Ecology, 92(7), 2011, pp. 1471–1480

Substrate concentration and enzyme allocation can affect rates of microbial decomposition

Donovan P. German,[1,4] Stephany S. Chacon,[2] and Steven D. Allison[1,3]

[1]*Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697 USA*
[2]*Department of Chemistry, University of California, Irvine, California 92697 USA*
[3]*Department of Earth System Science, University of California, Irvine, California 92697 USA*

Abstract. A large proportion of the world's carbon is stored as soil organic matter (SOM). However, the mechanisms regulating the stability of this SOM remain unclear. Recent work suggests that SOM may be stabilized by mechanisms other than chemical recalcitrance. Here, we show that the mineralization rate of starch, a plant polymer commonly found in litter and soil, is concentration dependent, such that its decomposition rate can be reduced by as much as 50% when composing less than ~10% of SOM. This pattern is largely driven by low activities of starch-degrading enzymes and low inducibility of enzyme production by microbial decomposers. The same pattern was not observed for cellulose and hemicellulose degradation, possibly because the enzymes targeting these substrates are expressed at constitutively high levels. Nevertheless, given the heterogeneous distribution of SOM constituents, our results suggest a novel low-concentration constraint on SOM decomposition that is independent of chemical recalcitrance. These results may help explain the stability of at least some SOM constituents, especially those that naturally exist in relatively low concentrations in the soil environment.

Key words: carbon cycling; carbon dioxide extracellular enzymes; cellulose; hemicellulose; microorganisms; starch.

Introduction

The amount of carbon (C) stored in soils is ~3300 Pg (1 Pg = 1×10^{12} kg), nearly four times the amount in living plants around the globe (Jobbágy and Jackson 2000, Tarnocai et al. 2009). However, the mechanisms regulating the decomposition of this soil organic matter (SOM) remain incompletely understood, especially in response to human activities. For instance, humans have the potential to alter the amount of C in soils through climate change, fertilizer application, and land clearing for agriculture and development (Trumbore 1997, Mellilo et al. 2002, Hyvönen et al. 2007, Bardgett et al. 2008). Given the role of the C cycle (atmospheric CO_2 in particular) in regulating climate change, it is critical to improve our understanding of the factors that influence the world's largest stocks of organic C.

Many factors influence SOM stability (i.e., residence time) in soils, including sorption to mineral surfaces and occlusion within small pores (Sollins et al. 1996, Zimmerman et al. 2004, Kleber et al. 2007, Grandy and Neff 2008), population dynamics and competitive interactions among soil biota (Allison 2005, Ekschmitt et al. 2005), recalcitrance of chemical bond structures to degradation (Sollins et al. 1996, von Lützow et al. 2006), and the heterogeneous distribution of SOM constituents (Ekschmitt et al. 2005, Allison 2006). Although these physical, chemical, and biological mechanisms are broadly recognized and important, research on SOM decomposition often overlooks the physiology of the microorganisms responsible for most SOM decay and biological mechanisms that can impede microbial attack on SOM (Kleber 2010). Nearly all widely used simulation models characterize decomposition rate as a k value that is constant for a given type of SOM but that varies with abiotic factors such as temperature and moisture (Parton et al. 1987, Potter et al. 1993, Schimel and Weintraub 2003, Friedlingstein et al. 2006). However, it has long been known that microbes are the proximate drivers of decomposition through their production of extracellular digestive enzymes and metabolism of low molecular weight SOM (Skujins 1976, Burns 1982, Sinsabaugh et al. 1991). Therefore, prediction of decomposition rates should take into account the physiology and enzymatic function of microbial decomposers (Schimel and Weintraub 2003).

Like all organisms, microbial decomposers can only express a finite number of metabolic pathways, each of which requires an input of energy and resources (e.g., N and C). In *Escherichia coli*, for example, lactose operon expression declines with decreasing lactose concentration, to a point that expression ceases entirely below lactose concentrations of ~1 mmol/L (Dekel and Alon 2005). Thus, a threshold can be reached below which it becomes too costly to express a particular enzyme

Manuscript received 27 October 2010; revised 15 February 2011; accepted 22 February 2011. Corresponding Editor: S. D. Frey.

[4] E-mail: dgerman@uci.edu

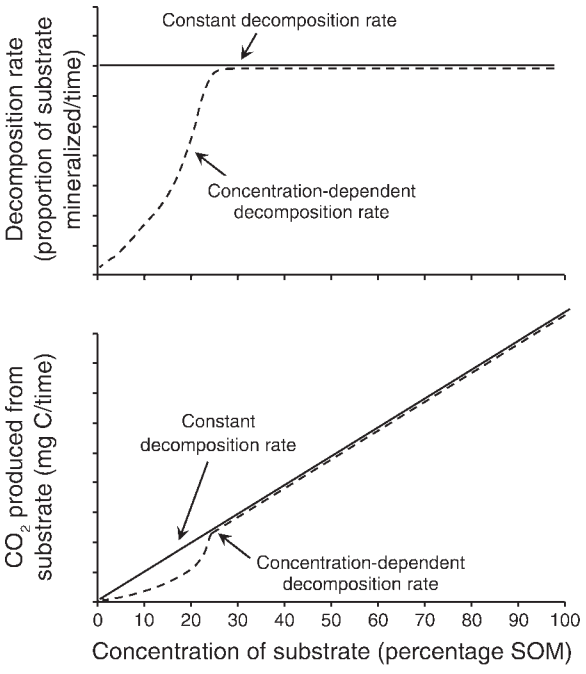

FIG. 1. A conceptual model of the effects of substrate concentration on the decomposition rate of that substrate. The null hypothesis is that decomposition rate (proportion of substrate mineralized per unit time) for a single substrate should be constant regardless of substrate concentration (upper panel, solid line). Therefore, the amount of C mineralized from a given substrate (OM, organic matter) should vary linearly with the concentration of that substrate (lower panel, solid line). However, the resource allocation hypothesis (low-concentration constraint) holds that decomposition rate is concentration dependent, such that a substrate will decompose at a progressively slower rate once the concentration of that substrate falls below a threshold (upper panel, dashed line). Thus, the amount of CO_2 produced from that substrate begins to decrease nonlinearly below some threshold substrate concentration (lower panel, dashed line). The model may apply to multiple scales and, hence, is depicted without values for the *y*-axis.

relative to the expected benefit of substrate metabolism. Similar thresholds for resource allocation may be important in soil because microbial decomposers encounter an array of C substrates (Kelleher and Simpson 2006, Lehmann et al. 2008) with concentrations that vary spatially and temporally (Allison 2006). The decomposition of these different SOM constituents (e.g., polysaccharides, lignins, proteins) may require microbial allocation to many distinct extracellular enzymes and metabolic pathways. However, investing resources in the degradation of low-concentration SOM substrates may not be energetically favorable for some microbial decomposers.

