic fractionation effect of up to 3.4 ‰ during soil microbial respiration (Blair et al. 1985; Mary et al. 1992).

Here, we test the hypothesis that soil microbial respiration is associated with an isotope fractionation effect. Because the extent of isotope fractionation is temperature dependent (Epstein et al. 1951; O’Neil 1986), even in biologically-mediated processes (e.g., Canfield et al. 2006), our approach involves incubating soils at different temperatures and measuring the stable carbon isotopic composition of respired CO₂. A fractionation effect occurring during the prolonged incubation of SOM respiration should produce a Rayleigh-type process (Clay et al. 2007). As a result, an asymptotic change in δ¹³C values of respired CO₂ should occur through time in response to a fractionation effect associated with microbial decomposition and different incubation temperatures should produce different asymptotic changes in δ¹³C values of respired CO₂.

Because plants contribute organic substrates to soils, which are readily decomposed by microbes (Jenkinson et al. 1991), an asymptotic change in δ¹³C values of respired CO₂ could also occur if the δ¹³C values of plant-produced organic matter are different than those of SOM. To distinguish a Rayleigh-type process from the preferential degradation of two sources with different δ¹³C values, we employed soils that were planted with C3 and C4 species. A Rayleigh-type process should produce similar asymptotic curves for soils planted with C3 and C4 species, whereas the preferential degradation of a mixture of two carbon
sources should produce inverted asymptotic curves that reflect the preferential degradation of organic substrates from the C3 and C4 plants (Fig. 1). For the study, we collected soil samples from a corn field that was converted to a pasture by planting *Phalaris arundinacea* (a C3 plant) and *Tripsacum dactyloides* (a C4 plant).

**Materials and Methods**

*Soil characteristics*

The studied soils were collected from planted grass monocultures at Iowa State University’s Rhodes Research and Demonstration Farm (42.00°N, 93.25°W) in Marshall County, Iowa. The climate is strongly continental with a mean annual temperature of 8.7°C and monthly mean air temperatures ranging from -8.1°C in January to 23.2°C in June. The mean annual precipitation is 880 mm, 14% of which occurs from November-February. Plots (15m×15m) of *Phalaris arundinacea* and *Tripsacum dactyloides* were established in 2001 on formerly tilled cropland long planted with corn without crop rotation. Since establishment, the soils had not been grazed, burned, hayed, or fertilized; and these studied soils are in the Nodaway series, which are fine-silty, mesic, Mollic Udifluvents (Oelmann 1981) that tend to be fertile, moist, and highly productive.

*Soil sample collection*

Soil samples were collected in plots cultivated with *P. arundinace* and *T. dactyloides* in August, 2006. For each plot of *P. arundinace* and *T. dactyloides*, ten soils were collected from the 0-15 cm depth using a soil auger, and then the collected soils were mixed together to have one sample per plot. All visible roots and fresh litter materials were removed from
the collected soil. Soil samples were passed through a 4-mm mesh sieve to remove large rocks and particles, homogenized by hand, and then air-dried.

*Soil incubation*

Well-mixed soil samples (10g dry weight equivalent) were incubated at a water potential of -0.03 MPa in triplicate in individual 75-ml vials. This water potential of -0.03 MPa was selected because it is the optimum moisture for decomposition of SOM (Harris, 1981). Once the optimal water potential was achieved, vials were weighed and then placed in incubators (Fisher Scientific, Pittsburgh, PA) that were set at three different temperatures (14, 24, and 34 °C). The vials were tightly capped with lids to keep constant moisture, but they were opened occasionally to allow the exchange of air. To ensure that moisture remained constant, samples were weighed every two days during the incubation period. When the weight was lower than the pre-incubation weight, water was added until the pre-incubation weight was achieved.

During the soil incubation experiment, both CO₂ production rates and carbon isotope compositions of respired CO₂ were measured on days 1, 3, 5, 11, 22, and 36. The unequal measurement intervals were selected to capture the typical asymptotic decrease in soil respiration rates during an incubation experiment (e.g., Schimel et al. 1994), as well as the predicted asymptotic change in δ¹³C values of respired CO₂. To measure CO₂ production rates, vials containing the soil samples were sealed with lids and flushed with CO₂-free air. The sealed vials were placed back in the incubator for a period of time that was sufficient to accumulate quantifiable amounts of CO₂ in the headspace. The vials were then taken out of
the incubator and flushed with CO$_2$-free air through a LI-820 IRGA (Infrared Gas Analyzer, LI-COR, Lincoln, NE) to measure the CO$_2$ flux. The IRGA data were calibrated with CO$_2$ standards of known concentrations to evaluate the amount of CO$_2$ respired in a certain interval, which were used to calculate SOM mineralization rates.

*Stable carbon isotope ratio measurements*

To determine $\delta^{13}$C values of respired CO$_2$, vials were sealed with lids and flushed with CO$_2$-free air. The sealed vials were placed back in the incubator to let CO$_2$ accumulate in the headspace. After five hours (at the beginning of the experiment) to several days (at the end of the experiment), the vials were taken out of the incubator, and 5 ml of headspace air was withdrawn with a gas-tight syringe. The variable duration involved in the collection of headspace air was necessary to ensure that enough CO$_2$ was available to produce reliable carbon isotope ratio determinations.

The withdrawn gas was injected into He-flushed exetainers (Labco Co, Houston, TX), which were placed in a Finnigan GasBench II instrument attached to a Finnigan Delta Plus XL isotope-ratio mass spectrometer (Thermo Scientific, Waltham, MA) to determine the carbon isotopic composition of respired CO$_2$.

To test the reliability of our method, incubation vials were flushed with helium gas containing known concentrations of CO$_2$ with known $\delta^{13}$C values. Vials were capped for several days to recreate the maximum period that was needed to collect respired CO$_2$ in the incubation experiment. After 1 (at the beginning of incubation experiment) to 10 (at the end
of incubation experiments) days, 5 ml of headspace air was withdrawn with a gas-tight syringe, and the isotopic composition of CO\textsubscript{2} was determined. Results indicate that the method used did not produce a measurable isotopic fractionation.

Besides determining $\delta^{13}$C values for respired CO\textsubscript{2}, $\delta^{13}$C values of SOM and plant tissues were also determined. For these determinations, leaf tissues from the planted grasses were collected, air dried, and ground. For the collected soils, aliquots of well-mixed samples were ground, and any carbonate was removed from the samples by HCl digestion. The $\delta^{13}$C values of the decalcified soil samples and the ground plant tissues were determined via flash-pyrolysis at 1100 °C with a Costech elemental analyzer fitted to a Thermo Finnigan Delta plusXL isotope ratio mass spectrometer. Analytical precision and accuracy were determined on the basis of repeated analysis of two internal lab standards calibrated against the internationally accepted V-PDB standard (standard deviation <0.08). Whereas the standards for determining the $\delta^{13}$C values of respired CO\textsubscript{2} consisted of CO\textsubscript{2} produced via phosphorolysis of pure carbonates of known CO\textsubscript{2} values, those for determining the $\delta^{13}$C values of SOM and plant tissues consisted of pure organic compounds of known $\delta^{13}$C values. The standards were measured simultaneously with the samples.

\textit{Carbon stable isotope calculations}

Carbon isotope ratios are reported in parts per thousand (‰) relative to the Vienna Pee Dee Belemnite standard (VPDB), according to the following equation

$$
\delta^{13}C(\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000
$$

(1)
Where $R_{\text{sample}}$ is the ratio of $^{13}\text{C}/^{12}\text{C}$ in the sample, and $R_{\text{standard}}$ is the ratio of $^{13}\text{C}/^{12}\text{C}$ in the standard.

The $\delta^{13}\text{C}$ values of respired CO$_2$ were fitted to the simplified Rayleigh equation:

$$\delta^{13}\text{C}_t - \delta^{13}\text{C}_0 = \varepsilon \ln\left(\frac{C_t}{C_0}\right) \quad (2)$$

Where $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_0$ are the carbon isotope ratios of the respired CO$_2$ at time $t$ and time 0, respectively, $C_t$ and $C_0$ are the soil carbon content at time $t$ and time 0, respectively, and $\varepsilon$ is the carbon isotope enrichment factor.

**Statistical analysis**

All statistical analyses were conducted using JMP 7.0 (SAS Institute, Cary, North Carolina, USA). Effects of temperature and incubation time on the $\delta^{13}\text{C}$ values of respired CO$_2$ were tested using two-way analysis of variance (ANOVA). Data were considered significantly different at $\alpha=0.05$ level.

**Results**

**Carbon isotope ratios**

Whereas SOM of soils covered with *P. Arundinacea* exhibited a mean $\delta^{13}\text{C}$ values of -21.2‰, SOM of soils covered with *T. dactyloides* exhibited a mean $\delta^{13}\text{C}$ values of -19.2‰. Leaf tissues of *P. Arundinacea* and *T. dactyloides* exhibited mean $\delta^{13}\text{C}$ values of -28.9‰ and -11.8‰, respectively (Table 1).
The $\delta^{13}C$ values of respired CO$_2$ asymptotically became less negative through time (Fig. 3). Soils covered with different vegetation (C3 and C4) significantly differed in terms of respired CO$_2$ $\delta^{13}C$ values, with those for soils covered with the C3 grass being more negative than those for the soils covered with the C4 grass ($P<0.0001$). Whereas respired CO$_2$ $\delta^{13}C$ values for the C3 soils ranged from -31.6‰ to -22.7‰, those for the C4 soils ranged from -26.3‰ to -16.6‰. The final respired CO$_2$ $\delta^{13}C$ values for the C3 soils (on day 36) were less negative than their SOM $\delta^{13}C$ value ($P<0.0001$), and those for the C4 soils were less negative than their SOM $\delta^{13}C$ values ($P<0.0001$).

The carbon isotope data for soil respired CO$_2$ were fitted to a Rayleigh-type equation to examine whether an isotopic fractionation occurred during SOM decomposition in the incubation process. Figure 4 shows the Rayleigh fractionation at three different temperatures (14, 24, and 34°C) for the studied C3 and C4 soils. The coefficient of determination $R^2$ ranged from 0.54 to 0.84, indicating that the Rayleigh model captured to a degree the obtained carbon isotope data. Table 2 showed the calculated enrichment factors ($\varepsilon$). A comparison of the estimated $\varepsilon$ values for the C3 and C4 soils reveals that they are statistically similar ($P > 0.05$). Moreover, whereas $\varepsilon$ at 14°C differed significantly relative to those obtained at 24 and 34°C ($P<0.05$); $\varepsilon$ values obtained at 24 and 34°C were similar (C3: $P =0.98$; C4: $P =0.79$).

**Discussion**

Our results suggest that $^{13}C$ values of respired CO$_2$ were strongly depleted in $^{13}C$ during the initial stages of incubation but they asymptotically became less negative with time (Fig. 3). A
similar pattern has been found in other soil incubation studies (e.g., Schweizer et al. 1999; Crow et al. 2006; Pendall and King 2007). This asymptotic pattern has traditionally been explained in terms of (1) a mixing of two carbon sources with different δ^{13}C values, (2) preferential microbial decomposition of SOM (Ehleringer et al. 2000), or (3) a shift in microbial community (Crow et al. 2006). These explanations, which are discussed below, assume, however, that the isotopic fractionation effect during heterotrophic decomposition is insignificant.

