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Highlights

- Lower percent C respired from starch, cellulose, and chitin at lower concentrations.
- Also lower percent C respired from substrate mixtures at lower concentrations.
- Respiration pattern for cellulose and chitin coincided with lower enzyme activities.
- Concentration dependence of respiration suggests soil carbon models need revision.

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Substrate concentration constraints on microbial decomposition

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ABSTRACT

Soil organic carbon is chemically heterogeneous, and microbial decomposers face a physiological challenge in metabolizing the diverse array of compounds present in soil. Different classes of polymeric compounds may require specialized enzymatic pathways for degradation, each of which requires an investment of microbial resources. Here we tested the resource allocation hypothesis, which posits that decomposition rates should increase once substrate concentrations are sufficient to overcome biochemical investment costs. We also tested the alternative hypothesis that mixing different substrates increases resource acquisition through priming effects involving generalist enzymes. Using a microcosm approach, we varied the soil concentration of seven distinct substrates individually and in mixture. We found that the percent carbon respired from starch, cellulose, chitin, and the mixture was significantly reduced at the lowest substrate concentration. The activities of β-glucosidase and N-acetyl-glucosaminidase that target cellulose and chitin, respectively, were also significantly lower at the lowest concentrations of their target substrates. However, we did not observe parallel declines in enzyme activity with starch or the mixture. Some enzymes, such as β-xylosidase, were consistent with specialist strategies because they showed the highest activity in the presence of their target substrate. Other enzymes were more generalist, with activity observed across multiple substrates. Together, these results suggest that the costs of biochemical machinery limit microbial decomposition of substrates at low concentration. The presence of enzymes with low substrate specificity was not sufficient to overcome this constraint for some substrates. Concentration constraints driven by microbial allocation patterns may be common in mineral soil and could be represented in new biogeochemical models based on microbial physiology.

1. Introduction

Soil holds the largest terrestrial organic carbon (C) reservoir (Gorham, 1991; Jobbágy and Jackson, 2000; Tarnocai et al., 2009). The majority of soil C is composed of polymeric biomolecules derived from plant and microbial metabolism (Kögel-Knabner, 2002). Overall concentrations of C in many soils are high, but soil C is chemically heterogeneous, and concentrations of specific chemical compounds are much lower (MacCarthy and Rice, 1991; Lehmann et al., 2008).

The decomposition of soil C compounds is controlled mainly by micro-organisms like bacteria and fungi (Swift et al., 1979; Schmidt et al., 2011). The chemical diversity of soil C means that these microbial decomposers face a fundamental tradeoff. They can either specialize and target a small number of chemical compounds or generalize and target a larger range of compounds (Nam et al., 2012). Specialization involves relatively little investment in biochemical machinery, but specialists can access only a fraction of the total resource pool. Generalists can access a broader range of resources but must synthesize and maintain a larger amount of biochemical machinery.

For generalists or specialists, the costs of resource acquisition must be offset by the resource flux from soil substrates (Koch, 1985; Dekel and Alon, 2005). For microbes decomposing polymeric soil compounds, these costs include extracellular enzyme synthesis. Enzymes are only beneficial if their substrates are available in high enough concentration to offset the costs of enzyme production. If substrate concentrations are too low, then enzyme production is not economical. Assuming there are no other enzymes that degrade the substrate, its decay rate should decline at sufficiently low concentrations due to lack of enzyme activity.

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Previous work found support for this *resource allocation hypothesis*, whereby starch decomposition rates were significantly reduced at lower starch concentrations (German et al., 2011a). However, the hypothesis was not supported with cellulose, which decomposed at the same rate regardless of concentration. Although starch and cellulose were mixed together in German et al. (2011a), it is not clear if the same results would be obtained in more complex mixtures more typical of soil organic matter. Differences among starch and cellulose responses also raise the question of whether the resource allocation hypothesis applies to soil compounds other than starch. If so, conventional models of the soil C cycle might need to be revised because they assume that decay rates for soil organic C depend on substrate chemistry but not substrate concentration (Todd-Brown et al., 2012).