In accordance with theory on resource allocation (Koch 1985), we hypothesized that decomposition rates would decrease at low substrate concentrations due to a reduction in the expression of enzymes targeting the substrate (the "resource allocation hypothesis"; Fig. 1, dashed lines). As a null hypothesis, we assumed that the decomposition rate (defined as the proportion of substrate lost per unit time) would be constant for a given substrate and therefore not vary with concentration (Fig. 1, solid lines). Under the null model, CO_2 production should vary linearly with the concentration of a particular substrate (i.e., with a constant slope).

Using field and laboratory approaches, we tested the resource allocation hypothesis using three polysaccharides (i.e., cellulose, hemicellulose, and starch), each of which are found in litter and soil, but are degraded by different enzymes. We chose polysaccharides for this experiment because they are degraded by hydrolytic enzymes with high substrate specificity, thus allowing for a direct test of our hypothesis. To our knowledge, the potential constraint of concentration on SOM decomposition has not been recognized or characterized, yet may provide an additional mechanism to explain the stability of SOM (Bol et al. 2009, Kleber 2010).

Materials and Methods

Field experiment

A field experiment was performed using soils from a black spruce (*Picea mariana*) forest located in central Alaska, USA (63°55′ N, 145°44′ W; see Plate 1). Soils at this site are acidic inceptisols (pH 5) classified as silt loams underlain by silt and gravel with 15% SOM in the organic horizon (Richter et al. 2000, Treseder et al. 2004). The goal of this study was to test whether carbon substrates at low concentrations degrade more slowly than at higher concentrations (Fig. 1). However, we wanted to avoid confounding substrate concentration with the total availability of energy or carbon. Thus, we constructed soil cores that contained two organic substrates: an unlabeled, high-concentration substrate, and a low-concentration ^{13}C-labeled substrate (Fig. 2). To control for the exact quantity and type of organic matter present in the soil cores, soil collected from the field site during the previous growing season (2008), which had been frozen at −20°C, was combusted in a muffle furnace at 550°C for three hours to remove the native SOM. Following combustion, the soil was divided into portions that received different organic substrates at a final concentration of 50 mg/g soil. Thus, all cores had the same amount of total C, but varied in the type of C present, and all featured realistic soil mineralogy for the field site. Three organic matter combinations were made, each containing a high-concentration background C substrate, and a low-concentration C substrate that composed progressively less of the total organic matter (Fig. 2). One combination contained ^{13}C-labeled starch at levels of 0.01, 0.1, 1, and 5% of the total organic matter, with cellulose composing the difference. Another combination had ^{13}C-labeled cellulose with the difference composed of unlabeled starch. As a control, we included cores with ^{13}C-labeled cellulose with the difference composed of unlabeled cellulose. Hemicellulose was not used in the field incubations, but was used

in the laboratory microcosm experiment (see *Laboratory microcosm experiment*). All labeled substrates were purchased from IsoLife BV (Wageningen, The Netherlands), and all other reagents were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Approximately 28 g of the soil–organic matter mixture was added to each core, which was constructed of 2.5×5 cm polyvinyl chloride (PVC) with 250-μm mesh on the bottom to prevent soil loss, but allowed water and solutes to pass through (see Plate 1). Each substrate–concentration combination was replicated four times. Thus, with three substrates, four substrate–concentration combinations, and $n = 4$ for each combination, we had a total of 48 cores. The cores were randomly placed in the ground at least 1 m apart in a 10×10 m plot and were allowed to incubate in the field for five months (May–September 2009), the length of the growing season at the field site. At the beginning of the experiment (May 2009), 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 mass : volume) in local well water.

Following the field incubation, each soil core was collected and placed in a 60-m L screw-cap vial and kept cold (4°C) for transport back to University of California, Irvine, California, USA. Upon arrival, soil samples were stored at −80°C until analyzed.

Laboratory microcosm experiment

Microcosms were established in septum-capped 40 mL vials containing 2 g of sterile sand, 100 mg total substrate, and 800 μL of microbial inoculum created by diluting (1:1000 w:v) fresh Alaskan soil (collected May 2009) in a sterile minimal-nutrient solution (Allison et al. 2009). As with the field experiment, the C substrates were a mixture of a ^{13}C-labeled substrate and a non-labeled background substrate, and the same substrates were used in the laboratory as in the field. With a total substrate addition of 100 mg, ^{13}C-labeled substrates were added at levels of 0, 0.01, 0.1, 1, 5, 10, and 20 mg, with the remainder composed of unlabeled cellulose (or unlabeled starch; Fig. 2). Each microcosm, therefore, contained the same amount of C, and each substrate–concentration combination was replicated four times. Four additional control microcosms were constructed that contained sand and inoculum, but no C substrate. CO_2 concentrations in the microcosms were measured every seven days, and the concentrations were used to calculate cumulative CO_2 respiration over a nine-week incubation period at 10°C, the mean daily temperature at the field site during the growing season (Allison and Treseder 2008). For each measurement, an 8-mL subsample of headspace gas was withdrawn by syringe and injected into an infrared gas analyzer (PP-Systems EGM-4, Amesbury, Massachusetts, USA). After measurement, vials were flushed with CO_2 free air (zero-air) and then closed. CO_2 concentrations never exceeded 3600 ppm for any seven-day period, so anaerobic conditions were unlikely to have occurred in the vials. The CO_2 concentrations of blank vials were subtracted from sample vials to calculate cumulative respiration of substrate C. The gas draw from week nine was used to measure the amount of ^{13}C respired under the different substrate–concentration combinations (see following section: *Stable isotopic measurements*).