A mixing of two soil carbon sources with different δ^{13}C values cannot adequately explain the obtained data. Because the isotopic signatures for the grasses in this study were distinct from those of bulk SOM (Table 1), respired CO$_2$ δ^{13}C values should have gradually approached those of SOM because microorganisms metabolize labile carbon more readily than older, more recalcitrant carbon. This old organic carbon was present in the studied soils before the grasses were planted. If this were the case, the initial δ^{13}C values of respired CO$_2$ would represent newer carbon inputs from the C3 and C4 grasses, followed by a gradual change in respired CO$_2$ δ^{13}C values as the incubation continues to increasingly reflect the relatively older SOM derived from the previous corn biomass. Consequently, the respired CO$_2$ δ^{13}C values for the C3 soils should initially be low, and these values should increase with time (Figs. 1, 3). The respired CO$_2$ δ^{13}C values for the C4 soils, however, should initially be higher, based on the δ^{13}C values of leaves and root of *Tripsacum dactyloides* (Table 1), and become more negative with time, as they approach to δ^{13}C value of SOM, which is the opposite trend that was observed in this study (Figs. 1, 3). Our results show a similar
asymptotic pattern of progressive $^{13}$C-enrichment of respired CO$_2$ for both C3 and C4 soils (Fig. 3).

Similarly, the observed gradual $^{13}$C-enrichment of respired CO$_2$ cannot be explained in terms of preferential microbial decomposition of SOM. Individual plant components can vary as much as 10‰ in their $\delta^{13}$C values, even within the same plant (O’Leary 1981). For example, lignin is depleted in $^{13}$C by 2.5‰ to 6.6‰ relative to bulk plant materials (Benner et al. 1987; Mary et al. 1992; Schweizer et al. 1999). Labile carbon substrates, such as cellulose and fatty acids, are usually slightly enriched in $^{13}$C in relation to bulk plant materials (Schweizer et al. 1999). As a result, the preferential decomposition of labile compounds produces $^{13}$C-enriched respired CO$_2$, which progressively becomes more $^{12}$C enriched as relatively more recalcitrant substrates are metabolized. In contrast, our data show the opposite pattern (Fig. 3), thereby ruling out the possibility that the preferential decomposition of labile substrates explains the observed data.

The observed initial depletion in $^{13}$C of respired CO$_2$ could reflect an isotopic fractionation effect caused by the decomposer community during mineralization of SOM. Fernandez and Cadisch (2003) tested the decomposition of pure biochemical compounds and bulk plant tissue by single fungal species, finding a variable $^{13}$C-depletion that changed with the time of decomposition. The largest fractionations (up to ~12‰ for lignin and ~7‰ for glucose) occurred during early stages of decomposition, followed by a progressive $^{13}$C-enrichment as the decomposition process continued. These findings strongly suggest the existence of a significant isotopic fractionation effect during SOM decomposition. Microorganisms initially
could either preferentially target positions occupied by $^{12}$C or selectively incorporate $^{13}$C into their microbial biomass (Henn and Chapela 2000). As a result, $^{12}$C-enriched CO$_2$ is released to the atmosphere, while $^{13}$C-enriched substrates accumulate either in SOM or in the microbial biomass. As the $^{12}$C-enriched substrates become less available, the residual $^{13}$C-enriched substrates are then utilized, thereby producing a subsequent $^{13}$C-enrichment of soil respired CO$_2$. A Rayleigh model adequately describes this process (Mariotti et al. 1981), and its application to our obtained isotope data reveals that the respired CO$_2$ δ$^{13}$C values (Fig. 4) can be explained in terms of an isotope effect produced during microbial decomposition of SOM.

In support of our conclusion that an isotope effect occurs during microbial decomposition, our data show that temperature influences the extent of isotope fractionation (Fig. 4). The obtained ε values were lower at low temperature (14°C) compared to those at higher temperatures (Fig. 4). This trend has been found in other studies addressing the effect of microbial processes. For example, increasing temperatures promotes microbial sulfate reduction rates, which results in decreased fractionation effect of stable sulfur isotopes (Canfield 2001). However, other studies found that within the microbial growth range of temperature, higher temperature could induce more fractionation effect against $^{34}$S. In terms of SOM decomposition, Garten et al. (2000) also identified that the extent of change in δ$^{13}$C values from forest litter inputs to mineral soil is significantly associated with mean annual temperature by comparing six forests over an elevation gradient. The largest isotopic discrimination was found to coincide with higher decomposition rates under higher temperature (lower elevation). However, this conclusion is inconclusive because other factors
such as moisture or litter quality could equally produce the observed altitudinal variation of SOM $\delta^{13}C$ values.

Our results show that both respired CO$_2$ $\delta^{13}C$ values and soil respiration rates asymptotically change through time. Our results also indicate that low temperatures produce both a stronger isotope fractionation and low soil respiration rates. These patterns suggest that microbial metabolism controls the extent of isotope fractionation with temperature possibly serving as the limiting factor on microbial growth at cooler conditions (e.g., Núñez-Regueira et al. 2006). As a result, a relatively small number of microorganisms were presumably metabolically active at the beginning of the incubation experiment, particularly if one takes into account that the experiments involved the addition of water to dry soils, which likely caused the slow revival of microorganisms at low temperatures. We hypothesize that the observed strong isotope fractionation resulted from the combined presence of few microorganisms because of drying and sufficient available carbon substrates in the soils. As the incubation process continued, we hypothesize that the microbial population increased and labile carbon substrates became less available, which resulted in a reduced fractionation effect. At higher temperatures, we observed lower CO$_2$ $\delta^{13}C$ values only during the first several days of incubation process after the soils were rewetted. This observation is consistent with our hypothesis, because it is likely that at the beginning of the incubation experiment the microbial population was low and/or carbon substrates were sufficient.

Although the Rayleigh fractionation model could largely explain the asymptotic change of carbon isotopic ratio of respired CO$_2$ during incubation periods (Fig. 4), deviations from the
model could result from some other mechanisms. For example, it is possible that soil microbial community changed during the incubation experiments as demonstrated by Crow et al. (2006), who identified a shift in the soil microbial community following the consumption of easily decomposed C pools. This presumed shift in the microbial community could account for the observed deviation from our Rayleigh model because it appears that the extent of isotope discrimination varies among different microbial species (Henn and Chapela 2000; Fernandez and Cadish 2003). This hypothesized shift in the soil microbial community could have occurred once readily available organic matter was depleted or could have been promoted by the rewetting of dried soil during the experiment. It is possible that the rewetting could have allowed fast growing colonizing microbial species to become prevalent at the beginning of the incubation experiments. As the experiment progressed, however, slowly growing species could have become more abundant. Furthermore, microbial community composition and function could differ significantly among different temperatures (Zogg et al. 1997), which could also account for the observed deviation from our Rayleigh model.

Because our results are based on incubation experiments, it is difficult to predict the extent of the inferred carbon isotopic fractionation in natural conditions. Unlike laboratory conditions, labile substrates are commonly available in most soils, and typical disruptions associated with the collection, sieving, and rewetting of soils are uncommon in natural conditions. We propose that the mineralization of labile substrates by soil microorganisms could result in a temperature-dependent fractionation effect. As a result, it is possible that the interpretation of
carbon isotope data to evaluate underground biogeochemical processes requires some caution, especially when the decomposition of complex SOM is involved.

**Conclusion**

Our results from soil incubation experiments suggest that an isotopic fractionation effect occurs during SOM decomposition. Microorganisms in the studied soils preferentially discriminated against $^{13}$C at the beginning of the incubation experiments and at lower temperatures. We also found that the extent of the fractionation effect correlates with respiration rates. Consequently, we hypothesize that these observations can be explained in terms of (1) the availability of labile substrates relative to the degree of mineralization and (2) a possible shift in the soil microbial community composition and function at different incubation periods and at different temperatures. Reduced availability of labile substrates relative to the degree of metabolic activity of soil microorganisms reduces the extent of discrimination against $^{13}$C. Species-specific discrimination may cause a shift in the carbon isotope ratio of respired CO$_2$ when microbial communities shift. Our results then suggest that the natural abundance of $^{13}$C should be used with caution to estimate the soil carbon dynamics, particularly because temperature could have an indirect effect on respired CO$_2$ $\delta^{13}$C values.

**Acknowledgements**

We thank Mark Mathison and Humberto Carvajal-Ortiz for their assistance in soil sample collection and Josh Heitman for helping us perform the soil water retention measurements.
This work was supported by NSF grant DEB-343766, with additional support from an Environmental Scholarship, Iowa State University.

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Aboka FO, Heijnen JJ, van Winden WA (2009) Dynamic $^{13}$C-tracer study of storage carbohydrate pools in aerobic glucose-limited Saccharomyces cerevisiae confirms a rapid steady-state turnover and fast mobilization during a modest stepup in the glucose uptake rate. FEMS Yeast Res 9:191-201


Zogg GP, Zak DR, Ringelberg DB, MacDonald NW, Pregitzer KS, White DC (1997)

Table 1. Carbon isotopic composition (in ‰) and 95% confidence interval (in parentheses) of leaf, and root samples of the studied grass species in 2005 and 2006.

<table>
<thead>
<tr>
<th></th>
<th>2005</th>
<th>2006</th>
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<tr>
<td></td>
<td>Leaf</td>
<td>Root</td>
</tr>
<tr>
<td><em>P. arandinaea</em></td>
<td>-27.98 (1.94)</td>
<td>-27.84 (2.52)</td>
</tr>
<tr>
<td><em>T. dactyloides</em></td>
<td>-12.18 (0.85)</td>
<td>-11.83 (0.14)</td>
</tr>
</tbody>
</table>
Table 2. Enrichment effect ($\varepsilon$) ± sd for soils from *Phalaris arundinacea* and *Tripsacum dactyloides* monocultures. Enrichment factors were derived for different incubation temperatures as illustrated in Figure 4. Values with the same letter indicate that those samples were statistically undistinguishable at the 0.05 probability level.

<table>
<thead>
<tr>
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<th>14°C</th>
<th>24°C</th>
<th>34°C</th>
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<tr>
<td><em>P. arundinacea</em></td>
<td>(-2.47)±0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(-1.03)±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(-1.20)±0.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. dactyloides</em></td>
<td>(-3.46)±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(-1.00)±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(-1.11)±0.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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Figure 1. Expected temporal variability in the $\delta^{13}$C values of respired CO$_2$ when soils with C3 and C4 plants are incubated, assuming an isotope effect associated with microbial respiration (a) or microbial decomposition of a mixture of two SOM source with different isotopic composition (b).

Figure 2. Mean SOM decomposition rates (mg CO$_2$-C g$^{-1}$ soil-C d$^{-1}$) ± sd (n=3) of soils planted with *Phalaris arundinacea* and *Tripsacum dactyloides* at three different temperatures on days 1, 3, 5, 11, 22, and 36.

Figure 3. $\delta^{13}$C values (‰) ± sd (n=3) of respired CO$_2$ of soil samples planted with C3 (*Phalaris arundinacea*) and C4 (*Tripsacum dactyloides*) monocultures. Isotopic determinations were measured for soils incubated at three different temperatures on days 1, 3, 5, 11, 22, and 36.

