Drying and substrate concentrations interact to inhibit decomposition of carbon substrates added to combusted Inceptisols from a boreal forest

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Abstract

Climate change is expected to alter the mechanisms controlling soil organic matter (SOM) stabilization. Under climate change, soil warming and drying could affect the enzymatic mechanisms that control SOM turnover and dependence on substrate concentration. Here, we used a greenhouse climate manipulation in a mature boreal forest soil to test two specific hypotheses: 1) rates of decomposition decline at lower substrate concentrations; and 2) reductions in soil moisture disproportionately constrain the degradation of low-concentration substrates. Using constructed soil cores, we measured decomposition rates of two polymeric substrates, starch and cellulose, as well as enzyme activities associated with degradation of these substrates. The greenhouse manipulation increased temperature by 0.8ºC and reduced moisture in the constructed cores by up to 90%. We rejected our first hypothesis, as the rate of starch decomposition did not decrease with declining starch concentration under control conditions, but we did find support for hypothesis two: drying led to lower decomposition rates for low-concentration starch. We observed a 3-fold reduction in soil respiration rates in bulk soils in the greenhouses over a four month period, but the C losses from the constructed cores did not vary among our treatments. Activities of enzymes that degrade cellulose and starch were elevated in the greenhouse treatments, which may have compensated for moisture constraints on the degradation of the common substrate (i.e., cellulose) in our constructed cores. This study confirms that substrate decomposition can be concentration-dependent, and suggests that climate change effects on soil moisture could reduce rates of decomposition in well-drained boreal forest soils lacking permafrost.

Introduction

Traditional models of soil C biogeochemistry assume that C substrates in soils have intrinsic decomposition rates, often known as *k*-values (Parton et al. 1987; Todd-Brown et al. 2012). Substrates that are more chemically or physically accessible to microbes are assumed to have higher intrinsic decomposition rates—for example, chemically simple compounds like glucose and amino acids have higher *k*-values than more complex substrates, such as lignin. These intrinsic decomposition rates can be modified by environmental conditions and are often assumed to decline with moisture limitation or increase with temperature (Gulledge and Schimel 2000; Rustad et al. 2001; Davidson and Janssens 2006; Bronson et al. 2008; Manzoni et al. 2011; Steinweg et al. 2012; Poll et al. 2013).

Despite this focus on substrate chemistry and environmental conditions, it has long been recognized that decomposition is also mediated by the abundance and activity of decomposer organisms (Swift et al. 1979). In line with this idea, recent conceptual and mathematical models have begun to revisit decomposition as an emergent property of microbe-substrate interactions (Ladd et al. 1996; Kleber et al. 2010; Schmidt et al. 2011; Wieder et al. 2011; Wieder et al. 2013). Constraints on microbial decomposers may therefore indirectly control substrate decay rates. For instance, decomposition of soil organic matter (SOM) depends on microbial production of hydrolytic and oxidative enzymes (Schimel and Weintraub 2003; Sinsabaugh 2010; German et al. 2011b). Thus, constraints on enzyme production and access to substrates can influence decomposition rates, independent of substrate chemistry. In addition, substrate concentration could affect decomposition rates by constraining the return on microbial investment in enzymatic machinery required for substrate metabolism (Nannipieri et al. 2002; Ekschmitt et al. 2005, 2008; Conant et al. 2011; German et al. 2011a). Studies dating back to the

1940's have tested for relationships between decomposition rate and substrate quantity (Broadbent and Bartholomew 1949), but constraints imposed by very low substrate concentrations have rarely been examined.

Previously, we proposed that certain SOM substrates should decompose at lower rates when present at low concentrations (German et al. 2011a; Allison et al. 2014). This model is potentially relevant in soils because SOM is composed of C compounds that may each be relatively low in concentration (Allison 2006). Substrates that require specific metabolic pathways for degradation may not be targeted by microbes unless substrate concentration is high enough to support the cost of expressing enzymes in the pathway. This idea is based on a simple extension of the Michaelis-Menten theory of enzyme kinetics:

$$\frac{d[S]}{dt} = \frac{V_{max}[E][S]}{K_m + [S]} \quad (1)$$

where *[S]* is substrate concentration, *[E]* is enzyme concentration, V_{max} is the maximum catalytic rate per unit enzyme, and K_m is the half-saturation constant. This equation can be rearranged to obtain the substrate decomposition rate in units of inverse time, similar to a *k*-value:

$$k = \frac{d[S]}{[S]dt} = \frac{V_{max}[E]}{K_m + [S]} \quad (2)$$

Finally, we assume that *[S]* is converted to *[E]* with efficiency ε if microbes are producing enzymes based on energy intake from the metabolism of *S*:

$$k = \frac{d[S]}{[S]dt} = \frac{V_{max}\varepsilon[S]}{K_m + [S]} \quad (3)$$

This model implies that the decomposition rate approaches $V_{max}\varepsilon/K_m$ as substrate concentration increases and approaches zero as substrate concentration declines due to a decline in the production of metabolic enzymes (Fig. 1). Although the right side of eq. 3 resembles the traditional Michaelis-Menten expression, our model is different because we are describing a fractional decomposition rate (in units of inverse time) rather than a reaction velocity. We also note that soil is a heterogeneous system, and our simple model ignores substrate and enzyme interactions with reactive particles (e.g. minerals) that are known to affect enzyme kinetic parameters (see review by Nannipieri and Gianfreda 1998).

The effect of substrate concentration could interact with climate conditions to determine decomposition rates (Ekschmitt et al. 2005; Or et al. 2007). If accompanied by substantial drying, climate warming could reduce microbial growth, enzyme production, and access to substrates (Geisseler et al. 2011; Manzoni et al. 2011), thereby disproportionately restricting the decomposition of low-concentration substrates within the soil matrix (Fig. 1). In our model, these mechanisms would be represented by declines in ε and/or an increase in K_m. Alternatively, warming and drying could reduce the thickness of water films (Or et al. 2007), thus increasing the effective concentration of enzymes and substrates. Such changes, especially when accompanied by warmer temperatures, could help mitigate the negative effect of restricted diffusion on decomposition, especially for low-concentration substrates.

