



## Phosphorus in the coarse soil fraction is related to soil organic phosphorus mineralization measured by isotopic dilution



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### ABSTRACT

A sound prediction of soil organic phosphorus ( $P_o$ ) mineralization would be helpful to improve fertilizer recommendations. However, in most mineral soils expensive and time consuming isotopic dilution experiments are required to assess  $P_o$  mineralization. A proposed method to estimate  $P_o$  mineralization is the quantification of P in the coarse fraction (CF,  $>53 \mu\text{m}$ ) of the soil. The aim of this study was to evaluate if P in the CF can effectively predict  $P_o$  mineralization. Soil samples from three North American long-term arable field experiments ( $>10$  years) with different textures (sand content 15–82%) and different fertilization treatments (non-P-fertilized, mineral P fertilizer, poultry litter) were analyzed. Soil samples were physically fractionated into CF and fine fraction (FF,  $<53 \mu\text{m}$ ). Total P ( $P_t$ ),  $P_o$ , and total carbon ( $C_t$ ) were determined in each fraction. Gross and net  $P_o$  mineralization rates as well as soil respiration were determined in a 13-day-incubation experiment. The cumulative gross  $P_o$  mineralization over 13 days ranged from 8.3 to 38.6 mg P kg<sup>-1</sup>, while  $P_t$  in CF varied between 9.7 and 90.7 mg P kg<sup>-1</sup> and  $P_o$  in CF between 6.1 and 17.7 mg kg<sup>-1</sup>. No association between  $P_t$  in CF and gross or net mineralized  $P_o$  was observed. However, a significant linear relationship between  $P_o$  in CF and net ( $R^2:0.68$ ,  $p < 0.01$ ) and gross mineralized  $P_o$  ( $R^2:0.72$ ,  $p < 0.01$ ) was observed after 6 days and 13 days of incubation. Additionally, gross and net mineralized  $P_o$  could also be predicted from C respiration, and these models were significantly improved by incorporating the  $C_t:P_o$  ratio of the CF. Our results suggest that the quantification of  $P_o$  in the CF as well as the measurement of respiration corrected by the  $C_t:P_o$  ratio of the CF are promising non-isotopic indicators of  $P_o$  mineralization rates.

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### 1. Introduction

Phosphorus (P) is a macronutrient involved in the energy metabolism and in the synthesis of cellular structures of plants (Raghothama et al., 2005). Consequently, the availability of P controls the growth and development of all crops. It is estimated that 5700 million hectares of soils worldwide do not have the adequate available P content required to achieve optimum crop production (Batjes, 1997). To alleviate this deficiency, usually mineral P

fertilizers are used at rates based on extraction methods calibrated by field trials, such as those developed by Bray and Kurtz (1945), Olsen et al. (1954) and Mehlich (1984). However, all of these soil P tests consider only the available inorganic P ( $P_i$ ) pool, without taking into account organic P ( $P_o$ ) which can represent up to 80% of total P ( $P_t$ ) (Condron et al., 2005; Harrison, 1987) and can become plant available through mineralization. Therefore, the measurement or estimation of  $P_o$  mineralization rates is important to better estimate P plant availability and P fertilizer recommendations.

This conversion from  $P_o$  to  $P_i$  can be expressed as gross or net  $P_o$  mineralization. Since  $P_i$  released by mineralization to the soil solution can be taken up again by microorganisms, i.e. immobilized, gross  $P_o$  mineralization is the sum of net  $P_o$  mineralization and microbial P immobilization (Bünemann, 2015). Consequently, net  $P_o$  mineralization can be derived as gross  $P_o$  mineralization minus

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the amount of immobilized  $P_i$ . The quantification of gross and net  $P_o$  mineralization is complicated by the high reactivity of phosphate ions, which are rapidly adsorbed by the soil mineral matrix after being released by mineralization from the organic pool to the soil solution. This reactivity makes it generally impossible to quantify net  $P_o$  mineralization by incubation-extraction methods as the ones used to measure net mineralization rates of other nutrients such as nitrogen (N) (Stanford and Smith, 1972) and sulfur (S) (Tabatabai and Al-Khafaji, 1980). An exception are soils with low reactivity, for which extraction methods could be appropriate (Grierson et al., 1998; Achat et al., 2010). Currently, the only method to quantify  $P_o$  mineralization on most mineral soils is based on isotopic dilution experiments under steady-state conditions, rendering gross and net  $P_o$  mineralization rates in the absence of flush effects (caused by, for example, recent inputs of labile C substrates or climatic variations) (Oehl et al., 2001; Bünemann, 2015). The isotopic dilution method has been used and improved in several studies (Oehl et al., 2001; Bünemann et al., 2007, 2012; Achat et al., 2009a; Randriamanantsoa et al., 2015) as recently reviewed by Bünemann (2015). However, such experiments are expensive and time consuming. Thus, there is a need to develop and evaluate non-isotopic methods to estimate the  $P_o$  mineralization potential of soils, based for instance on C mineralization (Achat et al., 2009a, 2010) or soil properties (Achat et al., 2013).