Our goal here was to test the underlying enzymatic mechanism of the resource allocation hypothesis. We frame this mechanism as the *substrate induction hypothesis*, which postulates that higher substrate concentrations increase associated enzyme activity per unit of substrate. Increases in microbial biomass, enzyme production, or specific enzyme activity could all contribute to this relationship. As a result, the fraction of C respired from the substrate should increase with increasing substrate concentration. If microbes specialize on particular substrates and enzymes have high substrate specificity, the substrate induction hypothesis should apply equally to substrates alone or mixed together.

As an alternative, we propose the *priming effect hypothesis*. Under this hypothesis, mixing substrates together would increase microbial respiration beyond the sum of respiration from individual pure substrates (Fontaine et al., 2004; Thiessen et al., 2013). Two mechanisms could contribute to this hypothetical pattern: constitutive enzyme production and enzyme promiscuity. Constitutive enzymes are produced even if they do not contribute to the degradation of a particular substrate. However, a constitutively-produced enzyme could catalyze degradation of its target substrate in a mixture, thereby increasing total substrate degradation by the enzyme producer. A similar phenomenon would occur if enzymes are active against multiple substrates. These promiscuous enzymes would contribute to additional degradation when there are multiple substrates in a mixture. We tested the substrate induction and priming effect hypotheses by measuring CO_2 respiration and extracellular enzyme activities in laboratory microcosms with substrates added in pure form and in mixtures.

2. Materials and methods

2.1. Laboratory microcosms

Soil was collected by auger to a depth of 10 cm from a temperate grassland ecosystem at Loma Ridge, Irvine, CA (33° 44′ N, 117° 42′W). The soil is classified as fine-loamy, mixed, superactive, thermic Typic Palexeralfs with a pH of 6.8 (German et al., 2012). Soils were combusted at 550 °C for 3 h to remove all organic matter while retaining the mineral material (German et al., 2011a). This treatment probably increased soil sorption potential by exposing mineral surfaces (Qualls, 2000). Microcosms consisted of septum-capped 40 ml vials containing 2 g combusted soil, substrates at varying concentrations, and 800 μl of microbial inoculum created by diluting (1:1000 w:v) fresh soil in a sterile, enriched nutrient solution. The enriched nutrient solution was made following the minimal nutrient medium of Allison et al. (2009), with the exception that we added 2 mg P ml^{-1} and 3 mg N ml^{-1} as K_2HPO_4 and NH_4NO_3, respectively, to provide excess P and N to all substrate treatments. Some of the substrates (i.e., chitin, protein, and DNA) would have otherwise provided more P and/or N than others, so additional P and N were added to avoid differential nutrient limitation across substrates.

To test the substrate induction hypothesis, we measured the percent C respired from 7 pure substrates commonly found in soils: lignin, starch, cellulose, xylan, chitin, DNA, and protein. Microcosms contained 10, 4, or 1 mg of each substrate (Fig. 1). To test the priming effect hypothesis, we used a mixture treatment that contained each of the 7 substrates added at 1/7 of their concentrations in the pure substrate microcosms (Fig. 1). Thus, the mixture treatment contained the same total substrate mass as the individual substrate treatments.

2.2. Microbial respiration

To quantify substrate degradation and mineralization, CO_2 concentrations in the microcosms were measured every 7 days, and the concentrations were used to calculate cumulative CO_2 respiration over a 10-week incubation period. Microcosms ($n = 6$ for each substrate and concentration) were incubated at 22 °C, which is 5 °C warmer than the mean annual temperature of the Loma Ridge grassland ecosystem (German et al., 2012). For each gas measurement, an 8 ml subsample of headspace gas was withdrawn by syringe and injected into an infrared gas analyzer (PP-Systems EGM-4). After measurement, vials were opened under sterile conditions, equilibrated with ambient air for ~5 min, and then closed. The CO_2 concentrations of blank vials were subtracted from sample vials to calculate cumulative respiration of substrate C. CO_2 concentrations in most vials never exceeded 1000 ppm, and only some cellulose and xylan vials briefly reached >5000 ppm, meaning that the microcosms were probably never anaerobic.

