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Dominance of either physicochemical or biological phosphorus cycling processes in temperate forest soils of contrasting phosphate availability

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ABSTRACT

The importance of organic phosphorus (P) mineralization for forest P nutrition may depend on the P status of the soil. Using an isotopic dilution technique, we measured organic P mineralization rates in two contrasting Cambisols under beech forest in Germany which had developed either on basalt, resulting in a silty-clayey soil high in available inorganic and total P, or on pleistocene sand, resulting in a sandy soil low in available inorganic and total P. To investigate if soil mixing during labeling causes artefacts in the assessment of microbial P immobilization and organic P mineralization we conducted a 38-day-long incubation experiment with labeling of pre-incubated soils either by mixing or by injection. Gross and net organic P mineralization were negligible and non-detectable against the great inorganic P availability in the soil developed on basalt, while biological and biochemical processes dominated P transformations by far in the sandy soil. A significant but transient pulse in soil respiration caused by mixing occurred in the silty-clayey soil, and it was accompanied by a transient increase in microbial P. Mixing of the sandy soil induced a small but persistent increase in respiration but a decrease in microbial P. Despite these effects on the microbial pool and activity, no evidence for an alteration of P transformation rates by soil mixing was found. This study suggests that labeling by injection might be feasible in P isotopic dilution studies, especially in low-P-sorbing sandy soils.

1. Introduction

In view of decreasing foliar phosphorus (P) concentrations of the main European tree species over the last 20 years (Jonard et al., 2015; Talkner et al., 2015), a better understanding of forest P nutrition is needed, and a major knowledge gap concerns biologically mediated soil P transformations. The mineralization of soil organic P can make an important contribution to potentially plant available P, especially in ecosystems with low availability of inorganic P and high amounts of soil organic carbon (C) such as forests (Bünemann, 2015). Since phosphate released by mineralization reacts quickly with the soil solid phase, organic P mineralization can in most mineral soils only be assessed using isotopic dilution principles in incubation experiments with ^{32}P- or ^{33}P-labeled soils.

So far, only a few studies have used isotopic dilution approaches to assess the relative and absolute contribution of physicochemical and biological/biochemical processes to P availability in forest ecosystems. For acid organic soils, a pioneering isotopic dilution study indicated net transfer from unlabeled organic P into both available inorganic and microbial P (Walbridge and Vitousek, 1987). The methodology was subsequently further developed (Oehl et al., 2001) and applied to arable soils, in which P availability was found to be dominated (70–80%) by physicochemical processes (Oehl et al., 2004). In forest soils, however, P dynamics may be largely controlled by microbial activity (Olander and Vitousek, 2004, 2005). Indeed, in a low-P sorbing Podzol with very low concentrations of total P (31 mg P kg^{-1}) under pine plantation forest in France, gross organic P mineralization contributed 70–90% of total isotopically exchangeable P, with the proportion increasing over the duration of the experiment (Achat et al., 2009). The majority of this flux was attributed to remineralization of microbial P. In mineral topsoils under forest in central Germany, biological/

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biochemical processes contributed 36–86% of total isotopically exchangeable P, depending on the soil type (Spohn et al., 2013). Further assessment of organic P mineralization rates by isotopic dilution on a range of soil types under forest is warranted, especially to clarify the effect of inorganic P availability on biological/biochemical processes.

Isotopic dilution studies to assess soil P transformation rates rely on a short-term isotopic exchange kinetics (IEK) experiment from which the baseline of isotopic exchange due to physicochemical processes only is extrapolated, and a parallel incubation experiment in which both physicochemical and biological/biochemical processes contribute to the isotopic dilution of phosphate in the soil solution (Oehl et al., 2001). The inverse of the specific activity ($^{33}P/^{31}P$) of phosphate in solution yields isotopically exchangeable P (the so-called *E*-value, in mg P kg^{-1}). The difference between *E*-values measured in the incubation experiment and *E*-values extrapolated from the IEK experiment corresponds to gross organic P mineralization. In addition, net organic P mineralization can be derived by subtracting microbial P immobilization from gross organic P mineralization (Bünemann et al., 2007).

Such experiments are conducted under steady-state conditions, i.e. with soil respiration rates and concentrations of phosphate in the soil solution being constant, and therefore render basal mineralization rates, i.e. in the absence of flush effects (Oehl et al., 2001). Typically, intensive soil mixing during labeling is used in order to reach a homogeneous distribution of the added tracer in the soil. This mixing can result in transient pulses in soil respiration (Achat et al., 2009; Bünemann et al., 2012). At the same time, very rapid microbial P immobilization has been observed in grassland soils with low availability of inorganic P and a large microbial P pool (Bünemann et al., 2012). This has raised the question whether the intensive soil mixing during labeling may stimulate microbial P uptake, inflating estimates of microbial P immobilization and leading to a transient deviation from steady-state conditions. This idea is supported by the fact that very small amounts of labile C substrates in the order of 5–15 μg g^{-1} can activate soil microorganisms, causing an up to fivefold release of C compared to the amount added (de Nobili et al., 2001). If microorganisms soon return to a state of resting or very low activity, this may explain why the recovery of the added P tracer in the microbial biomass has sometimes been found to be rather constant over time (Bünemann et al., 2004, 2012; Oberson et al., 2001). In addition, the potential destruction of aggregates during soil mixing may release P or increase P sorption (Borda et al., 2014; Sinaj et al., 1997).