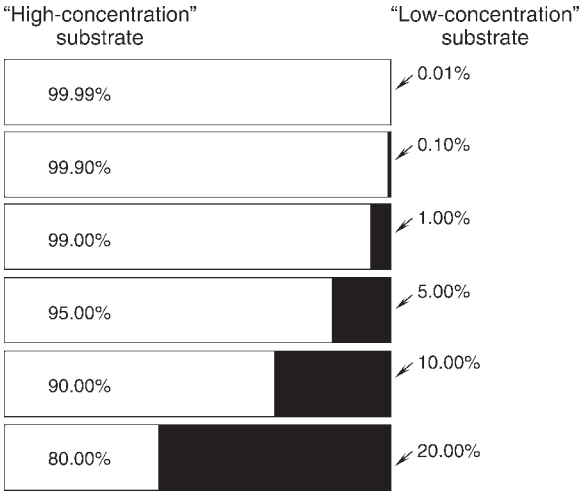

FIG. 2. Schematic representing the overall experimental design of this study. Organic matter present in field cores or laboratory microcosms was partitioned into two parts: a high-concentration, unlabeled C substrate (open portion of boxes), and a low-concentration, ^{13}C-labeled substrate (solid portions of boxes). A gradient of the substrates was established with increasing concentrations of the "low-concentration" substrate. Concentrations are in percentage of total C. Decomposition rate of, and digestive-enzyme activity against, the "low-concentration" substrate were hypothesized to follow substrate concentration (see Fig. 1). Note that this diagram is not to scale in order to show differences among the lower concentrations of the substrate. The two highest concentrations of the "low-concentration" substrate (10% and 20%) were only tested in the laboratory microcosm experiment, and not in the field.

Additional microcosms were constructed in the same manner, but were not used for gas analyses. These microcosms were frozen at weeks 2 and 10 of the microcosm experiment and used for assays of extracellular digestive-enzyme activities (see following section: *Extracellular digestive-enzyme activities*).

A second microcosm experiment was run using ^{13}C-labeled starch and hemicellulose (with unlabeled cellulose making up the difference) to confirm the results of the first experiment, and to test whether a pattern could be observed with hemicellulose. Gas samples from weeks 4 and 9 were sent for stable isotopic measurements to test whether it was problematic to wait until the ninth week to measure the isotopic signature of respired CO_2, as was done in the first laboratory microcosm experiment. In both experiments it took approximately one week for microbes to establish in the microcosms and generate detectable amounts of CO_2.

Moisture and organic matter contents

Moisture content of soils collected from the field site and from the incubated field cores was determined with 1-g samples dried at 105°C for 24 h. Total organic matter content was subsequently determined by combusting the dried soil at 550°C for 3 h. The difference in mass of the sample before and after combustion represented the total organic matter content.

Stable isotopic measurements

All stable isotopic analyses were performed in the Stable Isotope Facility at the University of California, Davis, California, USA. On week nine of the microcosm experiment (and weeks four and nine of the second microcosm experiment), 8 mL of gas was drawn from each vial with a syringe and transferred to 12-mL, septum-capped vials (Labco, Buckinghamshire, UK) filled with helium. The CO_2 samples were run through a Poroplot Q GC column (15 m × 0.53 mm ID [interior diameter], 25°C, 3 mL/min) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon, Cheshire, UK). Soil organic matter mixtures from the field cores were dried at 60°C for 24 h, mixed vigorously by hand, and ~20 mg of the core contents were placed in tin capsules and combusted in a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa (Sercon, Cheshire, UK) 20–20 isotope ratio mass spectrometer.

Stable-isotope abundances of CO_2 from the laboratory microcosm experiment and soil from the field cores are expressed in delta (δ) units, defined as parts per thousand (‰) relative to the standard as follows:

$$\delta = [(R_{\text{samp}}/R_{\text{stan}}) - 1](1000) \quad (1)$$

where R_{samp} and R_{stan} are the corresponding ratios of heavy to light isotopes (^{13}C:^{12}C) in the sample and standard, respectively. R_{stan} for ^{13}C was NIST 8560 (IAEA CH-7 for the soil samples). Standards were inserted in all runs at regular intervals to calibrate the system and correct for drift.

For the laboratory microcosm experiment, the precise proportion and isotopic signatures of the different C substrates present in each microcosm were known. Therefore, we could use the following linear mixing model to define the fractions of the different carbon substrates present in the microcosms:

$$\delta^{13}C_{\text{predicted}} = p_A\delta^{13}C_A + p_B\delta^{13}C_B \quad (2)$$

where p_X is the proportion of the available substrate composed of component X, and the $\delta^{13}C_X$ is the isotopic signature of that component. The decomposition ratio was calculated as the fraction of CO_2 carbon coming from the low-concentration substrate ($FCO_{2,\text{low}}$), as determined from the microcosm CO_2 isotopic signatures, to the fraction of low-concentration substrate C added to the microcosms (FC_{low}), as determined from the mixing model. The decomposition ratio allowed us to verify whether the decomposition rate of the ^{13}C-labeled substrate was constant over the range of substrate concentrations examined (null hypothesis), or if it deviated from the predicted line (Fig. 1, lower panel, dashed line). For the field cores, the decomposition ratio was calculated as the ratio of low-concentration substrate C in the initial material prior to field incubation (FC_i) to the fraction of low-concentration substrate C in the final material following the field incubation (FC_f). As with the microcosm experiment, if decomposition proceeded linearly with concentration, then the low-concentration substrate C of the final material would be identical to the starting material.