Figure 4. Rayleigh-type plots to determine the isotope effect associated with microbial decomposition of soils from *Phalaris arundinacea* and *Tripsacum dactyloides* monocultures. The soils were incubated for 36 days. A linear regression was fitted to the obtained data for each of the three temperatures (14, 24, and 34°C) at which the soils were incubated. The obtained slopes were transformed to enrichment factors through the use of equation 3 (see text).
Figure 2
Figure 3
Figure 4
CHAPTER 4. AN INNOVATIVE APPROACH TO ASSESS TEMPERATURE SENSITIVITY OF SOIL ORGANIC MATTER DECOMPOSITION USING ARTIFICIAL SOILS: EFFECTS OF CHEMICAL RECALCITRANCE AND CLAY-MINERAL COMPOSITION

A paper to be submitted to *European Journal of Soil Science*

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**Summary**

Knowledge about the temperature sensitivity of soil organic matter (SOM) is important to predict the response of soil carbon (C) dynamics to projected global warming. However, no consensus has been reached concerning whether or not the large stock of recalcitrant soil C is as sensitive to temperature as is labile soil C. Soil C is stabilized by three mechanisms: chemical recalcitrance, mineral interaction and physical accessibility. This study developed a novel approach using artificial soils having controlled compositions to assess how chemical recalcitrance (cellulose vs. lignin) and clay-mineral compositions (montmorillonite vs.
kaolinite) independently affect the temperature sensitivity of SOM decomposition at 2 - 32 °C. When only substrate composition was varied, the presence of cellulose was found to enhance the decomposition rate of lignin. High-cellulose treatments had high temperature sensitivities only at the lowest temperature, whereas high-lignin treatments had similar temperature sensitivities at all temperatures. When only clay-mineral composition was varied, SOM decomposition was greatest in soils containing kaolinite-montmorillonite mixtures (10%K:20%M) and was lowest in soils containing kaolinite only, at temperatures 12 °C and above. Clay mixtures and high-montmorillonite treatments had high temperature sensitivities at 2-12 °C, whereas pure-kaolinite treatments had high temperature sensitivities at 12-22 °C. The temperature sensitivities at the highest temperature (22-32 °C) were all low, regardless of cellulose:lignin or montmorillonite:kaolinite ratio ($Q_{10}$<1.1 on day 30 and 140). Artificial soils with controlled but flexible compositions may serve as simple, useful models for evaluating SOM dynamics with a minimum of confounding factors.

**Introduction**

Soil organic matter (SOM) contains two times more carbon (C) than does the atmosphere or living plants (Schlesinger, 1991), and soil CO$_2$ efflux is the second largest C flux in terrestrial ecosystems (Raich & Schlesinger, 1992). Even a 20% warming-induced increase in SOM decomposition would surpass CO$_2$ emitted to the atmosphere from fossil fuel combustion and land use changes. A possible positive feedback of SOM decomposition on global warming is a concern (Trumbore *et al.*, 1996; Conant *et al.*, 2008).

A principal uncertainty of soil C dynamics is whether global warming would induce significant C emissions from the large stock of recalcitrant SOM, to the same extent as
warming stimulates the decay of labile SOM. Despite the large number of studies addressing this issue, both in the field and laboratory, using both experimental and modeling approaches, no consensus has been reached about the temperature sensitivity of recalcitrant-C decomposition. There are two opposing views: first, that the temperature sensitivity of recalcitrant-C is similar to or even larger than that of labile C, so a possible positive feedback to global warming may be triggered (Mikan et al., 2002; Fang et al., 2005; Fierer et al., 2005); and second, that recalcitrant-C is less sensitive or insensitive to temperature change, so increases in soil CO$_2$ emissions due to global warming would be short-term (Liski et al., 1999; Giardina & Ryan, 2000; Eliasson et al., 2005; Bradford et al., 2008). Davidson & Janssens (2006) attributed the opposing views to both the diversity of SOM components, which exhibit a wide range of intrinsic temperature sensitivities, and to several environmental constraints, which obscure the intrinsic temperature sensitivity of substrate decomposition. SOM contains a mixture of organic compounds that vary in molecular weight, molecular structure, C:N ratio and in other ways, and that have different decomposition and turnover rates (Krull et al., 2003). Some environmental constraints, such as physical isolation within soil aggregates, chemical adsorption with mineral particles, drought, flooding and freezing, may further complicate the chemical and biochemical behaviors of the soil organic materials.

Sollins et al. (1996) argued that stabilization of SOM resulted from three general sets of characteristics: chemical recalcitrance, mineral interactions and physical accessibility. Chemical recalcitrance derives from molecular-level characteristics of organic substances. Mineral interactions refer to the inter-molecular interactions between organics and either
inorganic substances or other organic substances. Physical accessibility refers to the location of organic substances with respect to microbes and enzymes.

To better understand the potential response of SOM decomposition to global warming, we created artificial soils with controlled compositions that we carefully altered in single-factor experiments. We specifically examined the temperature sensitivity of SOM decomposition in response to variations in chemical recalcitrance and clay-mineral composition. Cellulose and lignin are two of the most abundant organic compounds in natural ecosystems (Hamer & Marschner 2002) and, because of their differences in molecular-level structures, represent organic substrates with different chemical recalcitrance. Montmorillonite, which is a 2:1 clay, has a much higher specific surface area than does kaolinite, which is a 1:1 clay (Burgos et al., 2002), and therefore has a stronger sorption capacity for organic matter. Montmorillonite and kaolinite were used as two clays representing different mineral adsorption capacities.

Artificial soils are widely used in the horticulture industry where various mixtures of sand, peat and other components are mixed to generate large amounts of potting media with desirable characteristics. Artificial soils also have been used as a tool to combat environmental threats of industrial wastes and to alleviate soil deficiencies in engineering (Adam, 2001; Kim, 1997; Guest et al., 2001). The use of artificial soils in scientific research of soil biogeochemistry is very limited, however Ellis (2004) and Adani & Spagnol (2008) used artificial soils as simplified soil matrix media. The advantage of using carefully constructed artificial soils for scientific research is the ability to control and replicate soil characteristics; to vary the parameters of interest while strictly controlling other soil characteristics; and thus to greatly enhance the capacity for controlled experimentation. By
using artificial soils with replicable characteristics, we can minimize the heterogeneity and complexity that characterize natural soils, and that make each soil experiment unique.

The objectives of this study were to (1) develop and test an innovative new approach for soil C dynamic research based on the use of artificial soils with controlled compositions, and (2) using this method, examine how SOM decomposition responds to temperature under varying C chemical recalcitrance and clay-mineral compositions.

**Materials and methods**

**Artificial soil components**

The artificial soil utilized was designed to mimic the characteristics of natural soils, while providing the control needed for scientific experimentation. Some critical characteristics needed to be appropriate for microbial activities, such as good texture and microstructure, soil water-holding properties and near-neutral pH. Other characteristics were designed to be flexible, such as organic matter compositions and clay-mineral compositions. The components of the artificial soils included natural sand containing a mixture of rock materials, acid-washed silica sand, montmorillonite, kaolinite, garden lime, humic acid, yeast extract, starch, cellulose, lignin and complete fertilizer (Table 1). Natural sands were combusted at 550 °C for 4 hours to remove possible organic matter. Kaolinite is a non-swelling 1:1 clay whose binding surfaces are primarily external; in contrast, montmorillonite is an expandable 2:1 clay with 10 to 30 times the ion-exchange capacity and 20-70 times the surface area of kaolinite, and 80% of the binding surfaces are interstitial (Bohn *et al.*, 1979). Montmorillonite and kaolinite have different pH values, so garden lime was used to adjust soil pH in the clay-mineral experiment. Humic acid is an essential organic matter in the soil,
and the addition of commercial humic acid to soils increases the content of available micronutrients, microbial growth and rates of organic matter mineralization (Ayuso et al., 1996; Sharif et al., 2002). Yeast extract is a general additive to culture media and was used to stimulate soil microorganisms at the beginning of the incubation experiment and to provide adequate N throughout the entire incubation process. Starch was used as a stimulus to soil microorganisms, by providing a labile C source at the beginning of the incubation experiment. Lignin and cellulose were mixed in different proportions to provide different levels of chemical recalcitrance. A complete 21-15-20 fertilizer was dissolved in distilled water and added to the artificial soils to provide additional macro- and trace nutrients.

One experiment, hereafter referred to as the “chemical-recalcitrance experiment”, was designed to test how the biochemical composition of SOM would affect the temperature sensitivity of SOM decomposition. A second experiment, hereafter called the “clay-mineral experiment”, was performed to test how different clay minerals would affect the temperature sensitivity of SOM decomposition. Both experiments utilized a soil matrix composed of course sand and clay with a sandy clay loam texture, a small amount of microbially labile organic compounds to stimulate microbial colonization, and fertilizer to eliminate nutritional constraints to microbes. The additional organic materials comprised 4.2% of the dry weight of the artificial soils; its composition was varied in the chemical-recalcitrance experiment. Clay minerals comprised 30% of the dry weight of the artificial soils; the proportions of kaolinite and montmorillonite/garden lime were varied in the clay-mineral experiment (Table 2). Two sets of artificial soils were created as shown in Table 2.

In the chemical-recalcitrance experiment, two blanks (inorganic blank and organic blank) and a natural wetland soil were incubated along with four treatments. The inorganic
blank contained only the inorganic materials and was used to examine if there was CO$_2$ coming from carbonates present within the sand. The organic blank contained all materials except cellulose and lignin, and was used to examine how much CO$_2$ was derived from the humic acid, yeast extract and starch. A wetland soil collected from the surface 10 cm of a natural wetland in Ames, Iowa, was incubated in parallel with these artificial soils for comparison. In the clay-mineral experiment (experiment 2), the blank contained 15% kaolinite and 15% montmorillonite/lime, and was sterilized after soil microbial inoculation. The artificial soil creation procedure is described in the appendix.

Soil incubation

Vials containing 10 g (dry weight equivalent) of microbially inoculated artificial soil were placed in four incubators (Fisher Scientific 146E, Waltham, MA) set at temperatures of 2, 12, 22 and 32 °C. Separate sets of four replicate samples were used to measure CO$_2$ production rates. The vials were loosely capped with lids to allow air exchange. To ensure that moisture remained constant, samples were weighed every two days and water was added to maintain the water potential at -0.03 MPa during the experiments.

Soils were incubated for about 140 days, and rates of CO$_2$ production were measured at intervals ranging from two days at the beginning to one month at the end of the incubation period. During each measurement, vials containing the soil samples were sealed with lids and flushed with CO$_2$-free air. The sealed vials were placed back in the incubator for a period of time that was sufficient to accumulate quantifiable amounts of CO$_2$ in the headspace, ranging from 30 minutes at the beginning of the experiment to several hours at the end. The vials were then taken out of the incubator and flushed with CO$_2$-free air through a LI-820 IRGA (Infrared Gas Analyzer, LI-COR, Lincoln, NE) to measure the amount of CO$_2$ produced. The
IRGA data were calibrated with CO$_2$ standards of known concentrations to evaluate the amount of CO$_2$ respired in a certain interval, which were used to calculate CO$_2$ production rates.

$Q_{10}$ calculation and statistical analysis

$Q_{10}$ values were calculated by the equations 1 and 2:

$$R_T = b * e^{kT} \quad (1)$$

$$Q_{10} = e^{10k} \quad (2)$$

where $R$ is the cumulative respired CO$_2$ (in mg CO$_2$-C) at temperature $T$ at a certain time within a treatment, and $b$ and $k$ are fitted parameters. Only cumulative respired CO$_2$ at two adjacent temperatures were used to calculate an individual $Q_{10}$ value, for example, $Q_{10}$ at 2-12 °C was calculated from the cumulative respired CO$_2$ at 2 and 12 °C.

