Geoderma 257-258 (2015) 86–93

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Gross organic phosphorus mineralization rates can be assessed in a Ferralsol using an isotopic dilution method

Lalajaona Randriamanantsoa [a,b], Emmanuel Frossard [a], Astrid Oberson [a], Else K. Bünemann [a,*]

[a] ETH Zurich, Institute of Agricultural Sciences, Group of Plant Nutrition, 8315 Lindau, Switzerland
[b] Laboratoire des Radio-Isotopes, Route d'Andraisoro, BP 3383, 101 Antananarivo, Madagascar

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form 5 January 2015
Accepted 7 January 2015
Available online 16 January 2015

Keywords:
Isotopic dilution
Soil organic phosphorus
Gross basal mineralization
Low P availability
Phosphate sorption
Ferralsol

ABSTRACT

Mineralization of organic phosphorus (P_o) may be of great importance for plant nutrition in soils containing very little available inorganic phosphorus (P_i). Gross organic P mineralization rates can be quantified by an isotopic dilution method using ^{33}P labeling of soil. However, its application remains a challenge in tropical soils in which the concentration of phosphate ions in the soil solution is below the detection limit of traditional colorimetric methods. This limitation can potentially be overcome by the hexanol concentration method, which uses hexanol to concentrate the blue-colored phosphomolybdate complex from large volumes. We applied the isotopic dilution method in combination with the hexanol concentration method to a Ferralsol from the highlands of Madagascar which had been preincubated in the presence or absence of plant residues for 90 days before the start of the experiment. The limits of detection (DL) and quantification (QL) of the gross P_o mineralization rate were 0.2 and 0.7 mg P kg^{-1} soil day^{-1}, respectively. Basal gross P_o mineralization rates after 7 days of incubation were 0.8 ± 0.5 and 1.7 ± 0.2 mg P kg^{-1} soil day^{-1} in non-amended and residue-amended soils, respectively. These rates are plausible, suggesting that the isotopic dilution method is applicable in highly weathered tropical soils with P_i concentrations in the soil solution below the detection limit of traditional colorimetric methods. Net P_o mineralization which sustains the plant available P pool remains to be quantified. Gross and net P_o mineralization rates should now be assessed in highly weathered soils under a range of land uses.

1. Introduction

Total phosphorus (P) concentration in soils ranges from 100 to 3000 mg P kg^{-1}, but only a fraction, often less than 0.1%, is immediately available to plants (Frossard et al., 2000). Plants take up P as phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) from the soil solution. Organic phosphorus (P_o) represents between 20% and 80% of the total P in the upper soil horizons (Dalal, 1977). The release of inorganic P (P_i) from P_o by mineralization can potentially increase P availability, especially under conditions of P deficiency which is widespread in tropical regions (Tiessen et al., 2001). Tropical soils with low amounts of total P and high sorption capacity for phosphate ions due to the presence of aluminum and iron oxides cover 2 billion ha in the tropics, of which 37% and 34% are Ferralsols and Acrisols, respectively (Fairhurst et al., 1999).

Soils in the highlands of Madagascar are predominantly Ferralsols. Legume cover crops such as *Stylosanthes guianensis* (stylo) are used in the Midwest of Madagascar to increase soil fertility, reduce soil erosion, suppress weeds and provide forage for the cattle during the dry season (Naudin et al., 2012). Stylo residues left as mulch on the surface or incorporated may affect soil P dynamics directly via addition of P or via reactions of organic compounds with the soil surface, or indirectly, e.g. via effects on soil structure or via a stimulation of microbial P immobilization and subsequent re-mineralization (Guppy et al., 2005).

Basal gross P_o mineralization is defined as the gross mineralization of P_o in a soil at constant respiration (Oehl et al., 2001). It represents the basal potential of a soil to release P_i from soil P_o to the soil solution. The direct measurement of net P_o mineralization rates by incubation in combination with chemical extraction methods for P_i may be possible in sandy soils with low P_i sorption (Grierson et al., 1998), but not in soils with high P sorption capacity in which P_i is sorbed rapidly onto soil particles after its release to the soil solution (Frossard and Sinaj, 1998). Alternatively, gross P_o mineralization rates can be quantified using isotopic dilution principles, where isotopically exchangeable soil P fractions are labeled with ^{32}P or ^{33}P so that the release of unlabeled P from P_o can be assessed (Walbridge and Vitousek, 1987). This approach has been further developed and used successfully in a range of temperate (Achat et al., 2009; Bünemann et al., 2012; Oehl et al., 2001, 2004) and mediterranean (Bünemann et al., 2007) soils.