Extracellular digestive-enzyme activities

Enzymes were assayed in soil–organic matter mixtures recovered from the field cores and in the microcosms used in the laboratory. For the field cores, a 5-g subsample from each core was taken prior to drying the soil for stable isotopic analyses. Homogenate was prepared by dispersing 1 g of this subsample in 125 mL of 50 mmol/L sodium acetate buffer, pH 5, consistent with the pH of the soil from the field site (King et al. 2002). Microcosms were prepared for enzyme assays as described by Allison et al. (2009).

Cellobiohydrolase (EC 3.2.1.91), β-glucosidase (EC 3.2.1.21), β-xylosidase (EC 3.2.1.37), α-glucosidase (EC 3.2.1.20), and N-acetyl-β-D-glucosaminidase (EC 3.2.6.1) activities were assayed in soil homogenates following a modified version of the protocol described by Allison et al. (2009) (Table 1). Briefly, 50 μL of fluorometric substrate solution 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 1 mol/L NaOH, and after a 10-min development period, the amount of fluorescence was determined in a fluorometer (Biotek Synergy 4, Winooski, Vermont, 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 (nmol product released·h^{-1}·[g dry soil]$^{-1}$) was calculated from the MUB standard curve following DeForest (2009). 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 mass loss of organic matter (%) was determined for the field cores using the equation:

$$1 - (SOM_f/SOM_i) \times 100 \quad (3)$$

where SOM_f is the final amount of organic matter remaining in the cores following the field incubation, and SOM_i is the initial amount of organic matter present

TABLE 1. Enzymes measured in this study, including their functions, substrates, and classification.

Enzyme	Enzyme function	Assay substrate†	Assay substrate concentration (μmol/L)
Cellobiohydrolase	hydrolyzes 1,4-β-D-glucosidic linkages in cellulose and cellotetraose	4-MUB-β-D-cellobioside	100
β-glucosidase	hydrolyzes 1,4 linked β-D-glucose residues from β-D-glucosides	4-MUB-β-D-glucoside	200
β-xylosidase	hydrolyzes 1,4 linked β-D-xylose residues from β-D-xylosides	4-MUB-β-D-xyloside	200
α-glucosidase	hydrolyzes 1,4-linked α-D-glucose residues from α-D-glucosides	4-MUB-α-D-glucoside	200
N-acetyl-β-D-glucosaminidase	hydrolyzes 1,4 linked N-acetyl-β-D-glucosaminide residues in chitooligosaccharides (chitin-derived oligomers)	4-MUB-N-acetyl-β-D-glucosaminide	400

† MUB is the abbreviation for methylumbelliferone, a fluorescent dye.

in the starting material at the beginning of the experiment.

Enzyme activities, percentage of mass loss, and cumulative CO_2 evolved in the laboratory microcosms were evaluated using regression, with substrate concentration as the independent variable. The exact model used (linear, logarithmic, or exponential) varied by analysis and is noted in the *Results*. Because the soil samples for stable isotopic analyses were mixed by hand, there was some heterogeneity in the distribution of SOM in the soil matrix. Hence, some of the analytical replicates from the stable isotopic analyses of the field cores and starting material contained low C concentrations (<300 μg C per replicate), which led to unreliable stable isotopic measurements for those replicates. To control for this, isotopic data from analytical replicates that had C concentrations <300 μg C were removed from the analysis, resulting in three to six analytical replicates per core. The decomposition ratio was plotted as a function of substrate concentration and examined with regression analyses. Normality was confirmed for all analyses before running the regressions, and homogeneity of variance was confirmed with Levine's test.

RESULTS

Field experiment

Among the different combinations of substrates investigated in the field experiment, the decomposition of starch decreased as starch concentration declined ($F_{1,15} = 16.69$, $P < 0.001$, $R^2 = 0.54$; Fig. 3). However, there was no relationship between decomposition and substrate concentration for ^{13}C-labeled cellulose in a matrix of unlabeled cellulose ($F_{1,15} = 0.360$, $P = 0.558$, $R^2 = 0.03$) or ^{13}C-labeled cellulose in a matrix of unlabeled starch ($F_{1,15} = 3.45$, $P = 0.084$, $R^2 = 0.18$) In support of the resource allocation hypothesis, β-glucosidase and cellobiohydrolase activities significantly decreased with decreasing cellulose concentration (increasing starch concentration; Fig. 4). In contrast, α-glucosidase showed no relationship with starch concentration, and α-glucosidase and N-acetyl-β-D-glucosaminidase activities were undetectable at the highest starch concentrations (95–99.99%). The opposite would be predicted for α-glucosidase by the resource allocation hypothesis. Low enzyme activities could indicate a lower microbial abundance in the high starch treatments, although the mass loss of SOM from the field cores showed no relationship with starch concentration ($F_{1,47} = 0.66$, $P = 0.421$, $R^2 = 0.01$).

Laboratory microcosm experiment

The microcosms revealed similar results to the field cores for starch decomposition: The rate of starch degradation decreased with decreasing starch concentration (Fig. 5). This was confirmed at weeks four ($F_{1,40} = 68.85$, $P < 0.001$, $R^2 = 0.63$) and nine ($F_{1,40} = 46.05$, $P < 0.001$, $R^2 = 0.55$) of the second microcosm experiment, showing that the pattern was identical early and late during the microcosm incubation. Cellulose ($F_{1,46} = 0.097$, $P = 0.759$, $R^2 = 0.01$) and hemicellulose ($F_{1,46} = 0.195$, $P = 0.661$, $R^2 = 0.01$) degradation in the

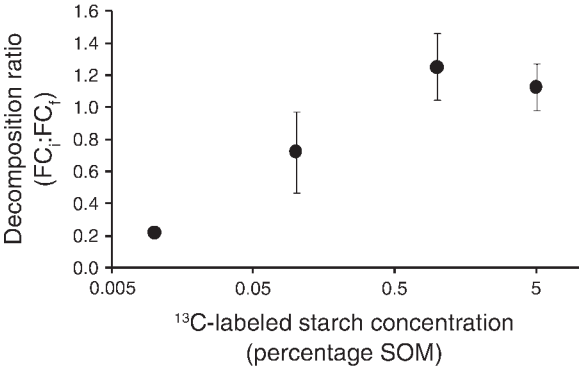

FIG. 3. Decomposition ratio plotted as a function of starch concentration for field-incubated cores [FC = fraction of low-concentration substrate C in the starting material before field incubation (i, initial) or following field incubation (f, final)]. A logarithmic model provided the best fit, showing a significant relationship between starch concentration and decomposition ratio ($F_{1,15} = 16.69$, $P < 0.001$, $R^2 = 0.54$). Values are means ± SE. Cellulose composed the remainder of the soil organic matter (SOM) in each microcosm, as depicted in Fig. 2.