In this study, we examined how warming and drying affected rates of microbial decomposition in boreal forest soils. Although there is consensus on warming of the boreal zone in the coming century, some areas of boreal forest are predicted to become warmer and wetter, whereas others are predicted to become drier with the changing climate (IPCC 2014). Therefore, although microbial decomposition will probably increase on average with this warming trend

(Bergner et al. 2004; Bronson et al. 2008), it is possible that rates of decomposition could decline in drier regions of the boreal zone (Allison and Treseder 2008).

Specifically, we tested two hypotheses related to climate and substrate concentration effects on microbial decomposition. First, we tested whether substrate decomposition rate declines with substrate concentration under field conditions, as we observed previously in a study with soils from a recently-burned boreal ecosystem (German et al. 2011a), and in a laboratory investigation with mineral soils from California (Allison et al. 2014). Second, we hypothesized that warming and drying would have a disproportionate negative effect on the decomposition of low-concentration substrates due to reductions in microbial growth and enzyme production (Fig. 1). These tests were designed to understand the mechanisms underlying SOM response to climate change in boreal forest ecosystems.

Materials and Methods

Greenhouse experiment

Our study took place in a mature black spruce (*Picea mariana*) forest located in central Alaska (63°55'N, 145°44'W). We used five pairs of 2.5 m x 2.5 m plots (i.e. $n = 5$ replicates) that were established in a 1 km^2 area of forest by Allison and Treseder (2008) as part of a climate change manipulation. Briefly, one plot from each pair was assigned to a soil warming (greenhouse) treatment, whereas the other served as a control. Plots in each pair were located 3-5 m apart and contained similar vegetation. Soils at the site are Inceptisols with a pH of 4.9±0.2 and organic matter content of 42±4% (Treseder et al. 2004; Allison and Treseder 2008). Manipulated soils

were warmed passively during the growing season with closed-top greenhouses that were established in May 2005 (Allison and Treseder 2008). We conducted our experiment in the sixth growing season (2010) of the greenhouse treatment. Our experiment spanned the entire growing season (May – September 2010), and soil temperatures were measured in paired control and greenhouse plots using Onset HOBO dataloggers that were buried at 5 cm depth and recorded temperature every 30 min.

To test for an effect of substrate concentration on decomposition rate, we constructed soil cores that contained two organic substrates: an unlabeled, high-concentration substrate (cellulose), and a low-concentration ^{13}C-labeled substrate (starch) (German et al. 2011a). Both substrates are plant-derived polymers that require hydrolysis by extracellular enzymes prior to microbial uptake. To control the quantity and chemistry of organic matter, we added the organic substrates to combusted soils. Soils for combustion were collected from the field site (0-10 cm depth), stored on ice, and combusted in a muffle furnace at 550°C for three hours. Following combustion, the soil was divided into portions that received specific organic substrates at a final concentration of 50 mg g^{-1} soil. ^{13}C-labeled starch was added at levels of 0, 0.01, 0.1, 0.5, 1, 5, and 10% of the total organic substrate, with cellulose composing the difference. ^{13}C-labeled starch was purchased from IsoLife BV (Wageningen, Netherlands), and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Approximately 28 g of the soil-organic substrate mixture was added to each core. The cores were 2.5 cm diameter x 5 cm depth PVC with 250 μm mesh on the bottom to prevent soil loss, but allow water and solutes to pass through. Each substrate-concentration combination was replicated in each plot pair. Thus, with seven starch concentrations, five replicates, and paired greenhouse and control treatments, we had a total of 70 cores. The cores were randomly placed in the ground at least 50 cm apart in

each 2.5 m x 2.5 m plot and were allowed to incubate in the field from 8 May – 1 September 2010. At the beginning of the experiment, each core was inoculated with soil microorganisms by adding 1 mL of inoculant, which was made by diluting fresh soil from the field site (1:1000 w:v) in local well water (German et al. 2011a).

Following the field incubation, the contents of each soil core were placed in a 60-mL screw-cap vial, mixed vigorously by hand, and immediately subsampled for the following analyses: ~1 g was placed in a 15 mL centrifuge vial for water content determination; an additional 5 g was transferred to a 15 mL centrifuge vial for enzyme analyses; and the remainder was retained for stable isotope and C concentration measurements. All samples were kept cold (4°C) for transport to UC Irvine, and were stored at -80°C until analysis.

Water content determination

The water content of soils from the field-incubated cores was determined with 1 g sub-samples dried at 105°C for 24 hours. The difference in mass between the sample before and after drying represents the water content.

Stable isotope and C concentration measurements

Soil-organic substrate mixtures from the constructed cores were dried at 60°C for 48 hours and homogenized in a ball mixer mill (8000D mixer/mill, Spex SamplePrep, Metuchen, NJ, USA). Initial soil-organic substrate mixtures that were not placed in the field (i.e., the starting material for the constructed cores) were also dried and mixed at this time. After mixing, approximately

20 mg of the soil-organic substrate mixture from the cores or the starting material (n=6 analytical replicates per sample) were placed in tin capsules and combusted in a PDZ Europa ANCA-GSL elemental analyzer (which measured C concentration) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. All stable isotopic analyses were performed in the Stable Isotope Facility at the University of California, Davis, CA, USA.

Stable isotope abundances of soil from the constructed cores are expressed in delta (δ), defined as parts per thousand (‰) relative to the standard as follows:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (4)$$

where R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$) in the sample and standard, respectively. $R_{standard}$ for ^{13}C was IAEA CH-7, which was inserted in all runs at regular intervals to calibrate the system and correct for drift.