2.3. Extracellular enzyme activities

Microcosms were established in the same manner as described for the CO_2 measurements and were vented every 7 days under

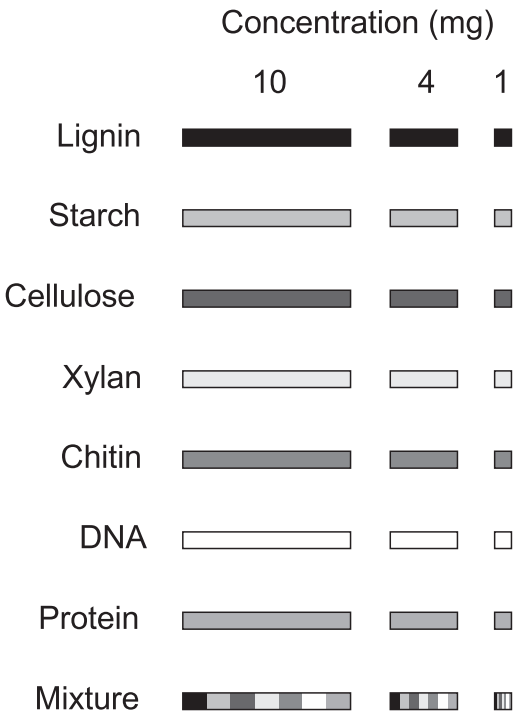

Fig. 1. Microcosm experimental design. Each bar represents one microcosm replicate with indicated substrate. Mixture microcosms contain equal amounts of all substrates, with a total substrate addition that is equal to the amount of the pure substrates.

sterile conditions to maintain an aerobic environment. Microcosms from each treatment (n = 4–6 for each substrate and concentration) were frozen at −20 °C after incubating for 2, 5, and 10 weeks. The activity levels of extracellular enzymes were measured in the microcosms within 3–4 months of being frozen. Although freezing can affect enzyme activity levels (Lee et al., 2007), all samples experienced the same freezing conditions, meaning that comparisons among treatments should be valid. Microcosms were prepared for enzyme assays by dispersing the entire contents of the vials in 60 ml ice cold 25 mM maleate buffer, pH 6.8 (German et al., 2012).

The oxidative enzymes, polyphenol oxidase and peroxidase, were measured following Bach et al. (2013) (Table 1). L-DOPA substrate (50 μl) was combined in each sample well with 200 μl soil homogenate. Homogenate control wells received 50 μl water and 200 μl soil homogenate. Substrate control wells received 50 μl L-DOPA substrate and 200 μl maleate buffer. For the peroxidase assay, sample and control wells also received 10 μl 0.3% hydrogen peroxide. There were 8 replicate wells for each sample and control. Samples were incubated in covered clear microplates for 1 h at 22 °C. The sediment in the soil homogenates settled to the bottom of the microplate during incubation, which interferes with absorbance readings in a spectrophotometer (Allison and Vitousek, 2004). Thus, following the incubation period, 150 μl of the control and assay solutions were transferred to empty wells (without sediment), and absorbance was measured at 460 nm. The micromolar extinction coefficient of the L-DOPA oxidation product (2-carboxy-2,3-dihydroindole-5,6-quinone) was determined to be 5.3, which is similar to published values (German et al., 2011b; Bach et al., 2013). This value was used to convert absorbance into enzyme activity.

The activities of the hydrolytic enzymes α-glucosidase, cellobiohydrolase, β-glucosidase, β-xylosidase, N-acetyl-β-D-glucosaminidase, acid phosphatase, and leucine aminopeptidase were assayed in microcosm homogenates using the fluorometric protocols outlined by German et al. (2011b) (Table 1). Fluorometric substrate solution (50 μl) was combined with 200 μl soil homogenate in a black microplate and incubated for 1 h at 22 °C. The reaction was stopped by the addition of 10 μl of 1 M NaOH, and the amount of fluorescence was determined immediately in a fluorometer (Biotek Synergy 4, Winooski, VT, USA) at 360 nm excitation and 460 nm emission. The each enzyme assay was replicated 8 times in each plate, and each plate included a known standard of the product (4-methylumbelliferone; MUB), substrate controls, and homogenate controls.

Enzymatic activity (nmol product released h^{-1} g^{-1} C) was calculated following German et al. (2011b). All reactions were run at saturating substrate concentrations as determined for each enzyme with soils from Loma Ridge, and linearity of the reactions was confirmed for the 1 h assay duration. Activities were expressed per unit initial substrate C as determined from the percent C by mass in each substrate (Sterner and Elser, 2002).

