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Estimating phosphorus availability for microbial growth in an emerging landscape

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ABSTRACT

Estimating phosphorus (P) availability is difficult—particularly in infertile soils such as those exposed after glacial recession—because standard P extraction methods may not mimic biological acquisition pathways. We developed an approach, based on microbial CO_2 production kinetics and conserved carbon:phosphorus (C:P) ratios, to estimate the amount of P available for microbial growth in soils and compared this method to traditional, operationally-defined indicators of P availability. Along a primary succession gradient in the High Andes of Perú, P additions stimulated the growth-related (logistic) kinetics of glutamate mineralization in soils that had been deglaciated from 0 to 5 years suggesting that microbial growth was limited by soil P availability. We then used a logistic model to estimate the amount of C incorporated into biomass in P-limited soils, allowing us to estimate total microbial P uptake based on a conservative C:P ratio of 28:1 (mass:mass). Using this approach, we estimated that there was <1 μg/g of microbial-available P in recently de-glaciated soils in both years of this study. These estimates fell well below estimates of available soil P obtained using traditional extraction procedures. Our results give both theoretical and practical insights into the kinetics of C and P utilization in young soils, as well as show changes in microbial P availability during early stages of soil development.

1. Introduction

Phosphorus (P) limitation in terrestrial ecosystems may be widespread (Elser et al. 2007 Wardle et al. 2004), but assessing P limitation is difficult for two main reasons: First, directly assessing ecosystem P limitation can only be achieved by manipulating P inputs and analyzing plant and/or soil responses through time (Vitousek and Farrington 1997). Second, accurately measuring biologically available P pools (available P) is problematic because measurements of "available P" typically utilize operationally-defined extraction procedures. For example, the amount of P that is liberated during an extraction with a particular chemical solution (e.g. Tiessen and Moir 1993) is implicitly assumed to represent available P, but these fractions may contain more or less P than is actually biologically available. Our limited ability to assess available P pools also challenges attempts to assess the extent to which P limits ecosystem processes in general, and to compare P limitation across ecosystems (Vitousek et al. 2010).

In an attempt to overcome these difficulties, we developed a bioassay approach—based on microbial growth and CO_2 production kinetics and constrained microbial carbon:phosphorus (C:P) ratios (Cleveland and Liptzin, 2007). This approach builds on a large body of previous work demonstrating that P can limit both the rate and extent of microbial growth in terrestrial and aquatic systems (Carlsson and Caron, 2001; Cleveland et al., 2002; King et al., 2008; Morris and Lewis, 1992; Souza et al. 2008) and that microbes have high affinity uptake systems that can scavenge P at levels below the detection limits of most methods for measuring P in natural systems (Button, 1985; Voegele et al., 1997; Zubkov et al., 2007). Our bioassay approach has two primary advantages over traditional methods commonly used to assess P availability and P limitation. First, using short-term laboratory assays, it provides estimates of the amount of P immobilized by microbes during growth, and thus serves as a direct measure of the amount of P available to microorganisms. Second, the method provides information describing relative P limitation in soils, and therefore may provide insight into the overall extent of ecosystem P limitation.

Here, we describe results from two sampling expeditions to a relatively pristine watershed in the High Andes of Peru. Using soil sampled at multiple sites in two years, we used this new technique to assess P availability and relative P limitation across this emerging (recently covered by a glacier) landscape. This remote site has been

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the focus of many recent ecological studies, partly because glaciers in the Sibinacocha valley have receded very rapidly over the past 80 years (Seimon et al., 2007) exposing large areas of soil that can remain devoid of plants for over 20 years (Nemergut et al., 2007; Schmidt et al., 2008). Preliminary studies of the P status of these soils indicated that they are low in available P and that phosphatase activities (~20 nmol $h^{-1} g^{-1}$) were the same as those in un-vegetated Colorado soils where heterotrophic microbial activity is P limited (King et al., 2008). Using soils collected from these sites, we examined P availability using both traditional methods and our new technique across this potentially P-limited landscape.

2. Methods

2.1. Study sites

We sampled soils in the recently deglaciated valley of the Laguna Sibinacocha Watershed (13°46′20″ S, 71°04′37″ W), Cordillera Vilcanota, Peru. Soils were collected during two separate expeditions to the sites in September 2001 and August 2003. At this recently deglaciated site, soils were collected at the edge of the glacial terminus ("0 m" samples) and 100 m from the terminus ("100 m" samples; uncovered for ~5 y). In addition, we sampled soil from a set of sites that had been exposed for >20 years ("Spit/Pass" sites) and from a vegetated site that remained ice-free during the Little Ice Age ("Boundary site"). Soil samples were frozen in the field at −10 °C (the low night-time soil temperature at the site; Schmidt et al. (2009)) and were transported on ice in thick-walled coolers to the laboratory at the University of Colorado, Boulder. General soil biogeochemical properties of all soils are provided elsewhere (King et al., 2008; Nemergut et al., 2007; Schmidt et al., 2008), and photographs and descriptions of the sites can be found elsewhere (Halloy et al. 2005; Schmidt et al. 2009; Seimon et al. 2009). When we returned to the watershed in 2003, we focused our attention on the rapidly receding Puca glacier and carried out more detailed microbial community structure work as reported elsewhere (Nemergut et al. 2007), as well as repeating the P addition experiments on soils that had been uncovered for <1 year (0 m) and 5 years (100 m). We used 4 spatially distinct replicates for each soil age and added more glutamate than in 2001 in order to enhance the effects of P on respiration kinetics.