We used the isotopic data to measure the decomposition rates (i.e. k-values, eq. 3) of starch and cellulose. Using the isotopic signature of the C in our cores, we calculated the fraction of starch in each core at the end of the field incubation (FS_f). The corresponding fraction of cellulose was therefore $1 - FS_f$. Based on mass loss, we calculated starch decomposition rate as:

$$k_{starch} = \frac{FS_i OS_i - FS_f OS_f}{t} \quad (5)$$

where OS_i is the total amount of organic substrate initially added to the core, FS_i is the initial fraction of organic substrate composed of starch, OS_f is the final amount of organic substrate present in the core, and t is incubation time. Cellulose decomposition rate is calculated analogously:

$$k_{cellulose} = \frac{(1 - FS_i)OS_i - (1 - FS_f)OS_f}{t} \quad (6)$$

Soil respiration

Bulk soil respiration rates were measured with an infrared gas analyzer (PP Systems EGM-4, Amesbury, MA, USA) by monitoring the change in CO_2 concentration over time in flux chambers. Two 25 cm diameter chamber bases were inserted into each plot in 2005. We measured fluxes in each chamber on 1 September, at the end of the 2010 growing season. For each measurement, we monitored CO_2 concentrations for 5–10 min after placing a lid over the chamber base (Allison et al. 2008). CO_2 concentrations in the chambers generally did not exceed 600 ppm during the measurement interval. Chamber volumes were corrected for moss and litter content, and the flux was calculated as:

$$f = \frac{mV}{ART} \quad (7)$$

where *m* is the change in CO_2 concentration in the chamber with time, *V* is the chamber volume, *A* is the cross-sectional area of the chamber, *R* is the ideal gas constant, and *T* is the chamber air temperature in Kelvin. Atmospheric pressure was assumed to be 1 atm.

Enzyme activities

Enzymes were assayed in soil-organic substrate mixtures from the constructed cores. Homogenate was prepared by dispersing 1 g of core material in 125 mL of 50 mM sodium acetate buffer, pH 5, consistent with the pH of the soil from the field site (King et al. 2002).

Cellobiohydrolase (CBH), β-glucosidase (BG), and α-glucosidase (AG) activities were assayed in soil homogenates following the protocol described by German et al. (2011b). This technique is thought to target extracellular enzyme activities but may include intracellular activity if the fluorimetric substrates are taken up by microbial cells (Nannipieri et al. 2012). Briefly, 50 μL of fluorometric substrate solution (CBH: 500 μM; BG: 1000 μM; AG: 1000 μM) was combined with 200 μL of soil homogenate in a microplate and incubated for one hour at 10°C. The reaction was stopped by the addition of 10 μL of 1M NaOH, and the amount of fluorescence was immediately determined in a fluorometer (Biotek Synergy 4, Winooski, VT, USA) at 360 nm excitation and 460 nm emission. The assay of each enzyme was replicated eight times in each plate, and each plate included a standard curve of the product (4-methylumbelliferone; MUB), substrate controls, and homogenate controls. Enzymatic activity (nmols product released h^{-1} g^{-1} dry soil) was calculated from the MUB standard curve following German et al. (2011b). All reactions were run at saturating substrate concentrations as determined for each enzyme with soils from the field site, and linearity of the reaction was confirmed for the one hour assay duration.

Statistics

The loss of soil C (%) was determined for each constructed core using the equation:

$$\left(1-\frac{C_f}{C_i}\right)\times 100 \quad (8)$$

where C_f is the final amount of C remaining in the core following the field incubation, and C_i is the initial amount of C in the core prior to incubation. Soil temperature and respiration rates, which were recorded in bulk soil within each plot, were compared among greenhouse and

control plots with paired *t*-tests. Soil moisture and C loss were pooled for all cores within the greenhouse and control plots, and were therefore compared with two-sample *t*-tests among the treatments. Pooling was justified because soil moisture (Greenhouse: $F_{1,38} = 0.02$, $P = 0.90$; Control: $F_{1,33} = 0.00$, $P = 0.98$) and C mass loss (Greenhouse: $F_{6,39} = 1.38$, $P = 0.25$; Control: $F_{6,30} = 2.65$, $P = 0.04$, with only the 0% and 0.01% concentrations treatments varying, $P = 0.0334$) did not show a consistent significant relationship with starch concentration. Enzyme activities were evaluated using 2-way ANOVA, with block as a random factor, and starch concentration and greenhouse treatment (and their interaction) as main effects. Tukey's HSD was used to compare enzymatic activities across starch concentrations within each treatment. Enzyme activities were compared among treatments at each starch concentration with two-sample *t*-tests, followed by a Bonferroni correction. The dependence of decomposition rate on substrate concentration was tested with non-linear regression, using the saturating function:

$$y = \frac{(a \times [starch])}{(b + [starch])} \quad (9)$$

where a represents the maximum decomposition rate, and b is the starch concentration at half of the maximum decomposition rate. We were justified in using the non-linear function because linear fits had R^2 values less than 0.10, and we expected a non-linear relationship between substrate concentration and decomposition rate (Fig. 1). The 0.01% and 10% starch treatments were excluded from the analysis for decomposition rate because the isotopic signatures of the 0.01% starch cores were too variable to analyze consistently, and starch concentrations $\geq$10% can inhibit decomposition in soils (German et al. 2011a). All statistics were run using SPSS statistical software version 20 (IBM, Armonk, NY, USA). Normality was confirmed for all analyses before running parametric tests, and data not meeting normality requirements were log transformed prior to analysis.

Results

Soil temperature, respiration, moisture, and C decomposition

The greenhouses significantly ($P = 0.038$) warmed the soil by 0.8°C in comparison to the control plots, and the bulk soil in the greenhouse plots showed significantly lower CO_2 efflux ($P = 0.042$) than the control soil (Table 1). The soil cores in the greenhouse treatment held only one-tenth of the moisture in the control plots ($P < 0.001$), yet there was no significant difference in soil C loss ($P = 0.157$) from greenhouse cores in comparison to control cores (Table 1).

Stable isotopic signatures and decomposition rate

The degradation of ^{13}C-labeled starch showed a statistically significant relationship ($P < 0.001$), albeit a weak one ($R^2 = 0.049$), with declining starch concentration in cores incubated in the control plots (hypothesis one; Fig. 2). The degradation of starch decreased more strongly ($R^2 = 0.222$; $P < 0.001$) with declining starch concentrations in cores incubated in the greenhouse plots (hypothesis two; Fig. 2). The degradation of cellulose showed significant effects of cellulose concentration and treatment, but there was no significant interaction (Table 2). Interestingly, with the exception of the 0.01% starch treatment (99.99% cellulose), the cores incubated in the greenhouses showed greater cellulose decomposition than those incubated in the control plots, with the overall effect of greenhouse treatment significant at $P = 0.041$ (Table 2).