All statistical analyses were conducted using JMP 8.0 (SAS Institute, Cary, NC). Effects of temperature, chemical recalcitrance or clay-mineral compositions were tested using two-way analysis of variance (ANOVA) on days 30 and 140. Data were considered significantly different at $\alpha=0.05$ level.

Results

The CO$_2$ production rates after 11 days of pre-incubation at room temperature tended to stabilize after an initial peak, indicating that microbial communities had established using the readily available organic materials (Data not shown). Results below reflect CO$_2$ production rates in temperature-controlled incubators, after an 11-day pre-incubation at room temperature.

Chemical-recalcitrance experiment
The cumulative amount of respired CO₂ in the chemical-recalcitrance experiment increased rapidly at the beginning of incubation period and became slower toward the end for all temperatures ≥2 °C (Supplemental Figure 1), as is typical of most soil incubation experiments (Leifeld & Fuhrer, 2005; Conant et al., 2008). Those treatments containing cellulose and at temperatures ≥12 °C had peak CO₂ production rates around day 10. No such peaks were observed in the pure-lignin treatment or at 2 °C (Data not shown). The cumulative respired CO₂ after 140 days of incubation were higher in the treatments with higher cellulose content (P<0.05, Figure 1E). The same trend was also found after 30 days of incubation at temperatures ≥12 °C (Figure 1C and G). Wetland soil had comparatively low CO₂ production rates, and was comparable to the all-lignin treatment (Supplemental Figure 1). Generally, higher temperatures induced higher CO₂ production (P<0.05, Figure 1B and F). A significant interactive effect was observed between chemical and temperature on both day 30 and 140, indicating a significant different temperature response of each treatment (Figure 1C and G). Q₁₀ plots were thereby created to better interpret the temperature response of SOM decomposition with different chemical recalcitrance (Figure 1D and H).

Temperature sensitivities (Q₁₀ values) of different treatments varied tremendously at the beginning of incubations, and converged toward 1 after about 140 days, probably due to depletion of labile C (Supplemental Figure 2). Decomposition of pure-cellulose was sensitive to temperature at 2-12 °C especially on day 30 (Q₁₀=4.5), and the Q₁₀ at 2-12 °C was higher than it was at higher temperature (12-22 and 22-32 °C) (Figure 1D and H). However, the decomposition of pure lignin was only sensitive to temperature (Q₁₀ significantly larger than 1) during the first 10 days (Supplemental Figure 2), and the temperature sensitivities were not different among different temperatures on day 30 and 140 (Figure 1d and h). Q₁₀ values
in the mixture treatments were higher at 12-22 and 22-32°C compared to pure-cellulose and pure-lignin treatments (Figure 1D and H). The natural wetland soil had somewhat different patterns than did the artificial soil treatments: the decomposition rates at all temperatures were sensitive to temperature ($Q_{10}>1$), and temperature sensitivities were highest at 22-32 °C for the duration of the incubations (Supplemental Figure 2).

Thirty days after pre-incubation, more than 25% of the cellulose was respired in the pure-cellulose treatment at temperatures $\geq 12$ °C, whereas only <2% of the lignin was respired in the pure-lignin treatment even at the highest temperature (Table 4). The mixture treatments (1.4%Lig:2.8%Cel and 2.8%Lig:1.4%Cel) produced CO$_2$ at intermediate rates, but not at the rates expected based on results from the single-chemical treatments. The expected cumulative CO$_2$ of the two mixture treatments on day 30 were calculated from pure-cellulose and pure-lignin treatments under the assumption that degradation of cellulose and lignin had no interaction. Expected percentages of cellulose and lignin C respired at 32 °C were higher than the measured percentages ($P<0.05$, Figure 3), indicating that the presence of cellulose induced faster decomposition of lignin at the highest temperature.

**Clay-mineral experiment**

The cumulative respired CO$_2$ in the clay-mineral composition experiment increased rapidly at the beginning of the incubation period and slowly toward the end at temperatures $\geq 12$ °C (Supplemental Figure 3), as in chemical-recalcitrance experiment. A peak in CO$_2$ production rates was observed around day 10 at temperatures of 12 °C and above (Data not shown), also as was observed previously. The clay-mineral composition significantly influenced cumulative CO$_2$ production rates. The CO$_2$ production rate of 10%K:20%M treatment was highest on day 140 ($P<0.05$, Figure 2E). At temperatures 12 °C and above, the CO$_2$
production rates followed the order of 10%K:20%M > 20%K:10%M = 0%K:30%M > 30%K:0%M (Figure 2C and G). Generally, higher temperatures induced higher CO₂ production \( (P<0.05, \text{ Figure 2B and F}) \) as described in chemical-recalcitrance experiment. Again, \( Q_{10} \) plots were created to better interpret the temperature response of SOM decomposition with different chemical recalcitrance (Figure 2D and H) because a significant interactive effect was observed between chemical and temperature on both day 30 and 140 (Figure 2C and G).

The temperature sensitivities of different treatments varied tremendously at the beginning of the incubations and converged to 1 after day 140, probably due to depletion of labile C (Supplemental Figure 4). On day 30, the pure-montmorillonite and mixture treatments had high temperature sensitivities at 2-12 °C and the pure-kaolinite treatment had high temperature sensitivities at 12-22 °C (Figure 2D and H). The \( Q_{10} \) values at 22-32 °C were all lower than at 2-12 and 12-22 °C \( (Q_{10}<1.1 \text{ on day 30 and 140, Figure 2D and H}) \).

**Discussion**

**CO₂ production rates**

The amounts of CO₂-C respired over 30 days after pre-incubation in the chemical-recalcitrance experiment were comparable to other studies on lignin and cellulose degradation (e.g. Donnelly *et al.*, 1990; Hamer & Marschner, 2002). In this controlled study, higher contents of cellulose induced higher rates of CO₂ production (Figure 1) and, in the treatments containing both cellulose and lignin, the presence of cellulose enhanced the decomposition of lignin at the highest temperature (Figure 3). Lignin decay in the absence of cellulose was minimal at all temperatures (Supplemental Figure 1). These results indicate
that lignin decomposition is promoted by the availability of a labile C substrate, as suggested by Hamer & Marschner (2002).

Cellulose is an important energy source for most soil microorganisms; lignin, in contrast, is considered to be resistant to microbial and enzymatic breakdown (Sollins et al., 1996), and its availability is a major factor that controls SOM decomposition rates (Donnelly et al., 1990, Marschner et al., 2008). Other studies have suggested that lignin can only be degraded co-metabolically (Haider & Martin, 1975; Hedges et al., 1985), because lignin-degrading organisms do not gain energy or assimilate from lignin degradation (Haider, 1994).

In the clay-mineral composition experiment, highest CO₂ production rates were found in 10%K:20%M, followed by 20%K:10%M and 0%K:30%M, with the lowest CO₂ production rates being found in the pure-kaolinite treatment at temperatures 12°C and above (Figure 2). This finding is somewhat contrary to the widely accepted notion that soil C is preserved by clays having higher surface area (Sørensen, 1975; Saggar et al., 1996). Montmorillonite has much higher surface area than kaolinite and therefore is more reactive and more capable of interacting with organic matter, and such mineral organic interactions could preclude decomposition by microbes (Sørensen, 1975, Baldock & Skjemstad, 2000; Satterberg et al., 2003). It therefore was expected that CO₂ production would decline with increasing montmorillonite content in this controlled experiment. However, higher surface area does not always lead to greater stability of associated organic matter. For example, Rong et al. (2007) reported that clay minerals stimulated the growth of *Bacillus thuringiensis*, and that the enhancing effect of montmorillonite was greater than that of kaolinite. Chen et al. (2009) found that montmorillonite presented a higher adsorption capacity for carbaryl (a pesticide) than kaolinite, and that degradation of adsorbed carbaryl by *Pseudomonas putida* was higher
with montmorillonite than kaolinite. Many other studies have also reported that
montmorillonite alone or with kaolinite improved the mineralization of organic material, or
pollutants, in pure bacterial cultures (Ortega-Calvo & Saiz-Jimenez, 1998; Chaerun et al.,
2005; Courvoisier & Dukan 2009). Ziervogel et al. (2007) argued that the extent to which
organic matter was degraded by microbial communities depended not only on the organic
matter chemical quality and its association with mineral surfaces, but also on the extent to
which microorganisms and their extracellular enzymes may be associated with mineral
surfaces and the consequences of such associations on enzyme activities. Sorbed enzymes are
believed to play important roles in organic matter mineralization in terrestrial (Sollins et al.,
1996) and in aquatic systems (Tietjen & Wetzel, 2003).

Thus, montmorillonite and kaolinite may have different sorption capacities for different
organic materials, microorganisms and their extracellular enzymes, and may differentially
affect substrate availability to microorganisms. The controlled study conducted herein
suggests that soil containing both kaolinite and montmorillonite promoted microbial
degradation of the added organic materials more so than did soils containing only kaolinite.

Temperature sensitivities
The temperature sensitivity was always higher at the beginning of incubation, when the
abundance of labile substrates was highest, and converged to 1 at the end (Supplemental
Figure 2 and 4), indicating either the exhaustion of decomposable materials or a build-up of
waste products. This process occurred fastest at high temperatures and more slowly at low
temperatures. The decomposition of pure cellulose was particularly sensitive to temperature
at low temperature (2-12 °C, Figure 1D); higher temperatures had lesser impacts on cellulose
decomposition rates. However, higher temperature was always favorable for lignin to
decompose within the temperature range of this experiment with the presence of cellulose (Figure 1D). Lignin is a recalcitrant substance because of its aromatic and disordered structure, which can only be decomposed co-metabolically (Hedges et al., 1985; Marschner et al., 2008). So the pure-lignin treatment was more sensitive to temperature at the very beginning of the incubation, when readily available organic matter was still present. Later on, lignin decomposition rates approached zero and $Q_{10}$ values approached 1 (Supplemental Figure 1 and 2). High temperatures apparently favored the co-metabolism of lignin and cellulose, as indicated by the relatively high $Q_{10}$ values at intermediate and high temperatures in the mixed chemical treatments.

Temperature sensitivities at low temperature were high, especially in the treatments with high cellulose contents and in mixtures of montmorillonite and kaolinite (Figure 1D and 2D). Similar results have been found in other field and laboratory experiments. Kirschbaum (1995) reviewed laboratory based $Q_{10}$ data and found that the temperature sensitivities of organic matter decreased with increasing temperature; this was indicated by the $Q_{10}$ values decreasing with temperature. Vanhala et al. (2008) studied short-term temperature sensitivity of boreal forest soil and also found that temperature sensitivity of the decomposition increased with decreasing temperature regime. Zheng et al. (2009) measured the temperature sensitivity of in situ soil respiration (including root respiration) in 15 ecosystems and collected previously published $Q_{10}$ values from 34 ecosystems. They found that ecosystems in colder regions and with higher soil organic C contents had relatively higher $Q_{10}$ values.