2.2. Soil P fractions

Our goal was to compare estimates of available P using a biologically defined method to more traditional, functionally-defined estimates of soil P availability. Therefore, "available P" was assessed using the initial steps of the modified Hedley fractionation procedure of Tiessen and Moir (1993). Briefly, 1 g soil was subjected to a resin extraction in water (Resin P_i) to extract inorganic Pi, followed by a bicarbonate extraction (Bicarb P_i). Organic bicarb extractable P (Bicarb P_o) was determined as the difference between total Bicarb extractable P (Bicarb P_t) and Bicarb P_i following digestion with ammonium persulfate and sulfuric acid (Tiessen and Moir, 1993). These three fractions (resin P_i and Bicarb $P_i + P_o$) are the most labile forms of P, and their sum is often used as a proxy for readily available P (Cross and Schlesinger, 1995). Bowman et al. (1978) and Levy and Schlesinger (1999) have shown that bicarb P ($P_i + P_o$) can be correlated with plant growth. Total P (P_t) in soil samples was determined by digesting 5 g of sieved, air-dried soil in H_2SO_4 and H_2O_2. Phosphate concentrations in all measured fractions were determined using the ammonium molybdate ascorbic acid method (Kuo 1996) on an Alpkem autoanalyzer (OI Analytical, College Station, TX). Total mineral P was determined using element analyses with a Philips PW1400 Wavelength Dispersive Spectrometer, X-ray fluorescence instrument. Operating conditions for the Rh X-ray tube were 40 Kv and 20 Ma. Samples were first dried and then ground to <70 μm. Samples were then mixed with a binder (corn starch) and pressed into a pellet. Quantitative analyses using USGS rock standards BHVO-1, GSP-1, or GH was performed using the fundamental parameters correction procedure in the Philips X40 V.4.0A software.

2.3. Microbial kinetics

The kinetics of C (glutamate) mineralization (with and without P additions) was measured in soils using previously described approaches (Cleveland et al., 2002; Colores et al., 1996). For each treatment, sieved (2 mm) and homogenized soil samples (5 g dry weight equivalent) were placed in sterile biometer flasks and glutamate (a source of available C and N for soil microbes, Scow et al. 1989) was added in a small amount of sterile water along with tracer concentrations of uniformly ^{14}C-labeled glutamate to yield final concentrations of 50 μg C per g of soil (2001) or 128 μg C per g of soil (2003). Soils were incubated at 22 °C (the high day-time soil temperature at the site; Schmidt et al. 2009) and the evolved CO_2 was trapped in 1 ml of 0.5 M NaOH in the sidearm of the biometer flask. Periodically, the NaOH was removed, mixed with 2.5 ml of scintillation cocktail (Scintiverse II Cocktail, Fisher Scientific, Pittsburgh, PA), and the mixture was counted using a scintillation counter. Fresh NaOH was immediately added back to the sidearm after each sampling. The influence of P on mineralization kinetics was determined by adding 500 μg of P per g of soil as 1 M potassium phosphate solution to a subset of the samples. The pH of the phosphate solution (7.8) was approximately the same as the pH of the soil (7.5–7.6; Nemergut et al. 2007) to avoid lowering the soil pH, which can increase the rate of CO_2 efflux from the soil.

2.4. Estimating soil P using microbial kinetics

Our overall approach for estimating microbial available P was to compare respiratory responses of the soil to glutamate additions, with and without added P (all other conditions being equal). Sigmoidal (logistic) CO_2 production kinetics are used to estimate microbial biomass C production under P limitation and subsequently the microbial P uptake can be estimated by assuming a conservative stoichiometry of C:P ratios in the final biomass produced. This overall approach provides a conservative and ecologically relevant estimate of the amount of P that was available to support microbial growth in these soils.

To estimate the biomass produced under P limitation, we invoked the well-established principle that the production of a primary metabolite (e.g. CO_2) by a microbial population is directly proportional to the biomass of organisms producing the metabolite (Anderson and Domsch 1978; Colores et al., 1996; Schlegel, 1992). Furthermore, the growth of microorganisms often follows logistic (sigmoidal) kinetics, indicating an initial growth phase followed by a deceleration and a cessation of growth due to nutrient limitation (Simkins and Alexander, 1984; Zwietering et al., 1990). Thus, we can express the production of CO_2 from a soil sample using the integrated logistic equation (Berman, 1974) in terms of CO_2 production or:

$$C(t) = C_k / \left(1 + e^{-r(t-i)}\right) \quad (1)$$

where C(t) is the total CO_2 produced (with units of μg C g^{-1}) at time t, r is the intrinsic rate of increase (equivalent to μ_{max} with units of h^{-1}), i represents inflection point of the CO_2 production curve (with units of h) and C_k is the total CO_2 produced per gram of soil up to the time that growth ceased. In other words, C_k is the total amount of CO_2 produced during the growth of the population up to the carrying capacity (K) of the soil. To estimate C_k, Eq. (1) was fit to curves of CO_2 accumulation over time using the non-linear regression package of Kaliedagraph® (Synergy Software, Reading, PA, USA).