Enzyme activities

We found a significant dependence of cellobiohydrolase activity (Fig. 3) on starch concentration and greenhouse treatment, but not on the interaction of the two. We also observed a significant dependence of β-glucosidase activity (Fig. 3) on starch concentration, but not on greenhouse treatment or the two-way interaction. Overall, the greenhouse cores had higher cellobiohydrolase and β-glucosidase activity at four starch concentrations (0.1, 0.5, 1, and 5% starch; Fig. 3), although the pairwise differences were not statistically significant according to post-hoc tests. We also measured α-glucosidase activities in all of the cores, but this enzyme activity was largely undetectable in the control cores, thus making comparisons among the greenhouse and control plots impossible. Regression of the α-glucosidase activity in the greenhouse plots against starch concentration showed no significant relationship ($F_{1,22} = 0.89$, $R^2 = 0.041$, $P = 0.357$). However, detection of α-glucosidase activity in the greenhouse plots but not in the control plots is consistent with elevated enzymatic activity under the drier conditions in the greenhouse treatments.

Discussion

We did not find strong support for our first hypothesis that low-concentration substrates would decompose at slower rates than high-concentration substrates under control conditions in boreal forest soils (i.e., the relationship was weak; $R^2 = 0.049$). However, the pronounced drying effect in our greenhouse treatments likely impeded the degradation of low-concentration starch, thus leading to support for our second hypothesis (Figs. 1 and 2). Interestingly, the enzymatic

activities were consistently elevated in the greenhouse treatment compared to the control treatments, also likely showing the effects of warming and drying on enzymatic production and/or stability.

We previously observed an effect of substrate concentration on decomposition rate in field and laboratory incubations with soils from a nearby boreal ecosystem that burned in a 1999 wildfire (Treseder et al. 2004; German et al. 2011a). Our current study shows that this pattern may not apply to mature boreal forest soils that contain significantly higher concentrations of organic substrate and/or moisture, but that drying within these environments may allow for substrate concentration effects to manifest. This finding is important because physical protection and soil microenvironment may influence SOM stability more than chemical recalcitrance of SOM (Schimel and Weintraub 2003; Ekschmitt et al. 2005; Kleber et al. 2010). Soils store nearly four times the amount of C found in the atmosphere (Gorham 1991; Jobbágy and Jackson 2000; Tarnocai et al. 2009), and the bulk of this C is considered "stabilized" (von Lützow and Kögel-Knabner 2009). Hence, understanding the regulation of stabilized SOM is important for making predictions of SOM decomposition and C cycling in response to climate change (Allison et al. 2010b).

In support of our second hypothesis, the substrate concentration constraint on starch decomposition rate was apparent in the greenhouse treatment. Our conceptual framework (eq. 3; Fig. 1) suggests that moisture limitation might increase the effective K_m for enzyme activity—restricted diffusion should limit enzyme-substrate interactions such that higher substrate concentrations are required to achieve the same decomposition rate. This mechanism may have operated in the greenhouse plots, even with an observed increase in potential activity of α-glucosidase. Drying may have also reduced the efficiency factor, ε, for enzyme activity (eq. 3).

Increases in effective K_m or declines in ε would push the dashed line of the greenhouse treatment downward in Fig. 1, relative to the control level, consistent with our observations.

We detected significant effects of starch concentration on cellobiohydrolase and β-glucosidase activities, with both enzymes showing their highest activities in the 0.5-1.0% starch range in the greenhouse and control plots (Fig. 3). This result is surprising because these enzymes degrade cellulose and its degradation products rather than starch. One possible explanation is that low to moderate starch concentrations increase microbial biomass and constitutive expression of cellulose-degrading enzymes. We consistently observed that cellulose loss was highest in the cores containing 0.01% and 0.1% starch in the greenhouse and control plots (Table 2). Along those lines, the addition of glucose (a degradation product of starch) has increased β-glucosidase activities in other soil microcosm experiments (Hernandez and Hobbie 2010). At concentrations above 1% of total SOM, starch appears to inhibit cellobiohydrolase and β-glucosidase production, both in this and our previous investigation (German et al. 2011a). Although the mechanism is unclear, this inhibition is consistent with other studies showing that elevated starch concentrations can impede C mineralization in some soils, (Schimel et al. 1992; Prescott and McDonald 1994). Taken together, these results suggest that the potential enzyme activities we measured are not tightly linked to substrate decay rates (Wallenstein and Weintraub 2008). Complementary measurements of enzyme gene frequencies and expression could potentially help uncover the mechanisms underlying differences in substrate decomposition (Nannipieri et al. 2012).

Although the decomposition rate of starch declined at lower concentrations in the greenhouse treatment (but not the control), the overall decomposition rate of starch+cellulose (measured as total C loss from the constructed cores; Table 1) did not vary with greenhouse

treatment. Moreover, cellulose decomposition in the cores was slightly higher in the greenhouse treatment relative to controls (Table 2). This pattern may be explained by elevated enzymatic activities in the greenhouse plots compensating for drier (less diffusive) conditions. The increase in enzyme activities could have resulted from increased enzyme production (Brzostek et al. 2012; Alster et al. 2013), reduced inhibitor concentrations, and/or reduced enzyme turnover (Burns 1982; Geisseler et al. 2011; Steinweg et al. 2012).

In contrast to the minimal effects of drying on overall C loss in the constructed cores, the respiration rates from the bulk soils in greenhouse plots were three-fold lower than in control plots (Table 1). This difference in response between cores and bulk soil could be driven by enzymes. Whereas enzyme potentials increased with drying in the constructed cores, there were no increases in the bulk soils that could offset the impacts of moisture limitation (Allison and Treseder 2008). Different responses cannot be explained by a greater magnitude of drying in the bulk soil: we observed a moisture reduction of 90% in the constructed soil cores versus a maximum reduction of ~40% previously observed for bulk soils (Allison and Treseder 2008). The constructed cores probably restricted lateral transport of water through the surface soil, thus resulting in greater drying.