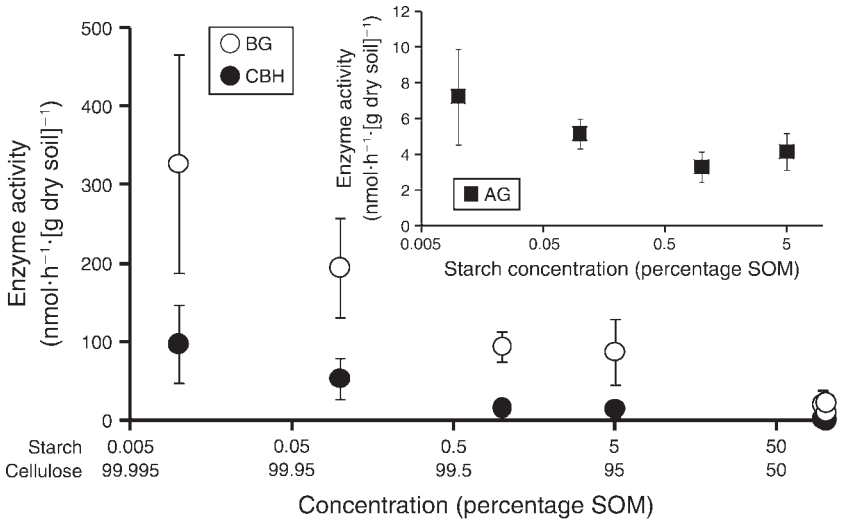

FIG. 4. The β-glucosidase (BG) and cellobiohydrolase (CBH) activities as a function of starch concentration in field-incubated soil cores. The remainder of the SOM in each core was composed of cellulose, and the cellulose concentrations are listed beneath the starch concentrations on the *x*-axis. The relationship between activity and starch concentration was best described by logarithmic models and was significant for BG ($F_{1,29} = 38.31$, $P < 0.001$, $R^2 = 0.58$) and CBH ($F_{1,24} = 35.30$, $P < 0.001$, $R^2 = 0.61$). In the inset panel, α-glucosidase (AG) was not detectable at the highest starch concentrations, and the relationship between activity and starch concentration was not significant for the concentrations presented (logarithmic $F_{1,15} = 3.10$, $P = 0.100$, $R^2 = 0.18$). Values in both panels are means ± SE.

laboratory microcosms showed no significant relationship with substrate concentration, following the null hypothesis. The total C respired in the laboratory microcosm experiment showed a significant negative relationship with starch concentration ($F_{1,47} = 35.26$, $P < 0.001$, $R^2 = 0.64$; Table 2). In support of the resource allocation hypothesis, α-glucosidase activity showed a significant positive relationship with starch concentration in the laboratory (Fig. 6). No relationship was found between cellulose concentration and β-glucosidase activities ($F_{1,27} = 0.780$, $P = 0.385$, $R^2 = 0.01$) or between hemicellulose concentration and β-xylosidase activities ($F_{1,46} = 0.030$, $P = 0.869$, $R^2 = 0.03$) in the laboratory.

DISCUSSION

We hypothesized that the decomposition rates of organic compounds would decrease with decreasing substrate concentration, both in terms of C mineralization and in terms of digestive-enzyme activities against these C substrates. Of the three substrates we tested, only starch was consistent with our hypothesis, whereas cellulose and hemicellulose decomposition more or less fit the null hypothesis. Consistent with the resource allocation hypothesis, β-glucosidase and cellobiohydrolase activities declined at low cellulose concentrations in the field incubation, but not in the laboratory microcosm experiments. Activity of α-glucosidase was positively related to starch concentration when a broad enough range of starch concentrations were included (e.g., concentrations greater than 10% of total SOM). Overall, our results suggest that SOM constituents that exist in low concentration may indeed escape decomposition, but this may not be true for all types of SOM compounds, especially abundant ones like cellulose and hemicellulose.

Our results are relevant for soil carbon cycling because "humic substances," which compose ~50% of SOM (Hayes et al. 1989, Orlov 1990, Lal 2004), may be composed largely of common plant and microbial polymers (e.g., cellulose, starch, lignin, chitin) and their degradation products (Kelleher and Simpson 2006), as opposed to highly diverse, chemically recalcitrant structures. Indeed, this may be true of SOM in general (Kleber et al. 2010). These findings are important

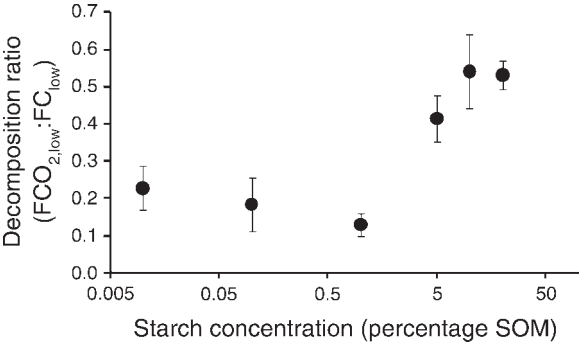

FIG. 5. Decomposition ratio plotted as a function of starch concentration for the laboratory microcosm experiment ($FCO_{2,low}$ = fraction of CO_2 C released from the low-concentration substrate; FC_{low} = fraction of low-concentration substrate C added to the micrososms. An exponential model provided the best fit, showing a significant relationship between starch concentration and decomposition ratio ($F_{1,21} = 15.18$, $P = 0.008$, $R^2 = 0.42$). Values are means ± SE. Cellulose comprised the remainder of the SOM in each microcosm.

