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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

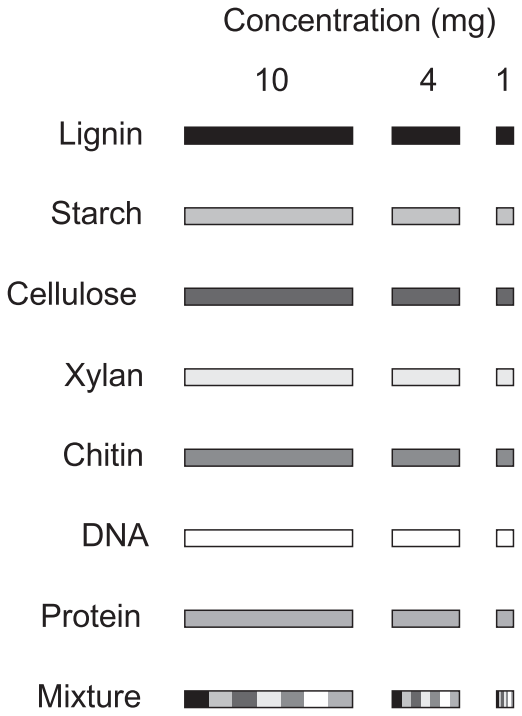

Fig. 1. Microcosm experimental design. Each bar represents one microcosm replicate with indicated substrate. Mixture microcosms contain equal amounts of all substrates, with a total substrate addition that is equal to the amount of the pure substrates.