Reduced rates of microbial decomposition are often observed under dry conditions (Davidson et al. 1998; Gulledge and Schimel 2000; Allison and Treseder 2008; Manzoni et al. 2011; Steinweg et al. 2012; Allison et al. 2013; Alster et al. 2013; Poll et al. 2013). This finding is logical because enzymes and degradation products must be able to diffuse within the soil matrix for adequate resource acquisition by microorganisms (Manzoni et al. 2011). Thus, decomposition may be attenuated if warming leads to drier conditions (Gulledge and Schimel 2000). In boreal forests, approximately 45-60% of the soils are well-drained and not underlain

by permafrost (Larsen 1980; Zhang et al. 2008; Allison et al. 2010a; Allison and Treseder 2011); these areas in particular may experience drying in conjunction with warming (Allison and Treseder 2008; Allison et al. 2010a), and in such areas, substrate concentration may represent an additional limitation on SOM decomposition.

Our experiment was conducted under field conditions, but our use of constructed cores almost certainly altered important physiochemical and biological properties. For example, combustion removes native organic matter and releases nutrient rich ash, which probably increased soil pH and nutrient availability in the cores. Also, the organic substrate composition in the cores was not representative of native SOM, which is much more complex. Starch and cellulose probably decompose more rapidly than most SOM compounds (Ratledge 1994), so the concentration dependence of substrate decomposition in native soils may differ. Finally, the composition of the microbial community in the cores was probably distinct from the native community due to our inoculation procedure, restricted access into the PVC core, increased nutrient availability and pH, and the unique C substrate composition. Despite these potential caveats, our design allowed for *in-situ* measurement of compound-specific decomposition rates through precise control over organic substrate composition, and a clear effect was observed under warming and drying.

Conclusions

Our study confirmed our second hypothesis that decomposition rate is more dependent on substrate concentration under dry conditions. Increased microbial enzyme secretion and/or reduced enzyme turnover under drying can lead to increased enzyme pool sizes, but more

enzymes may not always offset the negative impacts of drying on the decomposition of low-concentration substrates. Hence, ecosystem models of the boreal zone should account for heterogeneity in soil characteristics, and moisture in particular, when making predictions of the feedbacks between climate warming and C cycling.

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References

Allison SD (2006) Brown ground: a soil carbon analogue for the Green World Hypothesis? Am Nat 167: 619-627

Allison SD, Chacon SS, German DP (2014) Substrate concentration constraints on microbial decomposition. Soil Biol Biochem 79: 43-49 doi http://dx.doi.org/10.1016/j.soilbio.2014.08.021

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Change Biol 14: 1-13

Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK, Martiny JBH (2013) Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94: 714-725 doi 10.1890/12-1243.1

Allison SD, McGuire KL, Treseder KK (2010a) Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biol Biochem 42: 1872-1878

Allison SD, Treseder KK (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob Change Biol 14: 2898-2909

Allison SD, Treseder KK (2011) Climate change feedbacks to microbial decomposition in boreal soils. Fungal Ecol 4: 362-374 doi http://dx.doi.org/10.1016/j.funeco.2011.01.003

Allison SD, Wallenstein MD, Bradford MA (2010b) Soil-carbon response to warming dependent on microbial physiology. Nat Geosci 3: 336-340

Alster CJ, German DP, Lu Y, Allison SD (2013) Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol Biochem 64: 68-79

Bergner B, Johnstone J, Treseder KK (2004) Experimental warming and burn severity alter soil CO_2 flux and soil functional groups in a recently burned boreal forest. Globa Change Biol 10: 1996-2004

Broadbent FE, Bartholomew WV (1949) The effect of quantity of plant material added to soil on its rate of decomposition. Soil Sci Soc Am J 13: 271-274

Bronson DR, Gower ST, Tanner M, Linder S, Van Herk I (2008) Response of soil surface CO_2 flux in a boreal forest to ecosystem warming. Glob Change Biol 14: 856-867

Brzostek ER, Blair JM, Dukes JS, Frey SD, Hobbie SE, Melillo JM, Mitchell RJ, Pendall E, Reich PB, Shaver GR, Stefanski A, Tjoelker MG, Finzi AC (2012) The effect of experimental warming and precipitation change on proteolytic enzyme activity: positive

feedbacks to nitrogen availability are not universal. Glob Change Biol 18: 2617-2625 doi 10.1111/j.1365-2486.2012.02685.x

Burns RG (1982) Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biol Biochem 14: 423-427

Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, Evans SE, Frey SD, Giardina CP, Hopkins FM, Hyvönen R, Kirschbaum MUF, Lavallee JM, Leifeld J, Parton WJ, Steinweg JM, Wallenstein MD, Martin Wetterstedt JÅ, Bradford MA (2011) Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. Glob Change Biol 17: 3392-3404 doi 10.1111/j.1365-2486.2011.02496.x

Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Glob Change Biol 4: 217-227 doi 10.1046/j.1365-2486.1998.00128.x

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440: 165-173

Ekschmitt K, Kandeler E, Poll C, Brune A, Buscot F, Friedrich M, Gleixner G, Hartmann A, Kästner M, Marhan S, Miltner A, Scheu S, Wolters V (2008) Soil-carbon preservation through habitat constraints and biological limitations on decomposer activity. J Plant Nutr Soil Sci 171: 27-35 doi 10.1002/jpln.200700051

Ekschmitt K, Liu M, Vetter S, Fox O, Wolters V (2005) Strategies used by soil biota to overcome soil organic matter stability -- why is dead organic matter left over in the soil? Geoderma 128: 167-176

Geisseler D, Horwath WR, Scow KM (2011) Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiol 54: 71-78 doi 10.1016/j.pedobi.2010.10.001

German DP, Chacon SS, Allison SD (2011a) Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology 92: 1471-1480

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011b) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43: 1387-1397

Gorham E (1991) Northern Peatlands: role in the carbon cycle and probably responses to climatic warming. Ecol Appl 1: 182-195

Gulledge J, Schimel JP (2000) Controls on soil carbon dioxide and methane fluxes in a variety of Taiga forest stands in interior Alaska. Ecosystems 3: 269-282 doi 10.1007/s100210000025