2.4. Statistics

CO_2 concentrations from each vial were converted to mg C, and cumulative C respired during the 10-week incubation was determined. The data were expressed as the amount of C respired relative to the amount of initial C present in each microcosm (i.e., percent C respired). To evaluate substrate effects on respiration, the percent C respired data were square root transformed and analyzed by ANOVA with substrate, substrate concentration, and their interaction as main effects. Treatment means were compared with Tukey post-hoc contrasts. To test whether respiration fractions differed for substrates alone versus in mixture, the average percent C respired across the 7 pure substrates at each substrate addition level was compared to the percent C respired from the mixture treatment at the same substrate addition level. This comparison was performed by propagating the standard error on each of the single substrate means to obtain a 95% confidence interval (CI) for the overall mean. This CI was checked for overlap with the 95% CI of the 7-substrate mixture mean.

According to the substrate induction hypothesis, enzymes should show greater activity in the presence of an appropriate substrate. Thus, we used ANOVA on log-transformed enzyme activity levels with substrate, substrate concentration, sampling date, and all of their interactions as main effects. Enzyme activities were compared among substrates and among substrate concentrations with Tukey post-hoc contrasts.

3. Results

3.1. Percent carbon respired

We found significant interactions between substrate concentration and substrate type in the respiration analysis ($F_{14,120}$ = 2.716, P = 0.0017). Post-hoc tests showed significant differences among substrate concentrations for starch, cellulose, chitin, and the mixture treatment (Fig. 2). For each of these substrate treatments, a significantly greater percentage of C was respired from the 10 mg substrate concentration in comparison to the 1 mg substrate concentration. For chitin, a greater C percentage was also respired from the 4 mg substrate concentration compared to the 1 mg substrate concentration, but this was not the case for the other substrates (Fig. 2). There were no significant differences between the percent C respired from the mixture and the average of the pure compounds at any concentration level. The mixture means (±95% CI) were 2.7 ± 0.7, 3.8 ± 0.8, and 4.4 ± 1.3, and the pure compound means were 4.0 ± 3.5, 4.3 ± 2.7, and 4.6 ± 3.0 for the 1, 4, and 10 mg concentrations, respectively.

Table 1
Extracellular enzymes assayed in soil microcosms, including their functions, corresponding substrates, and substrate concentrations in the wells. L-DOPA = l-3,4-dihydroxyphenylalanine; MUB = methylumbelliferone.

Enzyme	Abbreviation	Enzyme function	Substrate	Substrate concentration
Polyphenol oxidase	PPO	Degrades lignin, aromatic polymers	L-DOPA	1000 μM
Peroxidase	PER	Catalyzes oxidation reactions	L-DOPA	1000 μM
α-1,4-glucosidase	AG	Starch degradation	4-MUB-α-D-glucoside	200 μM
Cellobiohydrolase	CBH	Cellulose degradation	4-MUB-β-D-cellobioside	200 μM
β-1,4-glucosidase	BG	Cellulose degradation	4-MUB-β-D-glucoside	400 μM
β-1,4-xylosidase	BX	Hemicellulose degradation	4-MUB-β-D-xyloside	400 μM
β-1,4-N-acetyl-glucosaminidase	NAG	Chitin degradation	4-MUB-N-acetyl-β-D-glucosaminide	400 μM
Acid phosphatase	AP	Mineralizes organic P into phosphate	4-MUB-phosphate	800 μM
Leucine amino peptidase	LAP	Peptide degradation	L-Leucine-7-amino-4-methylcoumarin	200 μM