**Strength and limitation**

One objective of this study was to develop an artificial soil that mimicked real soil while at the same time providing control over soil characteristics. Several studies have used artificial
soil matrices to conduct experiments. However, most of them were intended to create a medium that was simple enough to reduce confounding factors. For example, Ellis (2004) developed an artificial soil that contained sand, clays (kaolinite, bentonite), CaCO$_3$ and humic acid that could support realistic microbial populations and that successfully reduced substrate heterogeneity and biological complexity. However, that medium lacked an important soil component: a continuum of soil organic matter having varied chemical structures. Adani & Spagnol (2008) also used an artificial soil for an incubation experiment. Their artificial soil was a sandy mineral substrate containing sand, clay and the organic amendments used for incubation and was easy to utilize. However, it contained a very small fraction of clay minerals (10% of total minerals) and could not represent most natural soils. Our artificial soil was built as a sandy clay loam. Humic acid, a negatively charged colloid that is recalcitrant to biodegradation (Quall, 2004), was added to improve the artificial soil properties. Humic acid plays an important role in determining soil characteristics by influencing its chemical, physical and biological properties. Yeast extract and starch were added to provide readily available C and N to microbes, and complete fertilizer was added to provide other trace nutrients for microbial growth. Both labile organic matter and fertilizer served as a stimulus for microorganisms. Therefore, the inoculated microbes would grow rapidly on the readily available organic C at the beginning, and switch to consume the less labile and more recalcitrant organic C later on, as in natural-soil incubation experiments. Furthermore, we were able to control many essential soil characteristics, like pH, water potential, organic matter content, and vary only factors we were interested in (Table 3).

Our artificial soil also has limitations. Although it can be constructed to mimic natural soils with respect to organic matter chemical recalcitrance (by varying the composition of
added organic materials), and of mineral sorption (by varying the composition of clay
minerals), it may not accurately duplicate the micro- and macro-aggregate structure that have
developed through time, and under biotic influence, in natural soils, especially during the
early stages of incubations. Aggregates are particles composed of smaller particles, which
may themselves be aggregates (Emerson et al., 1986). Many binding agents, such as
polysaccharides released by bacteria and fungi, may stabilize aggregates (Sollins et al., 1996)
and aggregate structures may physically restrict microbes from accessing organic materials
(van Veen & Kuikman, 1990). Even though some aggregates were observed during mixing
of artificial soil, we doubt their ability to protect organic matter is similar to natural
aggregates. So caution is needed when interpreting our results because the inaccessibility of
soil organic matter might be an important mechanism to protect SOM in natural soils; with
respect to rising temperatures, a real soil may behave differently than did the artificial soils.

The intent of this work was not to build natural soils from pure chemical compounds, but
to create artificial soils that could serve as model systems for understanding the many
complex interactions that exist within natural soils, but without the many confounding and
frequently unknown, factors that make each soil unique. By so doing, it may be possible to
enhance our ability to identify and study the actual mechanisms by which soil attributes and
environmental variables influence organic matter dynamics.

**Conclusion**

Artificial soil provides a good medium to study the temperature dependence of SOM
decomposition in the perspective of chemical recalcitrance and soil clay-mineral
composition, although it might have difficulty in addressing that of SOM protected via
physical inaccessibility in naturally formed soil aggregates. Decomposition of high-cellulose organic matter was sensitive to temperature at low temperature and insensitive to temperature at high temperature, while decomposition of high-lignin organic matter had similar temperature sensitivities for the entire temperature range. Decomposition of organic matter associated with high-montmorillonite soil had high temperature sensitivities at low temperature, while that associated with high-kaolinite soil was sensitive to temperature at intermediate temperature. Artificial soils with controlled compositions allow experimenters to control essential soil characteristics while at the same time varying specific factors that they are interested in.

Acknowledgements

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References


Table 1 The essential components of the artificial soils that were constructed, their purchase information and their functions in the soils

<table>
<thead>
<tr>
<th>Components</th>
<th>Company</th>
<th>Catalog number</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural sand</td>
<td>Hallett Material Co.</td>
<td>NA</td>
<td>Ames, IA</td>
<td>Provide appropriate soil texture; provide mineral nutrients</td>
</tr>
<tr>
<td>Acid-washed sand</td>
<td>Sigma-Aldrich Co.</td>
<td>18649</td>
<td>St. Louis, MO</td>
<td>Provide appropriate soil texture</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>Sigma-Aldrich Co.</td>
<td>03584</td>
<td>St. Louis, MO</td>
<td>1:1 non-expanding clay</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>Sigma-Aldrich Co.</td>
<td>281522</td>
<td>St. Louis, MO</td>
<td>2:1 expanding clay</td>
</tr>
<tr>
<td>Garden lime</td>
<td>Earl May Nursery &amp; Garden Center</td>
<td>NA</td>
<td>Ames, IA</td>
<td>Adjust soil pH</td>
</tr>
<tr>
<td>Humic acid</td>
<td>Sigma-Aldrich Co.</td>
<td>53680</td>
<td>St. Louis, MO</td>
<td>Essential organic matter in soil; increase content of available micronutrients</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>Sigma-Aldrich Co.</td>
<td>Y1625</td>
<td>St. Louis, MO</td>
<td>Provide N source and stimulation of microbial growth</td>
</tr>
<tr>
<td>Starch</td>
<td>Walmart</td>
<td>NA</td>
<td>Ames, IA</td>
<td>Provide labile C source</td>
</tr>
<tr>
<td>Lignin</td>
<td>Sigma-Aldrich Co.</td>
<td>370959</td>
<td>St. Louis, MO</td>
<td>Provide relatively resistant C source</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Sigma-Aldrich Co.</td>
<td>C6288</td>
<td>St. Louis, MO</td>
<td>Provide intermediately available C source</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>Peters Excell</td>
<td>NA</td>
<td>Camarillo, CA</td>
<td>Provide additional macro- and trace nutrients</td>
</tr>
</tbody>
</table>
Table 2 Percentage (%) of artificial soil components by weight used in the two incubation experiments. The artificial soils were composed of core materials, which were the same for all treatments within experiments, and variable materials, which varied between treatments. The total amounts of each mixture add up to 100%.

<table>
<thead>
<tr>
<th>Components</th>
<th>Experiment 1 (Chemical recalcitrance)</th>
<th>Experiment 2 (Clay-mineral composition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural sand</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Acid-washed sand</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Garden lime</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Humic acid</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Starch</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0</td>
<td>1.4</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Soil pH and water potential at -0.03 MPa for each treatment in both experiments (chemical-recalcitrance and clay-mineral composition). Cel represents cellulose, Lig represents lignin, K represents kaolinite, and M represents montmorillonite. Standard errors are shown in the parenthesis (n=2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1 (Chemical-recalcitrance)</th>
<th>Experiment 2 (Clay-mineral composition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Water content at -0.03 MPa /%</td>
</tr>
<tr>
<td>0%Lig:4.2%Cel</td>
<td>7.31 (0.18)</td>
<td>26.13 (0.26)</td>
</tr>
<tr>
<td>1.4%Lig:2.8%Cel</td>
<td>7.28 (0.11)</td>
<td>25.54 (0.43)</td>
</tr>
<tr>
<td>2.8%Lig:1.4%Cel</td>
<td>7.27 (0.11)</td>
<td>25.26 (0.34)</td>
</tr>
<tr>
<td>4.2%Lig:0%Cel</td>
<td>7.29 (0.08)</td>
<td>24.78 (1.35)</td>
</tr>
<tr>
<td>Blank</td>
<td>7.38 (0.16)</td>
<td>24.79 (0.19)</td>
</tr>
<tr>
<td>Inorganic blank</td>
<td>7.16 (0.28)</td>
<td>23.20 (0.63)</td>
</tr>
<tr>
<td>Wetland</td>
<td>7.25 (0.17)</td>
<td>26.29 (0.58)</td>
</tr>
</tbody>
</table>
Table 4 Percentage of cellulose and lignin C (artificial soils) and percentage of total C (wetland soil) respired in 30 days after pre-incubation at four different temperatures. Cel represents cellulose, and Lig represents lignin. Standard errors are shown in the parenthesis (n=4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2°C</td>
<td>12°C</td>
<td>22°C</td>
<td>32°C</td>
<td></td>
</tr>
<tr>
<td>0%Lig:4.2%Cel</td>
<td>2.2</td>
<td>25.0</td>
<td>25.3</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.6)</td>
<td>(3.2)</td>
<td>(5.2)</td>
<td>(4.0)</td>
<td></td>
</tr>
<tr>
<td>1.4%Lig:2.8%Cel</td>
<td>1.3</td>
<td>11.1</td>
<td>17.8</td>
<td>21.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(5.7)</td>
<td>(3.1)</td>
<td>(1.4)</td>
<td></td>
</tr>
<tr>
<td>2.8%Lig:1.4%Cel</td>
<td>1.2</td>
<td>4.2</td>
<td>8.4</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.6)</td>
<td>(1.7)</td>
<td>(0.9)</td>
<td>(3.5)</td>
<td></td>
</tr>
<tr>
<td>4.2%Lig:0%Cel</td>
<td>0.2</td>
<td>0.9</td>
<td>1.0</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.2)</td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(0.5)</td>
<td></td>
</tr>
<tr>
<td>Wetland</td>
<td>1.8</td>
<td>2.1</td>
<td>2.7</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>(0.3)</td>
<td>(0.2)</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 1** Two-way ANOVA plots of the chemical-recalcitrance experiment on day 30 (A: chemical, B: temperature and C: chemical × temperature) and on day 140 (E: chemical, F: temperature and G: chemical × temperature) and Q_{10} plots created to better interpret the interactive effects of chemical and temperature on day 30 (D) and on day 140 (H). Different letters indicated that treatments were significantly different from each other. Cel represents cellulose and Lig represents lignin.

**Figure 2** Two-way ANOVA plots of the clay-mineral composition experiment on day 30 (A: clay, B: temperature and C: clay × temperature) and on day 140 (E: clay, F: temperature and G: clay × temperature) and Q_{10} plots created to better interpret the interactive effects of clay and temperature on day 30 (D) and on day 140 (H). Different letters indicated that treatments were significantly different from each other. K represents kaolinite and M represents montmorillonite.

**Figure 3** Measured and estimated percentage of original cellulose and lignin C (± se) resired in 30 days after pre-incubation at four different temperatures for two mixture treatments (1.4%Lig:2.8%Cel and 2.8%Lig:1.4%Cel) (n=4). The expected percentages were calculated from the pure cellulose and lignin treatment under the assumption that degradation of cellulose and lignin had no interaction. * indicates that the measured and expected percentages were significantly different (P<0.05). Cel represents cellulose, and Lig represents lignin.
**Supplemental Figure 1** Cumulative respired CO$_2$ (± se) at four temperatures of the chemical-recalcitrance experiment (n=4). Day 0 is the first day after the 11-day pre-incubation. Cel represents cellulose, and Lig represents lignin.

**Supplemental Figure 2** Calculated Q$_{10}$ (± se) of for each treatment of chemical recalcitrance experiment (experiment 1) at three different temperatures (2-12, 12-22, and 22-32 °C) (n=4). Day 0 is the first day after pre-incubation. Cel represents cellulose, and Lig represents lignin.

**Supplemental Figure 3** Cumulative respired CO$_2$ (± se) at four temperatures of clay-mineral composition experiment (experiment 2) (n=4). Day 0 is the first day after pre-incubation. K represents kaolinite, and M represents montmorillonite.