Hernandez DL, Hobbie SE (2010) The effects of substrate composition, quantity, and diversity on microbial activity. Plant Soil 335: 397-411

IPCC (2014) Climate Change 2014: Synthesis Report

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10: 423-436

King S, Harden J, Manies KL, Munster J, White LD (2002) Fate of carbon in Alaskan landscape project. Database for soils from eddy covariance tower sites, Delta Junction, Alaska. Open File Report 02-62. U. S. Geological Survey, Menlo Park, CA, USA

Kleber M, Nico PS, Plante A, Filley T, Kramer M, Swanston C, Sollins P (2010) Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. Glob Change Biol 17: 1097-1107

Ladd JN, Foster R, Nannipieri P, Oades JM (1996) Soil structure and biological activity. In: Stotzky G, Bollag J-M (eds) Soil Biochemistry. Marcel Dekker, New York, pp 23-78

Larsen JA (1980) The Boreal Ecosystem. Academic Press, Inc., London

Manzoni S, Schimel JP, Porporato A (2011) Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93: 930-938 doi 10.1890/11-0026.1

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: classical and molecular approaches. Biol Fert Soil 48: 743-762 doi 10.1007/s00374-012-0723-0

Nannipieri P, Gianfreda L (1998) Kinetics of enzyme reactions in soil environments. In: Huang PM, Senesi N, Buffle J (eds) Structure and Surface Reactions of Soil Particles. John Wiley & Sons, New York, pp 449-479

Nannipieri P, Kandeler E, Ruggiero P (2002) Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick RP (eds) Enzymes in the Environment. Marcel Dekker, New York, pp 1-33

Or D, Smets BF, Wraith JM, Dechesne A, Friedman SP (2007) Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. Adv Water Res 30: 1505-1527

Parton WJ, Schimel DS, Cole CV, Ojima DS (1987) Analysis of factors controlling soil organic-matter levels in great-plains grasslands. Soil Sci Soc Am J 51: 1173-1179

Poll C, Marhan S, Back F, Niklaus PA, Kandeler E (2013) Field-scale manipulation of soil temperature and precipitation change soil CO2 flux in a temperate agricultural ecosystem. Agricult Ecosyst Environ 165: 88-97 doi http://dx.doi.org/10.1016/j.agee.2012.12.012

Prescott CE, McDonald MA (1994) Effects of carbon and lime additions on mineralization of C and N in humus from cutovers of western red cedar - western hemlock forests of northern Vancouver Island. Can J Forest Res 24: 2432-2438

Ratledge C (1994) Biochemistry of Microbial Degradation. Kluwer Academic Publishers, Dordrecht

Rustad L, Campbell J, Marion G, Norby R, Mitchell M, Hartley A, Cornelissen J, Gurevitch J, Gcte N (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126: 543-562 doi 10.1007/s004420000544

Schimel JP, Helfer S, Alexander IJ (1992) Effects of starch additions on N turnover in Sitka spruce forest floor. Plant Soil 139: 139-143

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35: 549-563

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kogel-Knabner I, Lehmann J, Manning DAC, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. Nature 478: 49-56

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42: 391-404

Steinweg JM, Dukes JS, Wallenstein MD (2012) Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. Soil Biol Biochem 55: 85-92 doi 10.1016/j.soilbio.2012.06.015

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley, CA, USA

Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov S (2009) Soil organic carbon pools in the northern circumpolar permafrost region. Glob Biogeochem Cycl 23: GB2023

Todd-Brown KEO, Hopkins FM, Kivlin SN, Talbot JM, Allison SD (2012) A framework for representing microbial decomposition in coupled climate models. Biogeochem 109: 19-33

Treseder KK, Mack MC, Cross A (2004) Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecol Appl 14: 1826-1838

von Lützow M, Kögel-Knabner I (2009) Temperature sensitivity of soil organic matter decomposition—what do we know? Biol Fert Soil 46: 1-15 doi 10.1007/s00374-009-0413-8

Wallenstein MD, Weintraub MN (2008) Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biol Biochem 40: 2098-2106

Wieder WR, Bonan GB, Allison SD (2013) Global soil carbon predictions are improved by modelling microbial processes. Nature Clim Change 3: 909-912

Wieder WR, Cleveland CC, Townsend AR (2011) Throughfall exclusion and leaf litter addition drive higher rates of soil nitrous oxide emissions from a lowland wet tropical forest. Glob Change Biol 17: 3195-3207 doi 10.1111/j.1365-2486.2011.02426.x

Zhang T, Barry RG, Knowles K, Heginbottom JA, Brown J (2008) Statistics and characteristics of permafrost and ground-ice distribution in the northern hemisphere. Pol Geogr 31: 47-68.

Figure Legends

Fig. 1 Hypothesized dependence of decomposition rate on substrate concentration. The decomposition rate is hypothesized to decline with decreasing substrate concentration (solid line; German et al. 2011a), and the decline is predicted to be greater under drier conditions if enzyme-substrate interactions are limited by moisture (dashed line).

Fig. 2 Decomposition rate plotted as a function of starch concentration for cores incubated under greenhouse or control conditions. A non-linear function showed a significant relationship between starch decomposition and starch concentration in the greenhouse treatments ($y = (a \times [starch])/(b + [starch])$; $R^2 = 0.222$; $P < 0.001$), whereas a weaker (though still significant) relationship was detected in the control treatment ($R^2 = 0.049$; $P < 0.001$). Values are means ± SE. Cellulose composed the remainder of the organic substrate in each field core. The lowest (0.01%) and highest (10%) starch treatments were not used in the analysis. See Materials and Methods for an explanation of their exclusion

Fig. 3 Cellobiohydrolase (a) and β-glucosidase (b) activities as a function of starch concentration in greenhouse and control plots during the 2010 growing season. Values are mean and SE. Cellobiohydrolase showed significant effects of starch concentration and treatment, but not the interaction of the two (2-way ANOVA, Starch: $F_{6,24} = 5.01$, $P = 0.002$; Treatment: $F_{1,4} = 9.62$, $P = 0.036$; Starch x Treatment: $F_{6,24} = 1.02$, $P = 0.439$). β-glucosidase showed significant effects of starch concentration, but not treatment or the interaction of two variables (2-way ANOVA, Starch: $F_{6,24} = 4.59$, $P = 0.003$; Treatment: $F_{1,4} = 3.23$, $P = 0.147$; Starch x Treatment: $F_{6,24} = 0.45$, $P = 0.836$). See text for specific differences

Table 1. Soil temperature and soil CO_2 efflux at the plot level along with soil moisture and soil carbon (C) loss from constructed soil cores in control and greenhouse plots during the 2010 growing season in Alaskan boreal forest.