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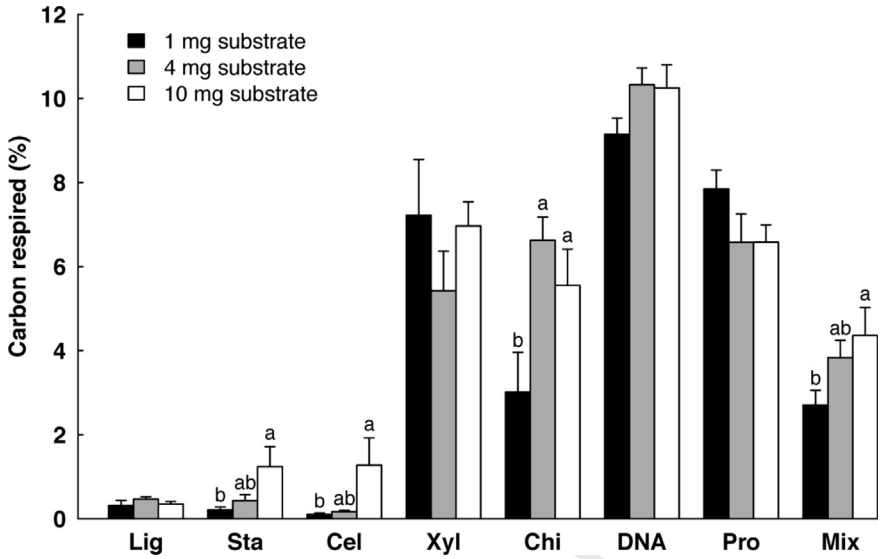

Fig. 2. Substrate respiration rates. Mean ± SE percent initial carbon respired after 10 weeks for each of the substrates ($n = 6$). Bars with the same letter or no letters within a substrate are not significantly different ($P > 0.05$, Tukey post-hoc tests on square root-transformed data).

3.2. Extracellular enzyme activities

With the exception of PER ($F_{7,339} = 1.862$, $P = 0.075$), all enzyme activities showed significant responses to substrate type ($P < 0.001$, except $P = 0.011$ for PPO; Fig. 3). Consistent with the substrate induction hypothesis, BX, NAG, AP, and LAP activities were greatest in microcosms containing their target substrates in pure form (Fig. 3). Aside from BX, though, these differences were not significantly significant at $P < 0.05$ (e.g. LAP activities were equally high in chitin and protein treatments, Fig. 3I). Other enzymes were not preferentially induced by their target substrates. AG activities were greater in DNA and protein treatments than in the starch treatment (Fig. 3C). The highest activities of CBH and BG were not in the cellulose treatment, but in the xylan and mixture treatments, respectively (Fig. 3D–E).

Although BG activity was not highest in the cellulose treatment, BG activities showed a strong interaction between substrate type and substrate concentration ($F_{14,346} = 7.027$, $P < 0.001$). Consistent with percent C respired (Fig. 2), post-hoc tests showed that BG activities were highest at the 10 mg concentration ($P = 0.024$) with cellulose as the substrate (Fig. 4A). In contrast, BG activities were lowest ($P < 0.001$) at the 10 mg concentration in the mixture treatment. NAG activities showed a similar interaction between substrate type and substrate concentration ($F_{14,346} = 2.109$, $P = 0.011$). NAG activity was highest in the 10 mg chitin treatment (Fig. 4B), also consistent with the respiration response (Fig. 2). However, NAG activity showed the opposite pattern in the mixture treatment. AG ($F_{14,346} = 2.268$, $P = 0.006$) and BX ($F_{14,351} = 3.127$, $P = 0.001$) also showed significant interactions between substrate type and substrate concentration, but the enzyme responses on the target substrates starch and xylan, respectively, were not clearly related to respiration responses. As with BG and NAG, all other enzymes showed a trend toward lower activities at higher concentrations of the mixture, with the trend significant for BX ($P = 0.004$ for 10 mg vs. 4 mg and $P < 0.001$ for 10 mg vs. 1 mg).

See Supplementary Datasets 1 and 2 in the online version of this article for all the data on percent C respired and enzyme activities.

4. Discussion

Our substrate induction hypothesis posited that percent C respired should increase with increasing substrate concentration. We found evidence for this hypothesis with some but not all substrates. Specifically, starch, cellulose, chitin, and the 7-substrate mixture all showed greater percent C respired at higher substrate concentrations (Fig. 2). In contrast, there was limited evidence to support the alternative priming effect hypothesis. Percent C respired from the mixture was not significantly greater than the average percent C respired from the pure substrates (Fig. 2). Although enzyme activities were often expressed on non-target substrates (Fig. 3), this lack of specificity did not prime substrate respiration in mixtures.

Substrate induction by starch is consistent with a prior analysis (German et al., 2011a), but our current analysis broadens this mechanism to a broader range of substrates. The previous study used isotope tracers to measure starch and cellulose degradation in soils from Alaskan boreal forest, but only found support for substrate induction with starch. Although our current study tested a similar hypothesis, there were differences in the experimental conditions and measurement techniques. Thus differences in methodology, soil properties, or microbial community composition could explain why we observed concentration effects on mineralization with more substrates in California soils.