**Supplemental Figure 4** Calculated Q$_{10}$ (± se) of for each treatment of clay-mineral composition experiment (experiment 2) at three different temperatures (2-12, 12-22, and 22-32°C) (n=4). Day 0 is the first day after pre-incubation. K represents kaolinite, and M represents montmorillonite.
Figure 1
Figure 2
Figure 3
Supplemental Figure 1
Supplemental Figure 2
Supplemental Figure 3
Supplemental Figure 4
Appendix

Artificial soil creation

1. Sands, humic acid, yeast extract, starch, montmorillonite, kaolinite (in the chemical-recalcitrance experiment), lignin and cellulose (in the clay-mineral experiment) were individually weighed and combined together according to the mass-based proportions shown in Table 2. The mixture was rotated with no water overnight in a slowly rotating rock tumbler (THU 130-GP, Thumler’s Tumblers, Auburn, WA) for complete mixing.

2. The parent mixture generated above was then separated in smaller containers (500 ml) for four different treatments and blanks. Lignin and cellulose were added in different proportion (see Table 2) to soils for the chemical-recalcitrance experiment; montmorillonite, kaolinite and garden lime were added to create the chemical-recalcitrance experiment, according to the proportions shown in Table 2.

3. The fertilizer was dissolved in distilled water to a concentration of 0.1 g ml⁻¹ and added to the artificial soil mixture to reach a moisture content (mass:mass) of 3%. Large water-formed aggregates were broken carefully with a metal scoop.

4. The individual mixtures were again rotated in a rock tumbler overnight. More distilled water was added to treatments, blanks and to air-dried wetland soil, but less than needed to reach the desired water content at potential of -0.03 MPa by observation. The water contents were recorded for water content control. Large aggregates were broken carefully using a metal scoop.

5. Twenty grams of soil (dry weight basis) of each treatment was taken out and air-dried to measure the water content at the potential of -0.03 MPa.
6. The rest of the soils were autoclaved at 121 °C for 30 minutes, and then rotated in a rock tumbler for two days.

7. The moist mixtures were separated carefully to 10 g (dry weight basis) and put in individual 75-ml vials; this step was completed within closed containers to prevent water evaporation.

8. Additional soil was used to measure pH values and soil organic C contents.

**Characteristics of artificial soils**

9. Soil water contents of all treatments were measured at potential of -0.03 MPa: Two sample replications of air-dried soils were packed into 2-cm tall, 5-cm diameter rings on a 1-bar ceramic plate; soil bulk density was not controlled. The samples were saturated from the bottom and then placed in the pressure chamber. Pressures of -0.03 MPa were applied and maintained for 24 hours to reach pressure equilibrium at each pressure. Samples were then removed from the chamber, weighed, dried for 24 hours at 105 °C, and reweighed to determine the soil water content ($\theta$, g g$^{-1}$) according to

$$\theta = \frac{\text{mass water}}{\text{mass solid}}$$

10. Soil pH was measured according to the method modified from Thomas (1996), 2 g of soil was weighed and put into a small beaker, and then 4 ml distilled water was added. The mixture was stirred mildly and then left to settle for 30 minutes before measuring pH.

11. Soil C contents were measured by the ISU soil and plant analysis laboratory using combustion method on ground dry soil samples.

**Microbial inoculation and pre-incubation**
12. Natural soils were freshly collected from the surface 15 cm from local forest, prairie and wetland sites. Fifty grams of each soil was weighed and mixed together in a beaker, and 150 g of distilled water was added and stirred carefully. This soil-water mixture was then put in a sonicator (Branson 5510, Branson Ultrasonic Co., Danbury, CT) for 10 minutes, and then the soil was allowed to settle for 2 hours. The supernatant was poured into another beaker and 0.3 ml of the supernatant was added to each vial to let the soil microorganisms grow in the artificial soils. Then soil moisture was adjusted to -0.03 MPa.

13. The inoculated artificial soils were placed at a room temperature of 22 °C for 11 days to let the microbiota colonize the newly made artificial soils, which contained nutrient and readily available organic matter.
CHAPTER 5. INTERACTIVE EFFECT OF SUBSTRATE AND CLAY-MINERAL COMPOSITION ON SOIL ORGANIC MATTER DECOMPOSITION USING ARTIFICIAL SOILS

A paper to be submitted to *European Journal of Soil Science*

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**Summary**

The temperature sensitivity of soil organic matter (SOM) decomposition has gained increasing interest because of its importance on soil carbon (C) dynamics in response to future global warming. The effect of increasing temperature on decomposition rates is determined by SOM molecular structures, known as intrinsic temperature sensitivity, and modified by environmental constraints, known as apparent temperature sensitivity. Artificial soils with controlled compositions were used to examine whether clay-mineral composition, as an environmental constraint, and substrate had an interactive effect on the temperature response of SOM decomposition. Results showed that soils with higher proportion of cellulose produced more CO\(_2\), and that soils containing montmorillonite produced more CO\(_2\).
than did soils containing only kaolinite at temperature $\geq 12^\circ C$. Temperature sensitivities of
SOM decomposition were higher at 2-12 $^\circ C$ than at higher temperatures, especially within
soils with high cellulose and montmorillonite contents. In contrast, pure-kaolinite treatments
had similar temperature sensitivities regardless substrate composition, and pure-lignin
treatments had low temperature sensitivities that did not vary with temperature or clay-
mineral composition. An interactive effect of substrate and clay-mineral compositions on
temperature sensitivities was found at 2-12$^\circ C$, indicating that mineral-organic C interactions
could influence the apparent temperature sensitivity of labile SOM decomposition in cool
environments.

**Introduction**

Soil organic matter (SOM) represents one of the largest reservoirs of carbon (C) in terrestrial
ecosystems. Approximately 81% of organic C resources that are actively involved in the
global C cycle are stored in soils, compared to about 19% in vegetation (Schlesinger, 1995).
Understanding the relationship between environmental changes and SOM decomposition is
critical for projecting soil C fluxes in a changing climate and its potential feedback on global
therefore has been extensively studied, yet no consensus has been reached (Davidson &
Janssens, 2006; von Lützow & Kögel-Knabner, 2009). Some studies have indicated that a
warmer climate may accelerate rates of SOM decomposition, that extra CO$_2$ emitted from
SOM may cause further warming, forming a positive feedback (e.g. Trumbore *et al.*, 1996;
Fierer *et al.*, 2005; Vanhala *et al.*, 2007; Conant *et al.*, 2008). Other studies have shown a
lower temperature sensitivity of the large stock of recalcitrant C compared with more readily
available labile C, suggesting that the response of SOM decomposition to global warming is unlikely to be substantial (Giardina & Ryan, 2000; Luo et al., 2001; Melillo et al., 2002; Eliasson et al., 2005; Bradford et al., 2008).

Davidson & Janssens (2006) explained the various responses of SOM decomposition to temperature by distinguishing between the intrinsic temperature sensitivity, the inherent kinetic properties of organic materials based on their molecular structures; and the apparent temperature sensitivity, the observed response to temperature under environmental constraints. The intrinsic temperature sensitivity could be well described by the Arrhenius equation, which shows higher temperature sensitivity of decomposition at low temperature and with increasing molecular stability of organic compounds (von Lützow & Kögel-Knabner, 2009). The apparent temperature sensitivities could be described by Michaelis-Menten kinetics, by which the reaction rates are modified by substrate availability and affinities of the enzymes for the substrates. So the apparent temperature sensitivity of SOM decomposition can either be larger or smaller than the intrinsic temperature sensitivity depending on the relationship between substrate availability and temperature (Davidson et al., 2006). According to Davidson & Janssens (2006), the substrate availability can be temporarily or indefinitely affected by several environmental constraints: physical protection by soil aggregates, chemical protection by mineral interaction, drought, flooding and freezing. Because substrate availability is limiting in most soils, it is informative to determine whether existing environmental constraints to SOM decomposition change with temperature. Physical and chemical protections within mineral soil matrices are important contributors to variability of soil C stocks, but the extent to which they will participate in positive or negative feedbacks to climate change is not clear. For example, both adsorption and
desorption processes are temperature sensitive and might both change with temperature such that their net effect might be minimal.

Artificial soil with controlled compositions was shown to be a good medium to examine the temperature sensitivity of SOM decomposition as influenced by substrate intrinsic molecular structures and by clay-mineral compositions (Chapter 4). In the current study, we used artificial soils to examine (1) how substrate chemical recalcitrance and clay-mineral compositions interact to impact the temperature sensitivity of SOM decomposition, and (2) whether the intrinsic temperature sensitivity of organic matter decomposition in vitro was influenced by mineral-organic C interaction as an environmental constraint.

**Material and methods**

*Artificial soil components*

The artificial soil utilized in this experiment was designed to mimic the characteristics of natural soils, while providing the control needed for scientific experimentation and a favorable environment for microbial activity (Chapter 4). The current experiment utilized a soil matrix composed of course sand and clay with sandy clay loam texture, a small amount of labile organic compounds to stimulate microbial colonization, and nutrients to eliminate nutritional constraints to microbes. The additional organic materials (cellulose and lignin) comprised 4.2% of the dry weight of the artificial soils, and clay minerals (kaolinite and montmorillonite/lime) comprised 30% of the dry weight of the artificial soils (Table 1). The experiment had the complete factorial design with three clay-mineral compositions (30%K:0%M, 15%K:15%M and 0%K:30%M) and three organic matter compositions (4.2%Lig:0%Cel, 2.1%Lig:2.1%Cel and 0%Lig:4.2%Cel), where K represents kaolinite, M
represents montmorillonite with lime, Cel represents cellulose and Lig represents lignin (Table 1). The blank contained all materials but cellulose and lignin, and the composition of clay (30% of total soil dry mass) was 15% kaolinite and 15% montmorillonite with lime.

To produce the artificial soils, all materials but cellulose, lignin, clays and garden lime (Table 1) were mixed and rotated overnight, without water, in a rock tumbler. Then the total mixture was separated into ten portions for use in the nine treatments and blank. Then the cellulose, lignin, clays and garden lime were amended according to the predetermined experimental design (Table 1). The ten distinct mixtures were supplemented with the fertilizer and some water, and again rotated overnight. Twenty grams soil (dry weight basis) was taken out to measure the water content at the potential of -0.03 MPa. At the same time, the rest of the soil mixture was autoclaved and rotated for two days. Then soil mixtures were divided into 10 g (dry weight basis) aliquots and put into individual 75-ml vials. Each treatment had sixteen vials, which gave four replicates at four temperatures. Additional soil was used measure pH values and soil organic C contents. A soil microorganism solution was prepared from local forest, prairie and wetland soils, and 0.3 ml of the solution was added to each vial. After that, soil moisture was adjusted to -0.03 MPa and vials were set at room temperature (22 °C) to pre-incubate for 11 days.