Soil variable	**Control**	**Greenhouse**	***t* (df)**	***P***
Temperature (°C)	9.14 ± 0.53	9.91 ± 0.35	3.05 (4)	**0.038**
CO_2 efflux (mg CO_2-C m^{-2} h^{-1})	153.73 ± 45.40	53.18 ± 14.78	2.94 (4)	**0.042**
Moisture (%)	33.20 ± 0.45	3.37 ± 0.43	64.05 (63)	<**0.001**
Soil C loss (%)	19.13 ± 1.13	21.31 ± 1.31	1.43 (69)	0.157

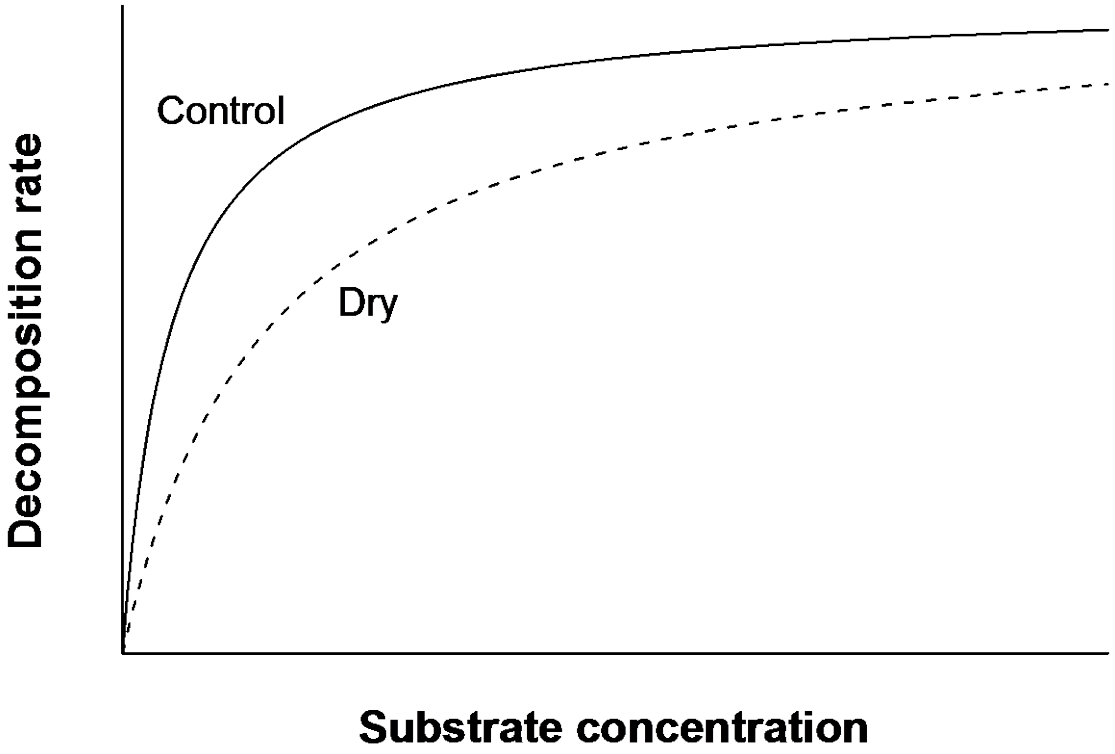
Values are mean ± SE. Statistical comparisons were made among control and greenhouse treatments for plot-level soil properties (i.e., temperature and CO_2 efflux) with paired-sample *t*-tests. Soil core variables (i.e., moisture and soil C loss) were compared among treatments with two-sample *t*-tests. *P*-values in **bold** indicate significant differences.

Table 2. Cellulose decomposition (% lost over four months) in control and greenhouse plots as a function of cellulose concentration.

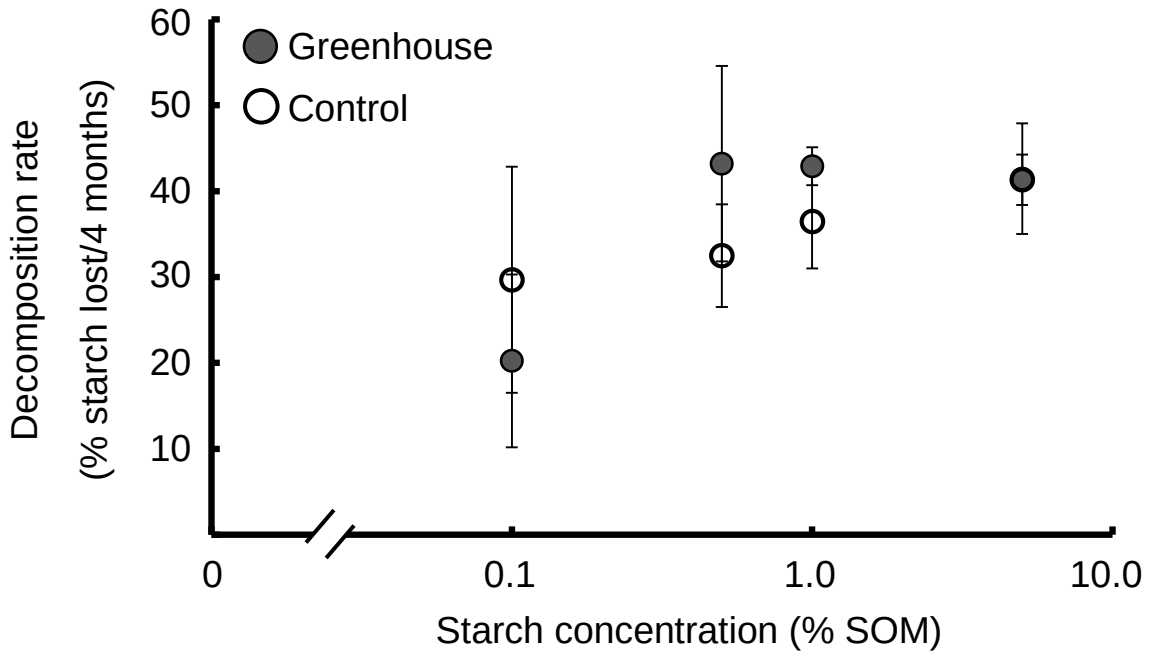
Cellulose concentration (% organic substrate)	Cellulose decomposition	Cellulose decomposition
	Control	Greenhouse
100	10.05 ± 0.97	14.75 ± 2.94
99.99	28.47 ± 3.13	21.70 ± 3.89
99.90	25.89 ± 3.04	27.05 ± 3.04
99.50	9.54 ± 1.94	13.81 ± 2.37
99.00	12.00 ± 5.52	12.95 ± 1.51
95.00	12.92 ± 0.72	14.30 ± 2.27
90.00	12.77 ± 1.62	16.18 ± 2.38
Average	16.51 ± 1.62	18.68 ± 1.45