Lower percent C respired in the 1 mg mixture suggests that increasing the diversity of chemical compounds does not alleviate concentration effects on substrate mineralization as expected under the priming hypothesis. Lower percent C respired in the 1 mg mixture probably reflects the average effect observed with individual substrates, including starch, cellulose, and chitin which showed the same pattern. However, all substrates in the mixture were 7-fold lower in concentration than in the individual treatments, so concentration constraints might have affected additional substrates at a lower threshold. Together, our results show that the total concentration of C, in addition to the concentrations of individual substrates, may constrain microbial respiration under the substrate induction hypothesis.

Given that we used relatively low substrate concentrations (<0.25% C by mass) and a mineral-rich soil matrix, our results might be more relevant for mineral soils than organic soils. Due to soil combustion, sorption might have limited enzyme–substrate interactions and facilitated substrate concentration constraints in our microcosms (Allison and Jastrow, 2006). On the other hand, mineral interactions may sometimes stimulate microbial growth and

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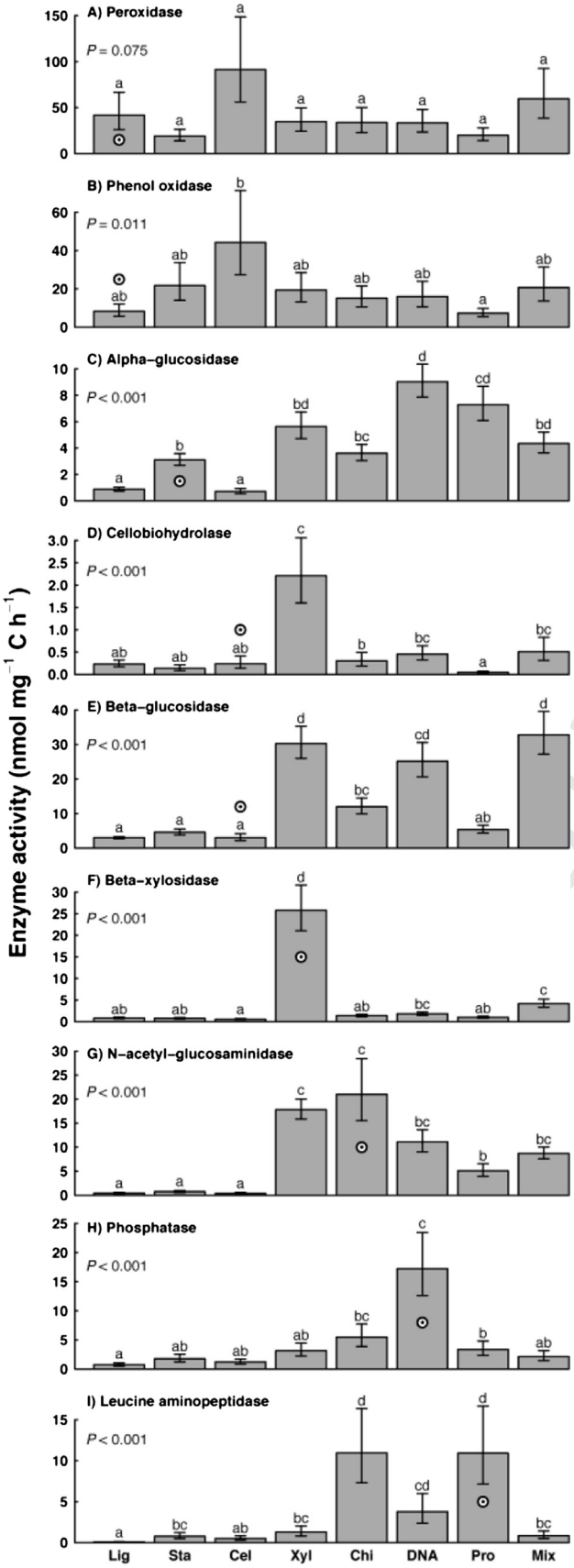

