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Short communication

Phosphatase activity does not limit the microbial use of low molecular weight organic-P substrates in soil

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Abstract

Plant roots and soil microorganisms contain significant quantities of low molecular weight (MW) phosphorylated nucleosides and sugars. Consequently, upon death these can represent a significant input of organic-P to the soil. Some of these organic-P substrates must first be dephosphorylated by phosphatases before being assimilated by the soil microbial community while others can be taken up directly from soil solution. To determine whether sorption or phosphatase activity was limiting the bioavailability of low MW organic-P in soil we compared the microbial uptake and C mineralization of a range of ^{14}C-labeled organic-P substrates [glucose-6-phosphate, adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP)] to that of the parent compounds (adenosine and glucose). In a fertile grassland soil we showed that at low organic-P substrate concentrations (<0.5 mM) phosphatase activity did not limit microbial uptake or mineralization in comparison to their non-phosphorylated counterparts. However, at high substrate concentrations (1–10 mM) the mineralization of the organic-P compounds was significantly lower than that of the non-phosphorylated compounds suggesting that phosphatase activity or microbial transporter capacity limited bioavailability. Sorption to the solid phase followed the series glucose < adenosine < G-6-P < AMP < ADP = ATP. However, sorption of the organic-P compounds to the solid phase did not appear to greatly affect bioavailability. The high adenosine mineralization capacity of the microbial biomass suggests that nucleosides may represent a significant source of C and N to the soil microbial biomass. We conclude that at low organic-P substrate concentrations typical of those in soil, neither phosphatase activity nor sorption greatly limits their bioavailability.

Keywords: Dissolved organic nitrogen; Extracellular enzyme activity; Nutrient cycling; Mineralization; Organic phosphorus; Phosphatases; Sorption

Organic-P represents 20–80% of the total P in most soils and consequently represents a significant reserve of potentially available P. Low phosphorus availability in soil, however, remains one of the largest constraints to agricultural production worldwide, particularly in areas where inorganic fertilizers are unavailable (Raghothama, 1999). Consequently, for designing sustainable agricultural systems much emphasis has been placed on the addition of organic residues to soil as a means of enhancing organic P reserves or for stimulating inorganic P release from previously unavailable soil reserves (Ryan et al., 2001; Toor et al., 2006). Under P deficiency conditions, both plants and microorganisms can release phosphatase enzymes into the soil which have the potential to mobilize at least some of this reserve (Joner et al., 2000; Singh and Walker, 2006).

Some compounds like inositol hexametaphosphate (phytate) may make up 50% of the organic P pool in soil (Turner et al., 2003; Vats et al., 2005). Inositol phosphates such as phytate tend to accumulate in soils due to their high insolubility, sorption capacity, and low bioavailability (George et al., 2005; Tang et al., 2006). While much research has focused on ways to access this largely inaccessible soil P reserve, it is apparent that very large amounts of organic-P must be cycled each year due to root and microbial turnover (Aerts et al., 1992; Chen et al., 2003). The dominant P compounds present in root and microbial cells includes phospholipids (e.g., phosphatidyl ethanolamine, phosphatidyl serine), nucleotides

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(e.g., adenosine triphosphate, guanosine diphosphate), nucleic acids (e.g., DNA, RNA), sugar-phosphates (e.g., glucose-6-phosphate, glyceraldehyde-3-phosphate) and phosphoproteins (e.g., dehydrin, heat shock protein 81; Berg et al., 2006). These compounds typically represent a substantial proportion of the water soluble organic-P fraction in soils and are likely to be highly bioavailable (Turner et al., 2002). For example, glycerophosphate has been shown to contribute significantly to the P uptake of Carex (Pérez-Corona and Verhoeven, 1996) and the availability of glucose-1-P, 3-phosphoglyceric acid (PGA) and ATP was equivalent to that of inorganic P when supplied to wheat under sterile conditions (Richardson et al., 2000). The bioavailability of low molecular weight (MW) organic-P, however, is likely to be largely dependent on (1) the amount of active phosphatases in soil solution, (2) the uptake and assimilation capacity of the soil microbial community, and (3) the amount and strength of sorption of the different compounds to the soil's solid phase (Jones and Edwards, 1998; Leytem et al., 2002; Berg and Joern, 2006). The aim of this study was therefore to investigate the relative importance of these three factors in controlling low MW organic-P mineralization in a fertile agricultural soil.