Values are mean ± SE. Decomposition rate showed significant effects of cellulose concentration and treatment, but not the interaction of the two (2-way ANOVA; Cellulose concentration: $F_{6,52}$ = 19.18, $P < 0.001$; Treatment: $F_{1,6}$= 4.39, $P = 0.041$; Concentration x Treatment: $F_{6,52}$= 0.189, $P = 0.979$).

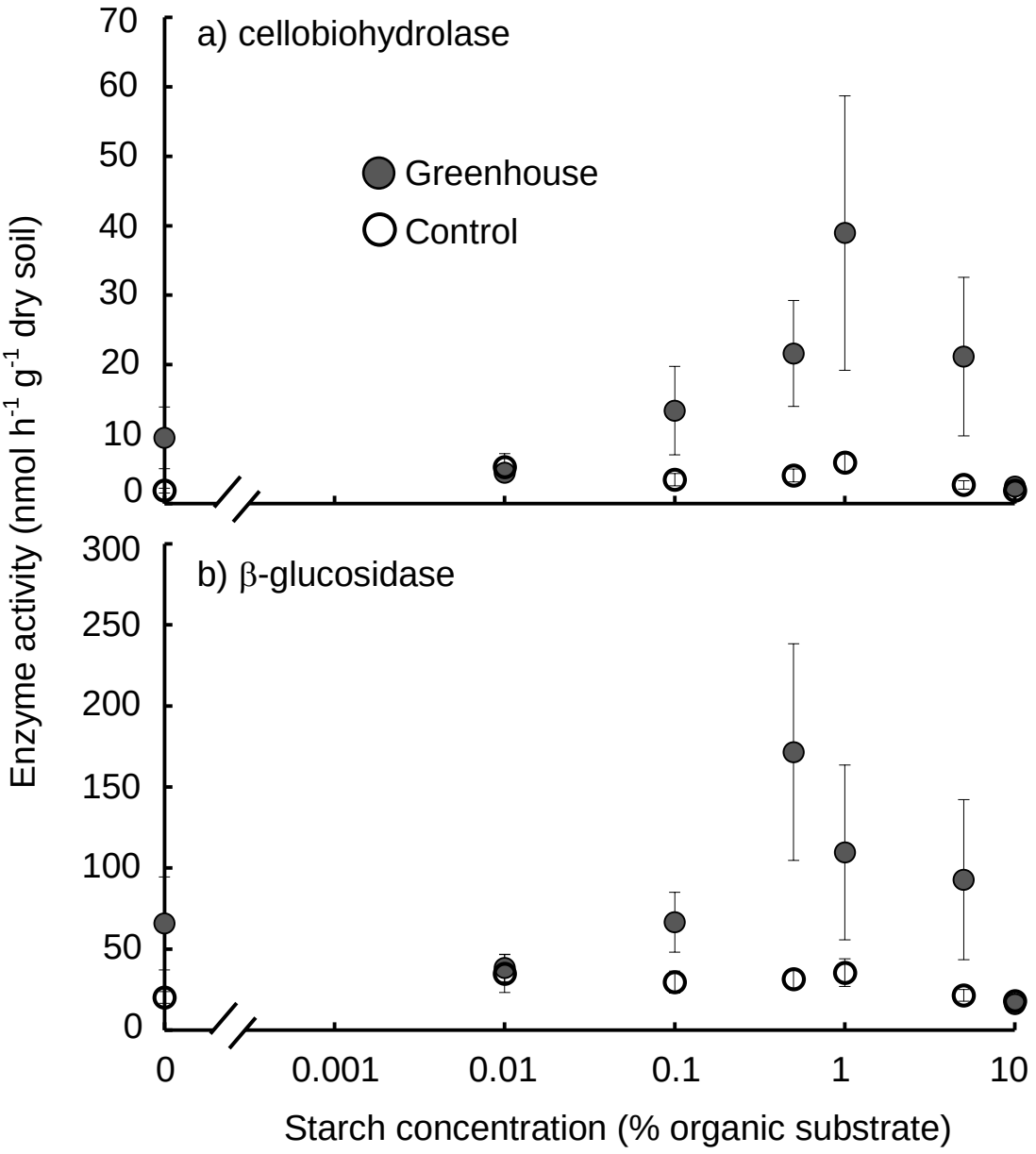
Figure



Figure


Greenhouse
Control
Decomposition rate
(% starch lost/4 months)
60
50
40
30
20
10
0
0.1
1.0
10.0
Starch concentration (% SOM)

Figure


a) cellobiohydrolase
b) β-glucosidase
Greenhouse
Control
Enzyme activity (nmol h-1 g-1 dry soil)
Starch concentration (% organic substrate)
0
10
20
30
40
50
60
70
50
100
150
200
250
300
0.001
0.01
0.1
1
10