Soil incubation

Vials were placed in four low temperature incubators (Fisher Scientific 146E, Waltham, MA) set at temperatures of 2, 12, 22 and 32 °C. Separate sets of four replicate samples were used to measure CO₂ production rates. The moisture remained constant at the potential of -0.03 MPa during the experiments by adding water at two-day intervals. The loosely capped lids allowed air exchange.
Soils were incubated for 80 days, and rates of CO$_2$ production were measured at intervals ranging from two days at the beginning to two weeks at the end of the incubation period. During each measurement, vials containing the soil samples were sealed with lids and flushed with CO$_2$-free air. The sealed vials were placed back in the incubator for a period of time that was sufficient to accumulate quantifiable amounts of CO$_2$ in the headspace, which ranged from 30 minutes (at the beginning) to several hours (at the end). The vials were then taken out of the incubator and flushed with CO$_2$-free air through a LI-820 IRGA (Infrared Gas Analyzer, LI-COR, Lincoln, NE) to measure the CO$_2$ flux. The IRGA data were calibrated with CO$_2$ standards of known concentrations, which allow the evaluation of the amount of CO$_2$ in each vial.

**Q$_{10}$ calculation and statistical analysis**

$Q_{10}$ values were calculated by the equations 1 and 2:

\[
R_T = b * e^{kT} \quad (1)
\]

\[
Q_{10} = e^{10k} \quad (2)
\]

where $R$ is the cumulative respired CO$_2$ (in mg CO$_2$-C) at temperature $T$ on a certain day within a treatment, $b$ and $k$ are fitted parameters. Only cumulative respired CO$_2$ at two adjacent temperatures were used to calculate an individual $Q_{10}$ value, for example, $Q_{10}$ at 2-12 °C was calculated from the cumulative respired CO$_2$ at 2 and 12 °C.

All statistical analyses were conducted using JMP 8.0 (SAS Institute, Cary, NC). Effects of temperature, substrate and clay-mineral compositions were tested using three-way analysis of variance (ANOVA) on days 30 and 80. In addition, student’s $t$-test was used to compare the $Q_{10}$ values between temperatures, substrates compositions and clay-mineral compositions. Data were considered significantly different at $\alpha=0.05$ level.
Results

Measured characteristics of the artificial soil are shown in Table 2. Measured C contents were very close to the designated C contents and pH values were maintained between neutral and slightly alkaline. Soil water contents were maintained at the potential of -0.03 MPa, as shown in Table 2.

The CO$_2$ production rates after 11 days of pre-incubation at room temperature tended to stabilize after an initial peak, indicating that microbial communities were established based on the readily available organic materials (Data not shown). Results below reflected CO$_2$ production rates after pre-incubation in temperature-controlled incubators.

The cumulative amount of respired CO$_2$ increased rapidly at the beginning of incubation period and became slower toward the end for all treatments containing cellulose and at 12°C and above (Supplemental Figure 1). Both substrate composition and temperature had significant influences on CO$_2$ production. Higher amount of cellulose or higher temperature induced higher CO$_2$ production rates on day 30 (Figure 1a and b) and on day 80 (Figure 2a and b). Both substrate and clay composition had interactive effects with temperature on CO$_2$ production on day 30 (Figure 1c and d) and on day 80 (Figure 2c and d). Within the same substrate, the pure-kaolinite treatments produced significantly less CO$_2$ than did other treatments at temperatures $\geq$ 12°C (Figure 1c and 2c). A significant three-way interactive effect of temperature, clay type and substrate was also found on day 30 and 80 (Data not shown).

Temperature sensitivities ($Q_{10}$ values) varied greatly during the incubation period (Supplemental Figure 2). On day 30, an interactive effect of substrate and clay compositions
on $Q_{10}$ was observed at 2-12°C (Figure 3): in pure-lignin treatments, $Q_{10}$ values were similar regardless of clay-mineral compositions (mean $Q_{10}=1.53$); while in pure-cellulose treatments, those containing montmorillonite (30%M: $Q_{10}=3.73$, 15%M: $Q_{10}=2.52$) were both significantly higher than that containing only kaolinite ($Q_{10}=1.12$) (student’s $t$-test, $P<0.05$). Temperature sensitivities at high temperature (22-32°C) were low ($Q_{10}<1.2$) except in the treatment containing 30% kaolinite and 4.2% cellulose.

**Discussion**

The effects of chemical recalcitrance and clay-mineral compositions on the CO$_2$ production rate and its temperature sensitivity were very consistent with our previous study (Chapter 4). This demonstrates that carefully constructed artificial soils can be used to conduct experiments that are repeatable at a later date.

*Effects of substrate composition*

Cellulose and lignin are both common organic compounds in natural soils. Cellulose, which constitutes 20-30% of litter mass, provides a significant C source to soil microbe (Berg & Laskowski, 2006). Cellulose-decomposing microorganisms are ubiquitous in soils, and numerous species of both bacteria and fungi are able to produce extracellular enzymes (Berg & Laskowski, 2006; Ulrich et al., 2008). Lignin, however, as a recalcitrant polyphenolic macromolecule, is decomposed in large part by the activity of extracellular enzymes, such as phenol oxidase and peroxidase, that catalyze the depolymerization and oxidation of phenylpropane alcohols from the lignin polymers (Kirk & Farrell, 1987). Because of its complex structure and limited energy production during decomposition, lignin can only be degraded co-metabolically (Hedges et al., 1985; Hamer & Marschner, 2002; Chapter 4). In
this study, when cellulose content was high, CO\textsubscript{2} production at 12 °C was substantially greater than it was at 2 °C, and this trend was maintained for 2 months. CO\textsubscript{2} production did not differ at temperatures of 22 and 32 °C (Figure 1). The lack of difference between 22 and 32 °C could not be due to substrate depletion because it started at the very beginning of incubation when substrates were still abundant. It is possible that cellulose has a low activation energy, so the extent to which the high temperature (22-32 °C) accelerate biochemical processes was not as great as at low temperatures (2-12 °C). It was also possible that the maximum growth and respiration rates of the soil microorganisms occurred at temperatures than≤32 °C, so no further increase in CO\textsubscript{2} production would be observed at 32 °C because peak metabolic activity had been achieved already.

\textit{Effects of clay-mineral compositions}

Clays are particularly important in protecting SOM from decomposition. It is thought that stable clay-organic complexes are responsible for formation of stabilized SOM in clay-rich soils (Saggar \textit{et al}., 1999). In fact, soil clay content is used as an abiotic factor modifying microbial decomposition activity or defining stabilized SOM pool size in SOM turnover models (Franko, 1996; Molina, 1996; Parton, 1996). If the surface area or cation exchange capacity were responsible for clay stabilization of organic matter we would expect that treatments containing higher montmorillonite content would have lower CO\textsubscript{2} production, because montmorillonite has higher specific surface area and cation exchange capacity than does kaolinite (Ravichandran & Sivasankar, 1997; Volzone \textit{et al}., 1999; Gupta & Bhattacharyya, 2008). However, a different result was observed: pure-kaolinite treatments (30%) had lower CO\textsubscript{2} production rates than did treatments containing montmorillonite, at 12, 22 and 32 °C. This result suggests that higher specific surface area or cation exchange
capacity might be favorable for microbial activities. Several mechanisms might have been involved in enhancing CO\textsubscript{2} production with montmorillonite: (1) surface sorption of extracellular enzymes on clay minerals can preserve enzymatic activities for a longer active lifetime in the environment (Naidja & Huang, 1996; Ziervogel et al., 2007), (2) association among clay minerals, organic substrates, microorganisms and their extracellular enzymes may result in higher substrate concentrations in the vicinity of the bacteria cells, thereby increasing their bioavailability (van Loosdrecht et al., 1990; Chaerun & Tazaki, 2005), (3) clay minerals could mitigate the toxic effects of compounds highly concentrated in the medium, by adsorption (Chaerun et al., 2005) and (4) clay minerals have large buffering capacities and could maintain the system at a more suitable pH for microbial growth (Stotzky, 1986). Based on a literature review including 201 surface soils, Müller & Höper (2004) also found a positive relationship between soil clay content and soil microbial biomass, and a negative relationship between soil clay content and metabolic quotient. Their findings indicated a clay-dependent capacity of soils to protect microbial biomass.

Montmorillonite and kaolinite also had different effects on temperature sensitivities of SOM decomposition. Pure-montmorillonite treatments tended to have high temperature sensitivity between 2 and 22 °C and low temperature sensitivity at 22-32 °C. In contrast, the temperature sensitivities of pure-kaolinite treatments were quite similar at all temperatures (Figure 2). The results indicated that the soils with montmorillonite were favorable for SOM decomposition when temperature increased up to 22°C. Substrates must be reversibly absorbed to clay minerals to act as a source for microbial utilization (van Loosdrecht et al., 1990), and when temperature was high, montmorillonite might decrease substrate availability by firmly and irreversibly absorbing substrates. In contrast, such a decrease in substrate
availability might not occur in pure-kaolinite treatments, probably because the adsorption to kaolinite was reversible for the entire temperature range (2-32°C).

**Interactive effect of substrates and clay-mineral compositions**

The interactive effect of substrate and clay-mineral compositions on temperature sensitivity of SOM decomposition was observed at 2-12°C (Figure 2). Soils with montmorillonite had high temperature sensitivity only when cellulose content was high, which indicated that the presence of montmorillonite favored decomposition of relatively labile organic matter when the temperature increased from 2 to 12°C. In contrast, soils containing only kaolinite did not affect the intrinsic temperature sensitivity, probably due to its low specific surface area. The temperature sensitivities of pure-lignin treatments were low and did not vary with temperatures and clay-mineral compositions. The reason was unknown, but may have been because the decomposition rate and temperature sensitivities of recalcitrant C were controlled by its intrinsic complex structures, and thus the effect of environmental constraints, such as mineral-organic C interaction, was relatively weak.

**Inference on soil C cycle under global warming**

Temperature sensitivities of SOM decomposition were influenced by both intrinsic chemical structures of the SOM and environmental constraints. Chemical interaction with mineral surface and physical protection within aggregates are two of the most important environmental constraints that influence substrate availability in mineral soils (Davidson & Janssens, 2006). The artificial soil with controlled compositions provided a good medium to examine intrinsic temperature sensitivity and apparent temperature sensitivity that was influenced by clay mineral-organic C interaction.

We infer that an increase in temperature will likely accelerate SOM decomposition.
where soil temperatures are low, especially in soils containing abundant labile SOM. This may raise concern on the C cycle in areas of high latitude, where the soil C content is high and SOM makes up an enormous stock of C globally (Kuhry et al., 2010), and where warming is expected to be greatest (Chapin et al., 2005). Recent findings in Alaska and northern Sweden provided strong evidence that SOM in permafrost areas was highly temperature sensitive (Schuur et al., 2009; Dorrepaal et al., 2009) and suggested that climate warming of about 1°C over the next few decades could induce a global increase in heterotrophic respiration of 38-100 Tg of C per year (Dorrepaal et al., 2009).