Fig. 3. Extracellular enzyme activities on different substrates. Mean ± SE enzyme activity per mg initial substrate carbon averaged over time and substrate concentration for each substrate (n = 36–54). P-values are from the overall ANOVA on log-transformed data. Means with the same letter are not significantly different ($P > 0.05$, Tukey post-hoc test). Target substrates are indicated with a target symbol. Chitin, DNA, and protein substrates contain nitrogen, and DNA also contains phosphorus. Other pure substrates are primarily carbon-based.

substrate metabolism (Van Loosdrecht et al., 1990). Also, if sorption were important in our experiment, we would have expected stronger concentration dependence for substrates with greater sorption potential, particularly protein (Kleber et al., 2007). Because we did not observe this pattern, sorption was probably not a major factor in our experiment. In any case, mineral soils store a large fraction of global soil C (Jobbágy and Jackson, 2000), and our results might help explain the ancient radiocarbon ages (>1000 yr) observed in many deep soil C pools (Trumbore, 2000).

In some cases, patterns in percent C respired were accompanied by comparable patterns in enzyme activity. BG and NAG both showed significantly lower potential activities per unit substrate C at lower concentrations of their target substrates (Fig. 4). This pattern suggests that microbes reduce investment in the biochemical machinery needed to degrade these substrates at low concentration, consistent with the substrate induction hypothesis. However, we did not observe concentration dependence for AG (Supplementary Dataset 2), even though percent C respired from starch was dependent on substrate concentration. Perhaps other steps in the intracellular pathway of starch metabolism were sensitive to starch concentration. Surprisingly, we observed higher enzyme activity at lower substrate concentrations for BG and NAG in the mixture, despite a lower percent C respired.

In the mixture treatment, it is possible that antagonistic interactions suppressed the activity of most enzymes at higher substrate concentrations. Previous studies have observed antagonistic interactions among bacteria and fungi as well as across bacterial populations (Mille-Lindblom et al., 2006; Romaní et al., 2006; Cordero et al., 2012). Compared to pure cellulose, C respiration was up to an order of magnitude higher in the mixture, suggesting a greater potential for microbial interaction. Mixing substrates stimulated BG activity compared to pure cellulose, particularly at the lowest substrate concentration (Fig. 4A). This enzymatic result is consistent with the priming effect hypothesis, although it did not translate into greater percent C respired. At the highest substrate concentration (10 mg), enzyme priming disappeared and BG activity was similar between pure cellulose and the mixture. This result suggests that BG activity was suppressed under high mixture concentrations, possibly due to competitive interactions or enzyme down-regulation. Similar responses may have occurred with NAG-producing microbes in the mixture (Fig. 4B), but there was no evidence for enzyme priming because NAG activity in the mixture was similar to activity in the lowest concentration of pure chitin.

The mechanism of concentration dependence in our experiment appears to be mediated through changes in enzyme production or V_{max} rather than through the enzyme half-saturation constant, K_m. Although substrate concentrations were relatively low in our experiment, they all exceeded the estimated K_m values of their associated enzymes. Measured K_m values ranged from 6.5 to 36 μmol l^{-1} for C-degrading enzymes in our field soil (German et al., 2012), which roughly corresponds to C concentrations less than 3 μg g^{-1}. Even in the 1 mg mixture treatment, each individual substrate concentration was >300 μg g^{-1}. Thus extracellular enzymes in our experiment were likely operating near V_{max}, and enzyme-mediated effects on respiration were probably due to lower investment in enzyme production. Note that our potential enzyme assays were conducted at saturating substrate concentrations and therefore reflect the total enzyme pool operating at V_{max}.

Our results are consistent with a combination of specialist and generalist strategies influencing enzyme activity. Perfect