TABLE 2. Cumulative CO_2 respiration as a function of starch concentration across a nine-week laboratory microcosm experiment.

Starch concentration (percentage of SOM)	Cellulose concentration (percentage of SOM)	CO_2 respired (μg)
0.00	100.00	52.65 ± 10.88
0.01	99.99	77.86 ± 10.82
0.10	99.90	84.42 ± 11.21
1.00	99.00	104.81 ± 15.00
5.00	95.00	57.50 ± 6.34
10.00	90.00	49.72 ± 10.81
20.00	80.00	55.34 ± 3.04
80.00	20.00	39.39 ± 9.75
90.00	10.00	45.51 ± 3.23
95.00	5.00	41.99 ± 8.18
99.00	1.00	28.12 ± 4.04
99.90	0.10	26.16 ± 6.68
99.99	0.01	46.66 ± 8.68

Notes: Values are means ± SE ($n = 4$); SOM is soil organic matter. A logarithmic model was used to examine the relationship between starch concentration and CO_2 production, which was significant ($F_{1,47} = 35.26$, $P < 0.001$, $R^2 = 0.64$).

because they imply that factors such as physical protection, enzymatic constraints, and soil microenvironment may influence SOM stability more than chemical recalcitrance (Schimel and Weintraub 2003, Ekschmitt et al. 2005, Kleber 2010). Given that the bulk of SOM is considered "stabilized" (von Lützow and Kögel-Knabner 2009), it is imperative to identify the full complement of mechanisms that lead to stabilization (von Lützow et al. 2006, Kleber 2010). Our data on starch degradation suggest that it is possible for common (but not necessarily abundant) polymers, like starch, to be stabilized in SOM based on concentration alone. Furthermore, based on the laboratory microcosm experiments, it appears that within our experimental design, starch concentrations >10% of total SOM (or 10 mg/g dry soil) may be necessary to induce greater α-glucosidase activity. Given the heterogeneity of SOM (Allison 2006), microbes may rarely encounter starch concentrations this high. For example, starch concentrations are <1% of total SOM in boreal forest soil near our sampling site (S. D. Allison, *unpublished data*).

Fungi are the primary decomposers in the soil environment (Paul and Clark 1996) and, similar to animals, fungi use glycogen (a branched form of starch) as a storage polysaccharide (Smith and Read 2008). Thus, at least intracellularly, most fungi likely metabolize starch, but it is clearly not abundant extracellularly within the soil matrix. Hence, low α-glucosidase activities coupled with low starch inputs may allow C substrates like starch to persist in SOM, even if they do not contain chemically "recalcitrant" structures (Kleber 2010). This observation is also consistent with Schimel and Weintraub's (2003) prediction that enzyme saturation may be more important in limiting C flow from a substrate than the chemical recalcitrance of the substrate itself.

Although similar patterns of increasing starch decomposition with increasing starch concentrations were observed in the field and in the laboratory, the two experiments showed different concentration thresholds for decomposition. For instance, rates of starch degradation rapidly declined when starch concentrations fell below 1% of SOM in the field cores, but this threshold was <10% in the laboratory microcosms. This difference in threshold value is likely due to the different environments inherent in the two experiments. The field cores were inoculated at the beginning of the field incubation, but they were open to additional microbial invasions during the course of the experiment. This may have allowed for more microorganisms specializing in starch decomposition to colonize the cores over time. On the other hand, the laboratory microcosms were inoculated once and were closed systems, only allowing microbes present in the soil inoculum to establish in the microcosms. The inoculum may have contained few starch degrading taxa, and thus, higher starch concentrations were required for starch degraders to establish and produce α-glucosidase. This explanation is corroborated by the observation that the decomposition ratios of the microcosms were below one for all starch concentrations, whereas they only fell below one at starch concentrations of <1% in the field.

Starch can be a common component of plant litter (Mooney 1972), but it may be used by a small subset of the microbial decomposer community (Schimel et al. 1992, Prescott and McDonald 1994). This suggestion is supported by our observation that α-glucosidase activities were two orders of magnitude lower than cellulolytic and hemicellulolytic activities, a pattern that has also been observed in other habitats (Tabatabai 1994, Gutknecht et al. 2010, Hernandez and Hobbie

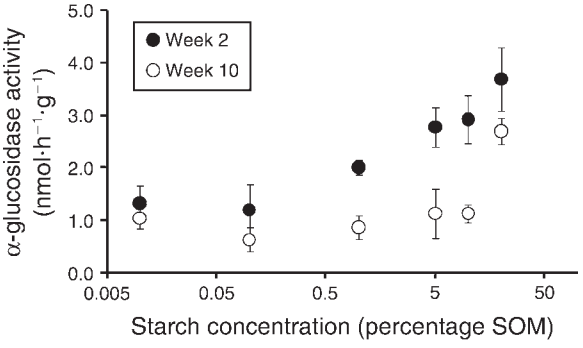

FIG. 6. The α-glucosidase activity as a function of starch concentration from the laboratory microcosm experiment. Values are means ± SE. The logarithmic regression among activity and starch concentration was significant for week 2 ($F_{1,23} = 23.34$, $P < 0.001$, $R^2 = 0.52$) and week 10 ($F_{1,23} = 29.22$, $P < 0.001$, $R^2 = 0.57$), and the α-glucosidase activity in the 20% starch concentration was significantly higher than this activity at the lowest starch concentrations at both time intervals ($P < 0.001$). Cellulose comprised the remainder of the SOM in each microcosm.

PLATE 1. Photos of the field site at Donnelly Flats, Fort Greely, Alaska: (top) view of Donnelly Flats from a bluff showing the boreal forest below and the Granite Peaks in the distance; (bottom) a soil core (with 1-mm mesh screen on top to prevent debris from entering the core) incubating in the ground during the growing season. Photo credits: top, S. D. Allison; bottom, D. P. German.