Mineral-organic C interaction could influence the apparent temperature sensitivities. Montmorillonite, but not kaolinite, could enhance labile SOM decomposition as temperatures increase, resulting in a high apparent temperature sensitivity at low temperature. Therefore, soil C stocks may not be simply controlled by clay content but, more precisely, by clay type. However, mineral-organic C interaction is not the only environmental constraint that influence the apparent temperature sensitivity of SOM decomposition. For example, the intrinsic temperature sensitivity of recalcitrant SOM decomposition is low and not greatly influenced by mineral-organic C interaction (Figure 2). Fontaine et al. (2007) found the stability of organic C in deep soil profiles was maintained. They suggested, however, that the increases the distribution of fresh C within the soil profile due to change in land use and agricultural practice could stimulate the loss of ancient buried C. In this case, the availability of labile C was the environmental constraint that could probably impacted the apparent temperature sensitivity of SOM decomposition.
Conclusion

Artificial soils with controlled compositions were utilized to examine the intrinsic and apparent temperature sensivities of SOM decomposition, and how they were influenced by mineral-organic C interactions. Soils with higher proportion of cellulose produced more CO$_2$, and soils containing montmorillonite produced more CO$_2$ than did soils containing only kaolinite at temperature $\geq 12^\circ$C. Both high-cellulose and high-montmorillonite treatments had high temperature sensivities at 2-12 $^\circ$C and low temperature sensivities at 22-32 $^\circ$C. In contrast, pure-kaolinite treatments had similar temperature sensivities regardless of substrate composition, and pure-lignin treatments had low temperature sensivities regardless of temperature or clay-mineral composition. An interactive effect of substrate and clay-mineral compositions on temperature sensivities was found at 2-12$^\circ$C, indicating that mineral-organic C interaction could influence the apparent temperature sensitivity of labile SOM decomposition at low temperatures.

Acknowledgements

We thank Dedrick Davis for his assistance in soil water potential measurement and Jose Guzman for his help with artificial soil creation procedure. This study received financial support from the Interdepartmental Graduate Program in Environmental Science and the Department of Ecology, Evolution and Organismal Biology at Iowa State University.

References


Table 1 Percentage (%) of artificial soil components by weight. The artificial soils were composed of core materials, which were the same for all treatments within experiments, and variable materials, which varied between treatments. The total amounts of each mixture add up to 100%.

<table>
<thead>
<tr>
<th>Components</th>
<th>Core materials (%)</th>
<th>Variable materials (factorial design, nine treatments) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural sand</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Acid-washed sand</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Kaolinite</td>
<td>30 15 0</td>
<td></td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>0 14.6 29.1</td>
<td></td>
</tr>
<tr>
<td>Garden lime</td>
<td>0 0.4 0.9</td>
<td></td>
</tr>
<tr>
<td>Humic acid</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>4.2 2.1 0</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>0 2.1 4.2</td>
<td></td>
</tr>
<tr>
<td>Fertilizer</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Soil organic C content, soil pH and water potential at -0.03 MPa for each treatment. K represents kaolinite, M represents montmorillonite, Cel represents cellulose and Lig represents lignin. Standard errors are shown in the parenthesis (n=2).

<table>
<thead>
<tr>
<th>Clay composition</th>
<th>Treatment</th>
<th>Organic C content (%)</th>
<th>Water content at -0.03 MPa (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>30%K:0%M</td>
<td>4.2%Lig:0%Cel</td>
<td>2.90 (0.05)</td>
<td>16.72 (0.04)</td>
<td>7.27 (0.18)</td>
</tr>
<tr>
<td>15%K:15%M</td>
<td>4.2%Lig:0%Cel</td>
<td>3.12 (0.07)</td>
<td>23.04 (0.04)</td>
<td>7.08 (0.10)</td>
</tr>
<tr>
<td>0%K:30%M</td>
<td>4.2%Lig:0%Cel</td>
<td>2.89 (0.01)</td>
<td>29.80 (0.05)</td>
<td>7.05 (0.03)</td>
</tr>
<tr>
<td>30%K:0%M</td>
<td>2.1%Lig:2.1%Cel</td>
<td>2.52 (0.27)</td>
<td>18.98 (0.16)</td>
<td>7.20 (0.11)</td>
</tr>
<tr>
<td>15%K:15%M</td>
<td>2.1%Lig:2.1%Cel</td>
<td>2.47 (0.02)</td>
<td>24.48 (0.44)</td>
<td>7.26 (0.04)</td>
</tr>
<tr>
<td>0%K:30%M</td>
<td>2.1%Lig:2.1%Cel</td>
<td>2.65 (0.14)</td>
<td>29.33 (1.58)</td>
<td>7.09 (0.16)</td>
</tr>
<tr>
<td>30%K:0%M</td>
<td>0%Lig:4.2%Cel</td>
<td>1.99 (0.04)</td>
<td>20.92 (0.19)</td>
<td>7.58 (0.29)</td>
</tr>
<tr>
<td>15%K:15%M</td>
<td>0%Lig:4.2%Cel</td>
<td>2.06 (0.04)</td>
<td>26.00 (1.05)</td>
<td>7.30 (0.09)</td>
</tr>
<tr>
<td>0%K:30%M</td>
<td>0%Lig:4.2%Cel</td>
<td>1.91 (0.08)</td>
<td>32.80 (0.01)</td>
<td>7.16 (0.10)</td>
</tr>
<tr>
<td>Blank</td>
<td>0.52 (0.02)</td>
<td>24.43 (0.02)</td>
<td>7.33 (0.10)</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 1** Significant effects (a: temperature, b: substrate, c: temperature×substrate and d: temperature×clay) detected by three-way ANOVA on day 30. The interactive effect of temperature, clay and substrate effect is also significant, this effect is interpreted as $Q_{10}$ values in Figure 3. Different letters indicate that treatments were significantly different with each other. K represents kaolinite, M represents montmorillonite, Cel represents cellulose and Lig represents lignin.

**Figure 2** Significant effects (a: temperature, b: substrate, c: temperature×substrate and d: temperature×clay) detected by three-way ANOVA on day 80. The interactive effect of temperature, clay and substrate effect is also significant, which is not shown here. Different letters indicate that treatments were significantly different with each other. K represents kaolinite, M represents montmorillonite, Cel represents cellulose and Lig represents lignin.

**Figure 3** $Q_{10}$ (±se) values calculated on day 30 at three temperature ranges. An interactive effect of substrate and clay mineral on $Q_{10}$ values was observed at 2-12 °C (n=4). K represents kaolinite, M represents montmorillonite, Cel represents cellulose and Lig represents lignin.

**Supplemental materials: Figure 1** Cumulative respired CO$_2$ (± se) at four temperatures (n=4). Day 0 is the first day after the 11-day pre-incubation. K represents kaolinite, M represents montmorillonite, Cel represents cellulose and Lig represents lignin.
Supplemental materials: Figure 2 Calculated $Q_{10}$ ($\pm$ se) of for each treatment at three different temperatures (2-12, 12-22 and 22-32 °C) (n=4). Day 0 is the first day after pre-incubation. K represents kaolinite, M represents montmorillonite, Cel represents cellulose and Lig represents lignin.
Figure 1
Figure 2

(a) Cumulative CO₂ (mg CO₂·g⁻¹ Soil-C) Least square means vs. Temperature (°C)

(b) Cumulative CO₂ (mg CO₂·g⁻¹ Soil-C) Least square means vs. Substrate

(c) Cumulative CO₂ (mg CO₂·g⁻¹ Soil-C) Least square means vs. Temperature (°C)

(d) Cumulative CO₂ (mg CO₂·g⁻¹ Soil-C) Least square means vs. Temperature (°C)
Figure 3
Supplemental materials: Figure 1
Supplemental materials: Figure 2
CHAPTER 6. GENERAL CONCLUSION

General Discussion

Knowledge about the temperature sensitivity of both recalcitrant and labile SOM is important to predict the response of soil C dynamics to projected global warming. The incubation experiment of real temperate prairie soils showed that the decomposition of relatively recalcitrant soil C pool was not as sensitive as of labile soil C pool. If that is true for all soils, the positive feedback of soil C dynamics to global warming might have been overestimated.

However, soils are extremely heterogeneous in terms of their characteristics, such as SOM composition, soil texture, clay composition, soil aggregates, soil water capacity, vegetation type, etc. So temperature sensitivities of SOM decomposition might differ among soils with different soil characteristics. The artificial soil provided a good medium to assess these factors individually and interactively. SOM chemical recalcitrance and clay-mineral composition are two of the most important factors that affect temperature sensitivity of SOM decomposition (Davidson & Janssens, 2006). The results from the incubation of artificial soils showed that the presence of cellulose enhanced the decomposition rate of lignin. Decomposition of high-cellulose organic matter was sensitive to temperature at low temperature and insensitive to temperature at high temperature, while decomposition of high-lignin organic matter had similar temperature sensitivities for entire temperature range. The results indicated that an increase in temperature might greatly accelerate SOM decomposition at low temperature, especially in soils containing abundant labile SOM. This may raise concern about the C cycle in areas on high latitude, where the soil C content is high and SOM makes up enormous stocks of C globally, and where warming is expected to be
greatest. Furthermore, SOM decomposition rate was greater in treatments containing both kaolinite and montmorillonite and lower in that containing pure kaolinite. Decomposition of organic matter associated with high-montmorillonite soil had high temperature sensitivities at low temperature, while that associated with high-kaolinite soil was sensitive to temperature at intermediate temperature. Therefore, the clay type, besides clay content, may also serve as an important parameter that controls the soil C stock and its response to global warming.

An interactive effect of substrate and clay-mineral compositions on temperature sensitivity was observed at low temperature: the temperature sensitivities of montmorillonite treatments were higher in pure-cellulose treatment than in pure-lignin treatment; in contrast, the temperature sensitivities of kaolinite treatments did not vary with substrate compositions. It is informative to consider how environmental constraints could impact on the apparent temperature sensitivities of SOM decomposition.

The stable C isotope composition of soil-respired CO₂ is an important parameter that has been used to evaluate soil C dynamics. The results indicated that an isotope effect occurs during SOM decomposition. Microorganisms in the studied soils preferentially discriminate against ¹³C at the beginning of the incubation experiments and at lower temperatures. Soil microbes discriminated more against ¹³C at low temperatures (14°C) relative to the isotope discrimination obtained at higher temperatures (24 and 34°C). Therefore, the natural abundance of ¹³C should be used with caution to estimate the soil C dynamics.

**Recommendations for Future Research**

Our studies mainly assess the response of SOM decomposition to global warming, with an explicit focus on the SOM decomposition impacted by SOM intrinsic chemical structures and external mineral-organic C interactions. Many other factors might also play important
roles on governing soil C dynamics in the foreseeable future warming. Soil microorganisms, for instance, directly mediate SOM decomposition processes, and therefore the responses and feedbacks of soil microbial communities to increasing temperature are of global importance. Does the microbial community structure change with increasing temperature? Does the substrate utilization differ in different temperatures with the same microbial community? Are microorganisms ubiquitously present or differ among different ecosystems? In spite of its importance for predicting the future SOM decomposition rates and magnitude of C stocks in the soil and in the atmosphere, the soil microbial community is rarely considered explicitly in models (Moorhead & Sinsabaugh, 2006; Allison & Martiny, 2008), and there is substantial debate over whether microbial community structure influences process rates despite the assumption that microorganisms are ubiquitously present (Kemmitt et al., 2008; Kuzyakow et al., 2009).

The artificial soil with controlled composition is a good medium for soil C dynamics experiments (Chapter 4 and 5). Soil microorganisms are an extremely complicated parameter and are heterogeneous among different soils or even within the same soil. In a relatively simple and controlled environment in artificial soils, it is possible to examine how temperature will affect soil microbial community structure, and how the temperature-affected soil microbial community structure will impact on substrate utilization. In addition, soil microorganisms from different ecosystems or different latitudes could be inoculated in the artificial soils to examine temperature responses of soil microbial communities of different origins and to test the assumption of ubiquitously present microorganisms.
References


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I dedicate this dissertation to those who have long supported and encouraged me to complete this degree

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