Oxygen isotope ratios of plant available phosphate in lowland tropical forest soils

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ABSTRACT

Phosphorus (P) cycles rapidly in lowland tropical forest soils, but the process have been proven difficult to quantify. Recently it was demonstrated that valuable data on soil P transformations can be derived from the natural abundance of stable oxygen isotopes in phosphate ($\delta^{18}O_P$). Here, we measured the $\delta^{18}O_P$ of soils that had received long-term nutrient additions (P, nitrogen, and potassium) or litter manipulations in lowland tropical forest in Panama and performed controlled incubations of fresh soils amended with a single pulse of P. To detect whether $\delta^{18}O_P$ values measured in the incubations apply also for soils in the field, we examined the $\delta^{18}O_P$ values after rewetting dry soils. In the incubations, resin-P $\delta^{18}O_P$ values converged to ~3.5‰ above the expected isotopic equilibrium with soil water. This contrasts with extra-tropical soils in which the $\delta^{18}O_P$ of resin-P matches the expected equilibrium with soil water. Identical above-equilibrium resin-P $\delta^{18}O_P$ values were also found in field soils that did not receive P additions or extra litter. We suggest that the 3.5‰ above-equilibrium $\delta^{18}O_P$ values reflect a steady state between microbial uptake of phosphate (which enriches the remaining phosphate with the heavier isotopologues) and the release of isotopically equilibrated cell internal phosphate back to the soil. We also found that soil nutrient status affected the microbial turnover rate because in soils that had received chronic P addition, the original $\delta^{18}O_P$ signature of the fertilizer was preserved for at least eight weeks, indicating that the off-equilibrium $\delta^{18}O_P$ values produced during microbial phosphate turnover was not imprinted in these soils. Overall, our results demonstrate that ongoing microbial turnover of phosphate mediates its biological availability in lowland tropical soils.

1. Introduction

Nutrient availability limits the productivity of many tropical forests (Vitousek, 1984; Cleveland et al., 2011; Wright et al., 2011). Phosphorus (P) is widely believed to be the primary limiting nutrient on the old, strongly weathered soils that characterize much of the lowland tropics, because many such soils have high P fixation capacities and contain low concentrations of soluble phosphate. Therefore, the maintenance of bioavailable P in sufficient amounts to sustain the productivity of lowland tropical

forests depends strongly on the continuous recycling of P within the soils (Vitousek, 1984; Cleveland et al., 2011; Turner et al., 2013). The aim of this project was to derive information on the processes that control phosphate cycling in tropical forest soils, based on natural variation in the isotopic composition of oxygen in soil phosphate ($\delta^{18}O_P$). This Technique has provided valuable information on soil P dynamics in different ecosystems worldwide (Zohar et al., 2010; Angert et al., 2011, 2012; Tamburini et al., 2012; Gross et al., 2013; Jaisi et al., 2014; Gross and Angert, 2015), but has not so far been applied in tropical soils.

We took advantage of two long-term and well-studied field experiments in lowland tropical forest in Panama: an experiment initiated in 1998 involving factorial P, nitrogen (N) and potassium (K) additions (Wright et al., 2011; Yavitt et al., 2011; Sayer et al., 2012; Mirabello et al., 2013; Schreeg et al., 2013; Turner and

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Wright, 2014), and a second experiment initiated in 2003 at the same site involving litter manipulation (removal and addition) (Sayer and Tanner, 2010; Vincent et al., 2010; Sayer et al., 2012). These experiments allowed us to isolate the role of nutrients and leaf litter status in the soil P cycle and study the processes that mediate soil P dynamics, while controlling other soil properties such as texture and organic matter content.

The use of $\delta^{18}O_P$ as a tracer for phosphate cycling in soils is based on the stability of the P–O bond, which under natural soil conditions can only be broken by biological reactions (Tudge, 1960; Dahms and Boyer, 1973; Kolodny et al., 1983). Thus, the involvement of various reactions in the soil P cycle can be determined from changes in $\delta^{18}O_P$, caused by the incorporation of new oxygen with a different isotopic composition. The major processes that affect the isotopic composition of bioavailable soil phosphate on short time scales are (Jaisi and Blake, 2014; Gross and Angert, 2015):

1) the exchange of phosphate molecules among soil P pools may cause isotopic dilution if their $\delta^{18}O_P$ values are different (Zohar et al., 2010; Angert et al., 2012). Such abiotic exchange reactions do not cleave the P–O bond of the phosphate.
2) the assimilation of phosphate by microorganisms which preferentially take up lighter isotopologues, and therefore cause the remaining phosphate in the soil to be enriched in ^{18}O (Blake et al., 2005).
3) Biological reactions that promote the exchange of oxygen between phosphate and water. These include:
   3.i) the hydrolysis of organic-P compounds by extracellular phosphatases, which induce strong kinetic effects and produces phosphate depleted in ^{18}O (Liang and Blake, 2006, 2009; Von Sperber et al., 2014).
   3.ii) the microbial turnover of phosphate, which produces phosphate in isotopic equilibrium with soil water as part of cell metabolism (Blake et al., 2005; Stout et al., 2014). This equilibrium is temperature dependent and is well described by the following equation (Longinelli and Nuti, 1973; Kolodny et al., 1983):

$\delta^{18}O_P = \delta^{18}O_{water} + (111.4 - T)/4.3$ (where T is the temperature in °C) (1)

where T is the temperature in °C and $\delta^{18}O_{water}$ is the isotopic composition of oxygen in soil water.