The biggest soil disturbance occurs already during sampling and homogenization, and this effect may be particularly large in forest soils which have remained undisturbed for decades. Therefore, ideally organic P mineralization would be assessed in undisturbed soil cores, as practiced in ^{15}N isotopic dilution studies (Rütting et al., 2011). For P, the feasibility of this approach may depend on the texture and P sorption properties of the soil. In a first step, this could be assessed on homogenized samples together with an evaluation of the potential for artefacts resulting from soil mixing at labeling.

Our main objectives were i) to assess the relative contribution of physicochemical and biological/biochemical processes to P availability in two forest soils of contrasting P status and ii) to assess the effect of soil mixing during labeling on soil P transformation rates. More specifically, we aimed to assess the potential for artefacts when assessing microbial P immobilization and organic P mineralization in soils labeled with ^{33}P by mixing, and to pre-evaluate if it might be possible to conduct future ^{33}P isotopic dilution studies without soil disturbance. We conducted an incubation experiment with mineral topsoil from two sites under beech (*Fagus sylvatica* L.) forest on contrasting parent materials (basalt vs. pleistocene sand). We hypothesized that biological/biochemical processes would dominate P cycling in a low-P sorbing sandy soil containing little total P, in which assessment of P transformation rates by labeling without mixing would be possible. In contrast, we expected that labeling by injection would fail in a high-P sorbing clayey soil with a large total P stock due to inhomogeneous distribution of the label, and that physicochemical processes would be more important than biological/biochemical processes, at least in relative terms.

2. Material and methods

2.1. Site description and soil sampling

Two sites under beech forest which are permanently monitored (BML, 1995) were selected for this study. Bad Brückenau (BBR) is located at about 800 m asl in Northern Bavaria, Germany (50°21′7.26″N, 9°55′44.53″E). Mean annual temperature is 5.5 °C and mean annual precipitation 1000 mm. The Dystric Skeletic Cambisol(Hyperhumic, Loamic) (FAO/ISRIC/ISSS, 1998) has developed on basalt, and the vegetation consists of a more than 120 year-old pure beech stand, with a diverse herbal layer (up to 31 species on an area of 400 m^2; Isaak Rieger, personal communication). Lüss (LUE) is located at 100 m asl in Lower Saxony, Germany (52°50′21.77″N, 10°16′2.37″E). Mean annual temperature is 8.4 °C and mean annual precipitation 775 mm. The Hyperdystric Folic Cambisol (Arenic, Loamic, Nechic, Protospodic) has developed on pleistocene sand. The vegetation is dominated by beech trees which are between 100 and 120 years old and shrubs or herbs are almost completely absent. The current annual N deposition at the two sites is 6–8 kg N ha^{-1} at BBR (Schönthaler and von Andrian-Werburg, 2008) and approximately 13 kg N ha^{-1} at LUE (NWFVA, 2008).

Soil samples were taken in October 2014 from the Ah1 horizon (0–7 cm) at BBR and from the Ahe horizon (0–7 cm) at LUE at several locations within an area of 9 m^2, sieved moist at 2 mm and combined to one composite sample per site. Small portions of these samples were dried at 40 °C or frozen at −20 °C, while the remainder was stored at 4 °C for a few days before starting the preincubation. The Ah1 from BBR contained 80, 550 and 370 g kg^{-1} of sand, silt and clay, respectively, while the corresponding numbers for the Ahe at LUE were 750, 190 and 60 g kg^{-1} (Jaane Krüger, personal communication). The maximum water holding capacity (WHC) of the sieved soils determined by placing the saturated soils on filter paper in funnels and letting the water drain for 24 h was 1.16 g H_2O g^{-1} dry soil for BBR, while it was 0.37 g H_2O g^{-1} dry soil for LUE.

2.2. Experimental design and set-up

An incubation experiment with ^{33}P labeling of soil from both sites was conducted. It had a two-factorial design with two soils (BBR and LUE) and two levels of soil mixing at labeling (mixed and unmixed). The incubation experiment was complemented by IEK experiments in order to determine the baseline of isotopic exchange due to physicochemical processes only. The incubation experiment lasted six weeks, with determination of ^{31}P and ^{33}P in water-extractable phosphate, resin-extractable and microbial P at seven sampling dates. In parallel to the incubation experiment, soil respiration was determined on similarly treated but unlabeled soils in weekly intervals.

In more detail, the gravimetric water content of the sieved soils was at 78% and 66% of the maximum WHC for BBR and LUE, respectively. Since the samples were taken neither immediately after rainfall events nor after a drought, we decided to conduct the incubation experiment at 75% of the WHC for both soils, which is in