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Control of soil phosphatase activities at millimeter scales in a mixed paper birch – Douglas-fir forest: The importance of carbon and nitrogen

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ABSTRACT

Organic P can serve as an important source of P for plants and microbes when mineralized by extracellular phosphatases. Substrate induction, end-product repression and/or resource limitation regulate activities of phosphatase in bulk soils. Yet, factors controlling enzyme activities in fine-scale microsites may differ from those observed at larger scales. Understanding such differences is needed to improve estimates of global models of biogeochemical cycling. Imprinting of soil profiles using cellulose sheets infused with chromogenic substrates allows study of extracellular enzymes at mm scales under naturally occurring soil temperatures, with minimal disturbance to soil microbial communities. In this study, we used a soil imprinting approach to investigate soil chemical characteristics associated with mm-scale regions of high *in situ* phosphatase activities in a mixed paper birch – Douglas-fir forest in the southern interior of British Columbia. In addition, we tested whether the addition of simple (ammonium chloride plus sodium acetate) and complex (cellulose, collagen, chitin) forms of carbon (C) and/or nitrogen (N) to 1 cm^2 microplots on soil profiles influenced *in situ* phosphatase activity. In unamended microplots, percent C was 30% higher on average ($P = 0.05$) and percent N was about 15% higher ($P = 0.05$) in high-phosphatase microsites. Extractable P did not differ between high and low-phosphatase microsites, regardless of the form of P measured. Within the first 24 h, no difference in imprintable phosphatase was observed between C and N addition treatments, but after 72 h, microplots receiving any substrate containing N had higher phosphatase activities than those receiving only water ($P < 0.001$). The results from both of our studies support a role for resource limitation in regulating phosphatase activities at this site because either (i) P became limiting in microsites with higher amounts of C and N, and/or (ii) microsites with higher C and N were the ones where these nutrients were in sufficient supply to allow microbes to excrete extracellular enzymes. We conclude that phosphatase excretion occurs in C + N-enriched soil microsites, but that any such phosphatase-active microsites located beyond the rhizosphere are unlikely to supply P to roots because of the low diffusion rates of orthophosphate.

1. Introduction

Phosphorus (P) plays an important role in driving primary productivity in terrestrial ecosystems. It is a structural building block of nucleic acids and phospholipids and is crucial to intracellular energy transfer in all organisms. Phosphorus initially enters the soil solution as soluble orthophosphate ions (PO_4^{-3}) after slow weathering of primary minerals (Filippelli, 2008). However, orthophosphate ions are highly reactive, and tend to co-precipitate with Fe, Ca, and Al, or become adsorbed onto the surfaces of soil particles (Gyaneshwar et al., 2002). The fraction of orthophosphate remaining in solution can be incorporated into organic matter after being taken up and assimilated by plants or microbes. Orthophosphate usually constitutes less than one percent of total phosphorus in soil at a given time (Sylvia et al., 2005) and, thus, P is one of the least available plant nutrients (Duff et al., 1994). Its availability in native soils is rarely adequate for optimal plant growth (Abel et al., 2002).

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Much of the P in soil, usually between 30 and 50%, (Sylvia et al., 2005), is sequestered in organic forms (Kogel-Knaber, 2006; Richardson et al., 2009). Plant roots, bacteria, and fungi produce a variety of extracellular phosphatases that mineralize organic P in soil, thereby releasing orthophosphate ions. In this manner, organic P can serve as an important source of P for plants or microbes (Tarafdar and Claassen, 1988; Hui et al., 2013). Phosphatases are classified as either acid or alkaline, based on their pH optimum (Vincent et al., 1992). Bacteria, fungi, and plant roots produce both acid and alkaline phosphatases (Tarafdar and Jungk, 1987; Duff et al., 1994; van Aarle and Plassard, 2010). Alkaline phosphatases are generally rather substrate specific (Duff et al., 1994), and are produced in lower quantities (Eivazi and Tabatabai, 1977; van Aarle and Plassard, 2010) than acid phosphatases. Since phosphomonoesters, such as phytate, comprise a significant portion of total organic P in soil (up to 70%; Richardson et al., 2009), acid phosphomonoesterases likely play a vital role in organic P mineralization, as well as mediating the overall availability of P to plants and microbes (Olander and Vitousek, 2000; Hui et al., 2013). Phosphodiesterases are important in some soils (Adams, 1992).

Some soil enzymes are constitutively produced at low concentrations, thereby allowing soil biota to detect the presence of macromolecules that can act as nutrient sources (Chrost, 1991; Allison and Vitousek, 2005); however, the excretion and/or activities of most extracellular enzymes appear to be regulated (see Geisseler et al., 2010; Sinsabaugh and Shah, 2012; Burns et al., 2013 for recent reviews). Substrate induction occurs when the presence of substrate induces enzyme activity. Conversely, end product repression occurs when a high concentration of the available form of the nutrient inhibits production of enzymes that release that nutrient from more complex substrates (Schimel et al., 1992; Allison and Vitousek, 2005). For example, addition of inorganic P suppresses activity of phosphomonoesterases in plant and microbial cells (Nannipieri et al., 2012).