We designed a series of controlled incubation experiments with labeled phosphate compounds to evaluate the role of each of these processes on phosphate $\delta^{18}O_P$ values in lowland tropical soils (Gross and Angert, 2015). We focused our measurements on phosphate that can be extracted with anion exchange resin (hereafter "resin-P"), which is considered to approximate the phosphate pool that is available for plant uptake (Cooperband et al., 1999; Qian and Schoenau, 2002). The incubations had three aims. The first aim was to detect whether a biologically mediated cleavage of the P–O bond of the added phosphate compound occurred or simply an exchange of the added phosphate with P from different soil P pools.

The second aim was to determine the role of organic-P mineralization and phosphate uptake by microbes. The third aim was to determine whether changes in the soil resin-P $\delta^{18}O_P$ values reflect a movement towards isotopic equilibrium with soil water, as was reported for the resin-P pool in a series of natural extra tropical soils (Zohar et al., 2010; Angert et al., 2011, 2012; Tamburini et al., 2012; Gross et al., 2013; Jaisi and Blake, 2014; Gross and Angert, 2015).

To detect whether the resin-P $\delta^{18}O_P$ values we measured at the end of the incubation period can be also found in soils in the field, we determined the $\delta^{18}O_P$ values for the same soils in such field circumstances. We hypothesized that in accordance with reports from other ecosystems, microbial biomass rapidly recycles phosphate added to the soil, altering the $\delta^{18}O_P$ values towards isotopic equilibrium with soil water both in the incubations and in the field. Our second hypothesis was that $\delta^{18}O_P$ responds to the addition of other nutrients (i.e. P, N and K) and litter manipulations. Specifically, we predicted that soil phosphate $\delta^{18}O_P$ values would (1) deviate from isotopic equilibrium in soils that received chronic P additions because of lower microbial demands for P (Turner and Wright, 2014) and (2) reflect the kinetic isotopic fractionation induced during mineralization of organic-P in soils that received double amounts of litter.

2. Methods

2.1. Study site

This study was performed at the Gigante Peninsula (9.063°N; 79.50°W) (Wright et al., 2011), which is part of the Barro Colorado Nature Monument (BCNM), Republic of Panama. The tropical monsoon (Köppen system) climate has a mean annual temperature of 27 °C and mean annual rainfall of 2600 mm (Windsor, 1990). Soils on the upper parts of the landscape are clay-rich Oxisols developed on Miocene basalt and are morphologically similar to the Typic Eutrudox (AVA and Marron soil classes) on nearby Barro Colorado Island (Dieter et al., 2010). Lower parts of the landscape are Inceptisols (Corre et al., 2010) with a shallow water table during the wet season. The soils are moderately acidic (pH 4.8–6.1 (Koehler et al., 2009)) with low readily available phosphate concentrations (<1 mg P kg^{-1}) low nitrate concentrations (~1 μg N g soil^{-1}) and a moderate potassium (K) concentration (~100 μg K g soil^{-1}), which are representative of forest soils in central Panama (Turner et al., 2013).

The fertilization experiment began in 1998 and is described in detail elsewhere (Yavitt et al., 2009; Wright et al., 2011). The experiment is remarkable in its duration and in the range of responses observed in terms of forest productivity, with significant effects involving N, P, and K (Wright et al., 2011). Briefly, fertilizer has been added four times per year at intervals of 45–60 days during the wet season between May and October to thirty two 40 m × 40 m plots which replicates each treatment in a full factorial design four times. Nitrogen is added as urea, P as triple superphosphate ($Ca(H_2PO_4)_2$), and K as KCl. Annual doses were 125 kg N ha^{-1}, 50 kg P ha^{-1} and 50 kg K ha^{-1}. To preclude movement of nutrients among plots, and reduce uptake by trees rooted in plots with different treatments, the minimum distance between plots was 40 m. Adjacent to the fertilization experiment, the Gigante Litter Manipulation Project (GLIMP) was established in 2000 (see Sayer and Tanner, 2010 for a detailed description of the experiment). Briefly, the litter was removed from five 45 m × 45 m plots once every three months (L$^-$ plots) starting in 2003 and immediately spread over five plots (effectively doubling the litter standing crop; L$^+$ plots). All GLIMP plots were trenched to a depth of 50 cm, lined with plastic and refilled to minimize transport of nutrients and water between treatments by roots and mycorrhizas.

2.2. Soil sampling

We sampled soils during the wet season when 90% of annual rainfall occurs (Windsor, 1990) and maximal biological activity is expected. The sampling was conducted in two campaigns, during August and September 2012, four and eight weeks after the most recent application of fertilizers. Soil samples were collected in each sampling campaign from the same three replicates of the full