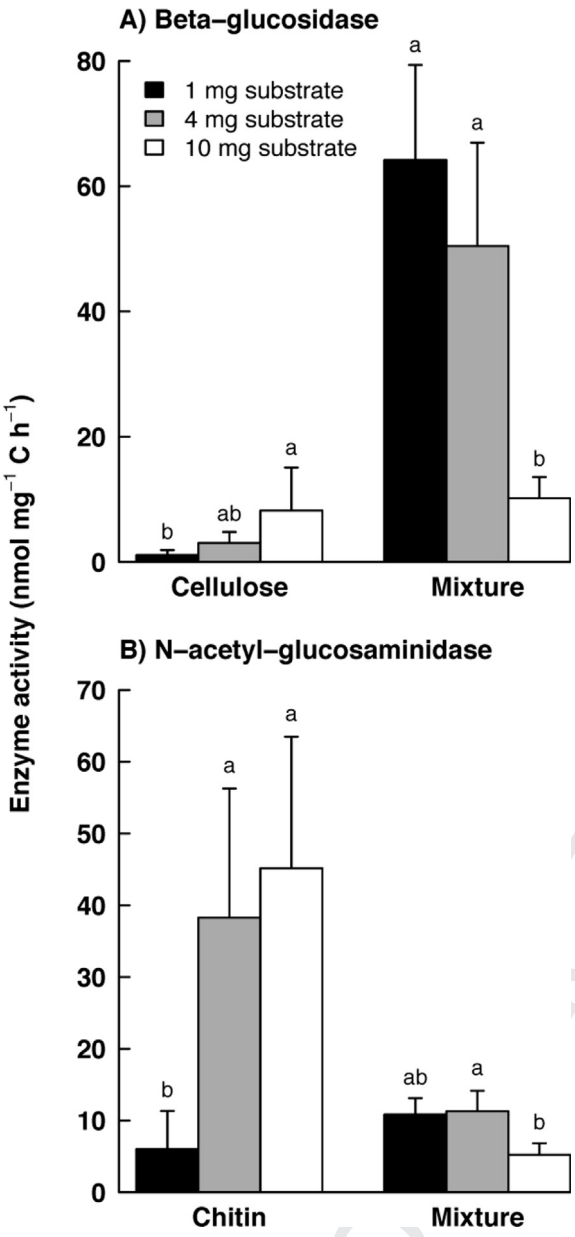

Fig. 4. Enzyme responses to concentration of selected substrates. Mean ± SE enzyme activity per mg initial substrate for A) β-glucosidase and B) N-acetyl-glucosaminidase on their target substrates and in the mixture ($n = 4–6$). Means with the same letter within a substrate are not significantly different ($P > 0.05$, Tukey post-hoc test on log-transformed data).

specialization would result in enzyme activity only in the presence of target substrates. For example, BX activity was markedly higher in the xylan treatment than in any other substrate treatment. Either BX-producing microbes are only active on xylan, or BX is regulated such that activity is only observed in the presence of target substrate. Thus xylan degradation may be a relatively narrow function carried out by microbial specialists (Schimel, 1995). In contrast, other enzyme activities were present across a range of substrates, consistent with more generalist strategies. AG and BG activities followed this pattern, at least in our California grassland communities. Yet our data also suggest that these activities are lower in substrate mixtures that support high levels of microbial activity.

In summary, our results suggest that low resource availability can constrain microbial investment in biochemical machinery, leading to lower fractional losses of substrate C. These results are relevant for predicting ecosystem responses to change in microbial substrate supply. Increases in substrate fluxes to soil, as might occur if elevated CO_2 increases net primary production (Norby et al., 2005), could lead to faster rates of substrate decomposition. This mechanism is distinct from the traditional priming effect, whereby increases in labile substrate inputs stimulate decomposition of more recalcitrant organic matter (Fontaine et al., 2007; Hungate et al., 2013). Rather, substrate induction occurs when substrate inputs reach a critical threshold sufficient to offset the metabolic costs of substrate acquisition.

Our findings have implications for biogeochemical models. Conventional models assume that substrate turnover is a function of chemical composition and climate conditions (Todd-Brown et al., 2012). In these models, higher substrate inputs correspond to greater soil C storage (Todd-Brown et al., 2013). In more recent microbial models, substrate loss rates are a function of microbial biomass, and soil C storage does not increase with greater substrate input (Allison et al., 2010). Adding substrate stimulates microbial activity and associated decomposition (Wieder et al., 2013). Our findings here support this feedback, as ten-fold increases in substrate concentration significantly increased percent C respired for several organic compounds and the substrate mixture. Thus our results are consistent with microbial but not conventional models and provide additional evidence that constraints on microbial physiology influence soil C cycling.

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Appendix A. Supplementary data

Supplementary data related to this chapter can be found at http://dx.doi.org/10.1016/j.soilbio.2014.08.021.

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