The soil used in this study was collected from the Ah horizon (0–15 cm) of an NPK fertilized freely draining Eutric Cambisol located in Abergwyngregyn, North Wales (53° 14′ N, 4° 01′ W). The soil supports a grassland sward consisting predominantly of perennial ryegrass (*Lolium perenne* L.) and clover (*Trifolium repens* L.) which is grazed throughout the year by sheep and maintained with a sward height of 2–5 cm. The soil has a sandy clay loam texture, pH in water of 5.70 ± 0.10, electrical conductivity of $0.14 \pm 0.01\ mS\ cm^{-1}$, total C content of $35 \pm 2\ g\ kg^{-1}$, total organic N content of $2.6 \pm 0.2\ g\ kg^{-1}$, microbial biomass-C of $0.75 \pm 0.04\ g\ kg^{-1}$, bulk density of $1.54 \pm 0.04\ g\ cm^{-3}$ and moisture content of $250\ g\ kg^{-1}$. Soil was collected from three representative sampling areas located 2 m apart. After collection, the samples were placed in gas permeable plastic bags, and stored at 4 °C before analysis (within 7 d).

To determine the rate of organic-P mineralization, 5 g of field-moist soil was placed in a 50 ml polypropylene tube and 500 μl of a 0.5 mM ^{14}C-labeled solution ($1.7\ kBq\ ml^{-1}$) of either [1-^{14}C]-glucose (Sigma-Aldrich Corp, Milwaukee, WI), [1-^{14}C]-glucose-6-phosphate (G-6-P; NEN-Dupont Corp, Boston, MA), [8-^{14}C]-adenosine (Sigma-Aldrich), [U-^{14}C]-adenosine monophosphate (AMP; NEN-Dupont), [8-^{14}C]-adenosine diphosphate (ADP; NEN-Dupont) or [8-^{14}C]-adenosine triphosphate (ATP; NEN-Dupont) were added to the soil. A NaOH trap was then placed inside the tube to collect $^{14}CO_2$ evolved and the tubes hermetically sealed and placed in an incubator in the dark at 10 °C (mean annual temperature at the field site). The NaOH traps were replaced after 1, 3, 6, 24, 48, 72 and 168 h and the amount of $^{14}CO_2$ captured determined by liquid scintillation counting. The $^{14}CO_2$ capture efficiency of the NaOH traps was >95% as determined with $NaH^{14}CO_3$ added to the soil (data not presented). The amount of ^{14}C label remaining in the soil's solution and exchange phase after 168 h was determined by extracting the soil with 100 mM citrate-phosphate buffer (pH 6) for 30 min followed by centrifugation (18,000*g*, 5 min) and recovery of the supernatant for ^{14}C determination. The half-life of the compounds in soil was determined using a double exponential first-order kinetic decay model (Lucas and Jones, 2006). The concentration-dependent kinetics of substrate mineralization was determined by adding varying concentrations of each ^{14}C-labeled compound (0–10 mM) to soil and monitoring $^{14}CO_2$ evolution after 1 h as described above. A Michaelis–Menten kinetic equation was then fitted to the mineralization results where

$$V = V_{max} \times C/(C + K_m) \quad (1)$$

and where V is mineralization rate ($\mu mol\ kg^{-1}\ h^{-1}$), V_{max} is the maximum rate of mineralization ($\mu mol\ kg^{-1}\ h^{-1}$), C is substrate concentration (mM) and K_m is the Michaelis constant describing the concentration at which half-maximal mineralization occurs (mM).

Solid phase sorption of each substrate was determined by shaking 1 g of soil with 5 ml of each ^{14}C-labeled compound (500 μM) for periods up to 1.5 h. After known shaking times, the soil suspension was centrifuged (18 000 *g*, 5 min) and the supernatant recovered for ^{14}C determination as described above. Sorption experiments were performed on both field-moist soil and soil sterilized by heating in a sealed container at 80 °C for 30 min just prior to performing the assays. As significant microbial uptake and subsequent mineralization to $^{14}CO_2$ occurred in the non-sterile, field soil (Kuzyakov and Jones, 2006), only the sorption results obtained from the sterilized soil are shown. The data presented here are for the statistical analyses (linear regression, *t*-tests, ANOVA) performed using Sigmaplot 8.0 (SPSS Inc., Chicago, IL) and Minitab 14 (Minitab Inc., State College, PA).

The results presented in Fig. 1 show that the mineralization of all the ^{14}C-labeled substrates in soil was rapid. Typically, the mineralization curves showed a bi-phasic response with a rapid mineralization phase (0–1 d) followed by a much slower mineralization phase (1–7 d). The initial mineralization phase corresponds to the depletion of the substrate in soil solution and rapid catabolic use whilst the second phase corresponds to turnover of substrate C immobilized in the microbial biomass (Lucas and Jones, 2006). The mineralization results conformed well to a double exponential first-order kinetic model for all substrates ($r^2 = 0.989 \pm 0.006$). From the rate constants we calculated that the half-life of the substrates in soil solution was rapid being on average 6.2 ± 0.8 h for the nucleosides and 0.72 ± 0.02 h for the sugars (Table 1). While the half-life of glucose and G-6-P were not significantly different from each other ($P > 0.05$), the half-life of ATP was significantly greater than that of adenosine, AMP and ADP ($P < 0.05$; Table 1). After 168 h in the soil, only 0.05% of the glucose and <0.10% of the adenosine