Finally, under resource limitation, enzyme activity is stimulated when concentrations of the available form of the nutrient are low relative to other nutrients (Burns et al., 2013). For example orthophosphate limitation has been shown to stimulate phosphatase excretion by plant roots (Duff et al., 1994, and references therein), bacteria (Lenburg and O'Shea, 1996), and ectomycorrhizal fungi (van Aarle and Plassard, 2010). Such a limitation can arise either from inherently low concentrations of the nutrient or from relatively high levels of other nutrients, especially carbon (Schimel and Weintraub, 2003; Allison and Vitousek, 2005; Hernandez and Hobbie, 2010).

Although these general principals are well established, many regulatory processes in soil occur at very fine mm to sub-mm scales, making them difficult to measure (Nannipieri et al., 2012). Schimel and Bennett (2004) suggested that the ability of plants to compete with microbes for available nitrogen depends on the existence soil micro-sites, which are spatially distinct, yet ubiquitous, throughout the soil. Each micro-site has unique features, such as the availability of labile and complex substrates, which control rates of mobilization and assimilation in that site. Fine-scale edaphic features are likely important for P mineralization as well, but to our knowledge, they have not been investigated. Because micro-scale characteristics may regulate macro-scale processes related to ecosystem functioning, an understanding of P cycling at fine scales is needed to improve estimates of global models of biogeochemical cycling, such as CENTURY (Schimel and Bennett, 2004; Wang et al., 2010).

Traditional lab enzyme assays suffer from several limitations (reviewed by Wallenstein and Weintraub, 2008; Nannipieri et al., 2012), and may not directly reflect enzyme activities occurring *in situ*. Imprinting of soil profiles using cellulose sheets infused with chromogenic substrates overcomes some of these limitations by allowing for the study of spatio-temporal variation of extracellular enzymes at mm scales, under naturally occurring soil temperatures, and with minimal disturbance to soil microbial communities (Dinkelaker and Marschner, 1992; Grierson and Comerford, 2000; Dong et al., 2007). The first objective of the current set of experiments was to determine whether mm-scale sites that differed in imprintable phosphatase activity also differed in activities according to traditional lab assays. The study reported here used soil imprinting to study acid phosphomonoesterase activities on soil profiles of a mixed Douglas-fir and paper birch stand in British Columbia, Canada. In these forests, soil P appears to be an important driver of microbial diversity (Twieg et al., 2009). In an earlier study, imprinting indicated that areas and intensities of fine-scale phosphatase activity increased with stand age in these forests, and correlated with the presence of some species of ectomycorrhizal fungi (Brooks et al., 2013). In the current study, our objective was to investigate soil chemical characteristics associated with mm-scale regions of high phosphatase activity at one of the sites used in the Brooks et al. (2013) study. By doing so, we would gain insight into the regulation of P cycling in forest soil microsites. Specifically, we (i) compared C, N, P and pH in microsites differing in phosphatase activity, and (ii) tested whether the addition of carbon (C) and nitrogen (N) to microplots on soil profiles *in situ* influenced phosphatase activity. An observation of higher C and N or lower inorganic P levels in high-phosphatase locations would be consistent with resource limitation (of P) or end product repression as controls of phosphatase activity at fine scales. Stimulation of phosphatase activities when C and N were added would be consistent with resource limitation (of P) and/or increased ability of the soil microbiota to synthesize extracellular enzymes.

2. Methods

2.1. Site description and study design

The study took place in a mixed Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), paper birch (*Betula papyrifera* Marsh) stand located at 50° 34′ 03″N, 118° 50′ 50″ W in the moist, warm variant of the Interior Cedar-Hemlock biogeoclimatic zone of southern interior British Columbia (Lloyd et al., 1990; Twieg et al., 2007, 2009). The stand has regenerated naturally after a wildfire in approximately 1945 (Twieg et al., 2007). The canopy consisted of approximately 38% Douglas-fir, 43% paper birch, and 4% Western redcedar (*Thuja plicata* (Donn ex D. Don) Spach; Twieg et al., 2009). Annual average temperature and precipitation for this region is 7.5 °C and 656 mm (Twieg et al., 2009). The soil is a Brunisol with sandy loam texture and moder humus form (Twieg et al., 2007).

Five root windows were installed at 5–15 m spacing during the summers of 2004 and 2005. The windows consisted of transparent acrylic panels (77 × 56 × 0.6 cm), each with a trap door (30 × 30 cm) in the center. Through the trap door, the forest floor, A and upper B horizons could be accessed. When not in active use, soil was back-filled against the windows. They are fully described in Brooks et al. (2013).

2.2. Soil sampling

For two weeks in the beginning of May 2011, soils were sampled from the upper mineral soil horizons through the trap door. Sampling was targeted to areas differing in phosphatase activity, as determined by the imprinting technique of Dong et al. (2007) and Jones et al. (2011). In brief, acid phosphomonoesterase-reactive imprinting sheets were created by soaking chromatography paper