2010). Cellulose and hemicellulose, on the other hand, compose approximately 40% and 10% of litter mass, respectively (Berg and McClaugherty 2008), and together compose at least 25% of SOM in boreal forest soil near our sampling site (S.D. Allison, *unpublished data*). Given the abundance of cellulose and hemicellulose, many microbial decomposers may preferentially degrade these polymers when present at any concentration, as long as they are within a favorable environment (Ekschmitt et al. 2005). Detectable β-glucosidase (~18 nmol·h^{-1}·g^{-1}) and cellobiohydrolase (~1 nmol·h^{-1}·g^{-1}) activities in microcosms and field cores containing only starch and no cellulose (data not shown) support this contention. In addition, Hernandez and Hobbie (2010) found that β-glucosidase activities were similar in microcosms containing either starch or cellulose as the main C source.

In support of the resource allocation hypothesis, β-glucosidase and cellobiohydrolase activities decreased with decreasing cellulose concentration in the field cores. Consistent with these enzymatic patterns, the 100% cellulose treatments (mean ± SD; 19.41% ± 2.24% mass loss) lost significantly more SOM ($t = 6.33$, $P = 0.001$, df = 6) than the 0.01% cellulose treatments (4.39% ± 4.19% mass loss). However, the overall regression of SOM mass loss on cellulose concentration was not significant. Thus, cellulose-degrading enzyme activities matched differences in SOM mass loss at the extreme ends of our treatments, but not across the range of substrate concentrations tested in the field cores. This variation may reflect the difficulty of accurately measuring extracellular enzymes in soil, where potential enzyme activities do not always correlate with decomposition rates (Allison and Vitousek 2004, Allison et al. 2007, Hernandez and Hobbie 2010). Factors such as enzyme stabilization on mineral surfaces, spatial separation of enzymes from substrates, and microscale heterogeneity often reduce the correspondence between potential activity and decay rates (Burns 1982, Tabatabai 1994).

Total respiration in laboratory microcosms increased with hemicellulose concentration, and ^{13}C-labeled hemicellulose was mineralized at a rate higher than predicted by concentration alone at all hemicellulose concentrations tested. This high level of hemicellulose degradation may be explained by the consistent high level of β-xylosidase activities observed in the microcosms. Therefore, in contrast with starch, extremely low concentrations of cellulose or hemicellulose would be required for stabilization of these substrates in SOM. Because >90% of the ~12 000 fulvic acid constituents found in soil from our field site exist at relative abundances of <0.00017 (S. D. Allison, *unpublished FT-ICRMS data*), low concentration may remain a potential stabilizing mechanism for cellulose and hemicellulose, or at least for their degradation products (Kelleher and Simpson 2006). This potential remains to be tested.

In conclusion, we found support for the hypothesis that the decomposition rate of starch in soil changes with substrate concentration. Constrained decomposition at low concentration may be particularly important for substrates that only induce increased enzyme expression at relatively high (>10% of SOM) concentrations, as we observed for α-glucosidase activities and starch. We only investigated a small number of chemical compounds, but low concentrations could limit the decomposition of other low-concentration plant-derived

SOM constituents such as mannan and its degradation products and polysaccharides containing L-rhamnose. Kleber et al. (2010) suggest that the turnover of SOM is best viewed as the interaction between substrates, microbial decomposers, and abiotic factors. Our data demonstrate a potential constraint on decomposition that arises because of limits on microbial allocation of extracellular digestive enzymes that target SOM substrates low in concentration. In the context of global change, warming is predicted to reduce microbial decomposer carbon use efficiency, which has the effect of reducing microbial biomass and extracellular enzyme expression in soils (Allison et al. 2010). Hence, in concert with the predictions of Allison et al. (2010), our study suggests that a decrease in extracellular enzyme expression could further stabilize SOM, and low-concentration organic C substrates in particular.

Acknowledgments

The authors thank Jennifer Talbot for help in the field and for providing critical feedback on earlier versions of the manuscript, and the UCI EEB Microbial Group for valuable comments on the study design. This project was funded by a University of California President's Postdoctoral Fellowship (to D. P. German), UC–Irvine University Research Opportunity Program and Minority Biomedical Research Support (to S. S. Chacon), and a National Science Foundation Advancing Theory in Biology grant (to S. D. Allison).

Literature Cited

Allison, S. D. 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Letters 8:626–635.

Allison, S. D. 2006. Brown ground: a soil carbon analogue for the Green World Hypothesis? American Naturalist 167:619–627.

Allison, S. D., T. B. Gartner, K. Holland, M. Weintraub, and R. L. Sinsabaugh. 2007. Soil enzymes: linking proteomics and ecological process. Pages 704–711 *in* C. J. Hurst, R. L. Crawford, J. L. Garland, D. A. Lipson, A. L. Mills, and L. D. Stetzenbach, editors. Manual of environmental microbiology. Third edition. ASM Press, Washington, D.C., USA.

Allison, S. D., D. S. LeBauer, M. R. Ofrecio, R. Reyes, A. M. Ta, and T. M. Trin. 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biology and Biochemistry 41:293–302.

Allison, S. D., and K. K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology 14:2898–2909.

Allison, S. D., and P. M. Vitousek. 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36:285–296.

Allison, S. D., M. D. Wallenstein, and M. A. Bradford. 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3:336–340.

Bardgett, R. D., C. Freeman, and N. J. Ostle. 2008. Microbial contributions to climate change through carbon cycle feedbacks. The ISME Journal 2:805–814.

Berg, B., and C. McClaugherty. 2008. Plant litter: decomposition, humus formation, and carbon sequesteration. Springer-Verlag, Berlin, Germany.

Bol, R., N. Poirier, J. Balesdent, and G. Gleixner. 2009. Molecular turnover time of soil organic matter in particle-size fractions of an arable soil. Rapid Communications in Mass Spectrometry 23:2551–2558.