The basic assumption of the isotopic dilution method is that P_i released from unlabeled P_o will decrease the specific activity ($^{33}P/^{31}P$) of isotopically exchangeable phosphate in a soil labeled with $^{33}P_i$ (López-Hernández et al., 1998; Oehl et al., 2001). The method is based

* Corresponding author at: ETH Zurich, Institute of Agricultural Sciences, Group of Plant Nutrition, Eschikon 33, 8315 Lindau, Switzerland.
E-mail address: else.buenemann@usys.ethz.ch (E.K. Bünemann).

http://dx.doi.org/10.1016/j.geoderma.2015.01.003
0016-7061/

on the comparison of the specific activity (SA) of soil solution P_i resulting only from physicochemical processes (baseline), and the SA obtained during incubation under the influence of physicochemical and biological processes. The baseline is obtained using the isotopic exchange kinetic (IEK) method, i.e. via extrapolation from a short-term batch experiment in which biological processes can be excluded (Fardeau et al., 1991). However, Oehl et al. (2001) cautioned that this extrapolation is not valid for strongly P sorbing soils with low concentrations of water-soluble P_i.

Such concerns are in line with earlier studies which showed that isotopically exchangeable phosphate tends to be overestimated in soils with high P sorption capacity and low concentrations of water-soluble P_i (Amer et al., 1969; Tran et al., 1988; Wolf et al., 1986). Several potential explanations have been given, namely i) a different behavior of the tracer (^{32}P or ^{33}P) to that of the tracee (^{31}P), ii) the absence of equilibrium conditions at the time of tracer additions, and iii) inaccurate determination of the concentration of water-soluble P_i due to values near or below the detection limit, possibly in combination with errors due to colloidal P, silicates and organic P hydrolysed during colorimetry. Based on the comparison of isotopic exchange kinetic experiments with ^{32}P and ^{33}P, Randriamanantsoa et al. (2013) concluded that both tracers have a similar behavior as ^{31}P in soils, although a small isotope mass effect was observed in a system with synthetic goethite, phosphate and water. Also the second concern was shown to be invalid based on the fact that the concentration of phosphate in the soil solution in acid tropical soils remained constant between 18 h and 5 weeks of equilibration (Bühler et al., 2003). Therefore, Frossard et al. (2011) concluded that the precise determination of the concentration of water-soluble P_i remains the main problem when trying to apply the IEK method to soils with high P sorption capacity and low water-soluble P_i.

The concentration of water-soluble P_i needs to be above the limit of quantification for the method used (Bühler et al., 2003). Unfortunately, concentrations of water-soluble P_i below 10 μg P L^{-1} cannot be measured reliably with standard colorimetric methods, even when using spectrophotometer cells of 10 cm length (Randriamanantsoa et al., 2013). If concentrations of water-soluble P_i cannot be quantified, then the isotopic dilution method to determine gross organic P mineralization rates cannot be used.

Some authors have attempted to overcome the problem of low concentrations of water-soluble P_i by measuring NH_4F/HCl extractable P_i (Walbridge and Vitousek, 1987), resin extractable P_i (Maertens et al., 2004) or Diffuse Gradient in Thin-film (DGT) extractable P_i (Six et al., 2012) instead. However, the isotopic dilution method requires that isotopic parameters for the baseline and in the incubated soil are measured in the same accessible soil compartment (Frossard et al., 2011). Our attempts to establish the baseline using a resin extraction (data not shown) failed because the time needed for this extraction was very long in relation to the very rapid exchange reactions controlling P_i isotopic exchange in soils. The same would apply to DGT. Alternatively, the limit of quantification of water extractable P_i could be lowered, e.g. by the hexanol concentration method, which uses hexanol to concentrate the blue-colored phosphomolybdate complex (Randriamanantsoa et al., 2013). This method has been used successfully to determine very low concentrations of water extractable P_i in the order of 1 to 5 μg P L^{-1} in Ferralsols and an Andosol from Madagascar (Randriamanantsoa et al., 2013) and in Lixisols from Burkina Faso (Kiba et al., 2012).

The objective of this study was to apply the isotopic dilution method to measure the rate of gross P_o mineralization (Oehl et al., 2001) in combination with the hexanol concentration method (Randriamanantsoa et al., 2013) to a soil with high sorption capacity for P_i and very low P_i availability. To test the plausibility of the measured gross P_o mineralization rate, we analyzed a soil which had been preincubated with or without plant residues for 90 days before the start of the experiment. We hypothesized that plant residue amendment would increase the microbial activity in the soil, which would result in an elevated gross P_o mineralization rate.

2. Materials and methods

2.1. Experimental design

This laboratory study assessed the rate of basal gross P_o mineralization in the upper horizon of a Ferralsol with two treatments (residue-amended and non-amended soil). It consisted of a preincubation period of 90 days, followed by IEK experiments to determine the baseline of isotopic dilution due to physicochemical processes and an incubation experiment to account for biological processes (Fig. 1). The IEK experiments as well as all measurements in the incubation experiment (determination of ^{31}P and ^{33}P in water-soluble P_i and soil respiration) were conducted with 4 replicates, and nanopure H_2O was used throughout.

2.2. Soil and residue sampling and preparation

The soil used for this study was sampled in October 2010 from the topsoil (0–10 cm) of a Ferralsol (FAO/ISRIC/ISSS, 1998) outside of a field experiment testing the effect of different fertilization regimes on upland rice located at Ivory, in the Midwest of the Malagasy Highlands (19°33′S, 46°24′E, 900 m above sea level). The site had been under weedy fallow vegetation (dominated by *Aristida* grasses) for at least 5 years. The soil was air-dried and sieved at 2 mm before storage in a closed bucket at 20 °C for 6 months. The topsoil had 0.26 g clay g^{-1} dry soil and 0.25 g silt g^{-1} dry soil and a high P sorption capacity (Randriamanantsoa et al., 2013). The water holding capacity (WHC) was 0.46 g H_2O g^{-1} dry soil.

Dry residues of *S. guianensis* cv. CIAT 184 (stylo) were collected at the same time as the soil from an adjacent farmer's field at Ivory. The residue-amended treatment mimicked a situation where legumes are integrated into the cropping system as green manure. Complete soil coverage can be reached by a mulch application of 10 t DM ha^{-1} (Naudin et al., 2012). In order to increase microbial activity, as in Randhawa et al. (2005), the soil was amended with stylo residues at a rate of 7.69 g DM kg^{-1} dry soil (equivalent to 10 t DM ha^{-1}, assuming a bulk density of 1.3 g cm^{-3}). The aboveground biomass of the collected stylo residues had been cut at the end of the rainy season (April 2010) and left in situ to dry before collection in October 2010. The residues were cut into small pieces and milled to a diameter of less than 1 mm. The stylo residues had low concentrations of macronutrients, especially P (Table 1). The residue amendment added 3.5 g C kg^{-1} soil and only 2.2 mg P kg^{-1} soil.

2.3. Analysis of soil and plant residue

Total C and N in soil and plant residues were determined by dry combustion using a NCS analyzer (FlashEA 1112 series NCS Analyzer, Thermo Fisher Scientific, Waltham, MA, USA). Total P in the stylo residues was determined by ashing 0.2 g of plant material at 550 °C for 4 h, dissolving the ash in 2 mL of 15 M HNO_3 and making the volume to 100 mL using nanopure water, followed by colorimetric measurement of P_i with the Malachite green method (Van Veldhoven and Mannaerts, 1987). Total P in the soil was determined by wet digestion with concentrated H_2SO_4/H_2O_2 (Anderson and Ingram, 1993). An aliquot was taken from the diluted digest to measure P_i using malachite green.

Soil P_i extractable with 0.5 M H_2SO_4 ($P_{i\_H_2SO_4}$) was determined by shaking 1 g dry soil in 25 mL of 0.5 M H_2SO_4 overnight (Saunders and Williams, 1955) and measuring $P_{i\_H_2SO_4}$ in the filtrate. Soil P_o was extracted with 0.25 M NaOH and 0.05 M Na_2EDTA (Bowman and Moir, 1993). An aliquot was taken from this extract to measure P_i. Another aliquot was used to determine total P by autoclaving it with a digestion mix (6 g of ammonium persulfate dissolved in 100 mL of 0.9 M H_2SO_4) at 121 °C and 1.1 bar for 1 h, followed by pH adjustment to 5.5 and colorimetric determination of P in the digest. Total P_o was calculated as the difference of total P and P_i in the NaOH–EDTA extract. Resin-