Burns, R. G. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology and Biochemistry 14:423–427.

DeForest, J. L. 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biology and Biochemistry 41:1180–1186.

Dekel, E., and U. Alon. 2005. Optimality and evolutionary tuning of the expression level of a protein. Nature 436:588–592.

Ekschmitt, K., M. Liu, S. Vetter, O. Fox, and V. Wolters. 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.

Friedlingstein, P., et al. 2006. Climate-carbon cycle feedback analysis: results from the C4MIP model intercomparison. Journal of Climate 19:3337–3353.

Grandy, A. S., and J. C. Neff. 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of the Total Environment 404:297–307.

Gutknecht, J. L. M., H. A. L. Henry, and T. C. Balser. 2010. Inter-annual variation in soil extra-cellular enzyme activity in response to simulated global change and fire disturbance. Pedobiologia 53:283–293.

Hayes, M. H. B., P. MacCarthy, R. L. Malcolm, and R. S. Swift. 1989. Humic substances II. In search of structure. Wiley-Interscience, Chichester, UK.

Hernandez, D. L., and S. E. Hobbie. 2010. The effects of substrate composition, quantity, and diversity on microbial activity. Plant and Soil 335:397–411.

Hyvönen, R., et al. 2007. The likely impact of elevated [CO_2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. New Phytologist 173:463–480.

Jobbágy, E. G., and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10:423–436.

Kelleher, B. P., and A. J. Simpson. 2006. Humic substances in soils: are they really chemically distinct? Environmental Science and Technology 40:4605–4611.

King, S., J. Harden, K. L. Manies, J. Munster, and L. D. White. 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, California, USA.

Kleber, M. 2010. What is recalcitrant soil organic matter? Environmental Chemistry 7:320–332.

Kleber, M., P. S. Nico, A. Plante, T. Filley, M. Kramer, C. Swanston, and P. Sollins. 2010. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. Global Change Biology 17:1097–1107.

Kleber, M., P. Sollins, and R. Sutton. 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85:9–24.

Koch, A. L. 1985. The macroeconomics of bacterial growth. Pages 1–42 *in* M. Fletcher, and G. D. Floodgate, editors. Bacteria in their natural environments. Academic Press, London, UK.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304:1623–1627.

Lehmann, J., D. Solomon, J. Kinyangi, L. Dathe, S. Wirick, and C. Jacobsen. 2008. Spatial complexity of soil organic matter forms at nanometre scales. Nature Geoscience 1:238–242.

Mellilo, J. M., P. A. Steudler, J. D. Aber, K. Newkirk, H. Lux, F. P. Bowles, C. Catricala, A. Magill, T. Ahrens, and S.

Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298:2173–2176.
Mooney, H. A. 1972. The carbon balance of plants. Annual Review of Ecology and Systematics 3:315–346.
Orlov, D. S. 1990. Soil humic acids and general theory of humification. Moscow State University Publisher, Moscow, Russia.
Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of factors controlling soil organic-matter levels in great-plains grasslands. Soil Science Society of America Journal 51:1173–1179.
Paul, E. A., and F. E. Clark. 1996. Soil microbiology and biochemistry. Second edition. Academic Press, San Diego, California, USA.
Potter, C. S., J. T. Randerson, C. B. Field, P. A. Matson, P. M. Vitousek, H. A. Mooney, and S. A. Klooster. 1993. Terrestrial ecosystem production: a process model based on global satellite and surface data. Global Biogeochemical Cycles 7:811–841.
Prescott, C. E., and M. A. McDonald. 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. Canadian Journal of Forest Research 24:2432–2438.
Richter, D. D., K. P. O'Neill, and E. S. Kasischke. 2000. Postfire stimulation of microbial decomposition in black spruce (*Picea mariana* L.) forest soils: a hypothesis. Pages 197–213 *in* E. S. Kasischke, and B. J. Stocks, editors. Fire, climate change, and carbon cycling in the boreal forest. Springer-Verlag, New York, New York, USA.
Schimel, J. P., S. Helfer, and I. J. Alexander. 1992. Effects of starch additions on N turnover in Sitka spruce forest floor. Plant and Soil 139:139–143.
Schimel, J. P., and M. N. Weintraub. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35:549–563.
Sinsabaugh, R. L., R. K. Antibus, and A. E. Linkins. 1991. An enzymic approach to the analysis of microbial activity during plant litter decomposition. Agriculture, Ecosystems and Environment 34:43–54.
Skujins, J. J. 1976. History of abiontic soil enzyme research. Pages 1–49 *in* R. G. Burns, editor. Soil enzymes. Academic Press, London, UK.
Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis. Third edition. Academic Press, San Diego, California, USA.
Sollins, P., P. Homann, and B. A. Caldwel. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74:65–105.
Tabatabai, M. 1994. Soil enzymes. Pages 775–833 *in* R. W. Weaver, S. Angle, and P. Bottomley, editors. Methods of soil analysis. Part 2: microbiological and biochemical properties. Soil Science Society of America, Madison, Wisconsin, USA.
Tarnocai, C., J. G. Canadell, E. A. G. Schuur, P. Kuhry, G. Mazhitova, and S. Zimov. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochemical Cycles 23:GB2023.
Treseder, K. K., M. C. Mack, and A. Cross. 2004. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecological Applications 14:1826–1838.
Trumbore, S. E. 1997. Potential responses of soil organic carbon to global environmental change. Proceedings of the National Academy of Sciences USA 94:8284–8291.
von Lützow, M., and I. Kögel-Knabner. 2009. Temperature sensitivity of soil organic matter decomposition—what do we know? Biology and Fertility of Soils 46:1–15.
von Lützow, M., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions: a review. European Journal of Soil Science 57:426–445.
Zimmerman, A. R., J. Chorover, K. W. Goyne, and S. L. Brantley. 2004. Protection of mesopore-adsorbed organic matter from enzymatic degradation. Environmental Science and Technology 38:4542–4548.