The  $P_t$  concentration in the coarse fraction (CF) (soil fraction > 53  $\mu\text{m}$ ) has been suggested as a potential estimator of  $P_o$  mineralization (Ciampitti et al., 2011). These authors observed that the concentration of  $P_t$  in the CF ( $\text{mg } P_t\text{-CF kg}^{-1}$  soil) was positively related to the P accumulation in corn (*Zea mays* L.) plants growing in unfertilized plots on a Mollisol in Argentina, and suggested that this association is based on the rapid turnover rate of the  $P_o$  in the CF. However, there are no studies analyzing the association between  $P_o$  mineralization and  $P_t$  or  $P_o$  content in the CF to support this hypothesis. The CF has been previously named “particulate organic matter” (Cambardella and Elliott, 1992) or “particulate fraction” (Wyngaard et al., 2013). Here, we will refer to it as “coarse fraction” (CF), since this fraction is composed of both organic matter and mineral particles. As the determination of  $P_t$  or  $P_o$  in the CF is simple and economical, it could be a promising indicator to estimate potential  $P_o$  mineralization.

Other labile soil  $P_o$  fractions that vary depending on the climate, vegetation, mineralogy, and fertilization history can be characterized using enzyme addition assays (Koopmans et al., 2007; He et al., 2008; Keller et al., 2012; Requejo and Eichler-Löbermann, 2014; Annaheim et al., 2015). Bünemann et al. (2012) observed that an enzyme-labile P fraction (P hydrolyzed by phytase) was not associated with the measured gross and net  $P_o$  mineralization rates in a grassland soil from Switzerland. It should be evaluated for other land-uses and contrasting management practices if there is a relationship between enzyme-labile  $P_o$  fractions and  $P_o$  mineralization rates.

Others have suggested to use soil respiration (C–CO<sub>2</sub> released during soil incubations) as an estimator of gross (Achat et al., 2010) or net (Bünemann et al., 2012) mineralization of non-living  $P_o$  when microbial P remains constant, assuming that mineralization of non-living  $P_o$  is driven by the microbial need for energy. The empirical evidence that  $P_o$  in CF as well as soil respiration are associated with  $P_o$  mineralization should be validated by comparison with results from isotopic dilution experiments.

Our objective was to evaluate if  $P_t$  and  $P_o$  in the CF, enzyme-labile  $P_o$  fractions, and/or respiration can be used as indicators of gross and net  $P_o$  mineralization measured by isotopic dilution. To this end, we analyzed soils which were not fertilized with P or were amended with inorganic fertilizer (IF) or poultry litter (PL) for more than 10 years.

## 2. Materials and methods

### 2.1. Field experiments and soil sampling

Soil samples were collected from three long-term agricultural field experiments in the United States in August 2014 at 0–20 cm depth. These sites were located in Delaware (39°40'13"N 75°45'10"W, on a Hapludult, Woodstown series, according to the Natural Resources Conservation Service (1999)), Maryland (39°10'42"N 76°10'58"W, Hapludult, Mattapex series) and Iowa (42°01'22"N 93°46'35"W, Hapludoll, Nicollet/Webster series). These soils were classified by their particle size distribution as sandy loam (14.5% clay and 82.0% sand), silt loam (21.8% clay and 16.1% sand) and clay loam (29.2% clay and 42.0% sand), respectively. At each site, plots fertilized with inorganic fertilizers or PL (a mixture of excreta, organic bedding material, feathers and wasted feed) were sampled to obtain a wide range of values for  $P_o$  in CF and enzyme-labile  $P_o$  fractions.

The experimental design in these long-term experiments was a complete randomized plot arrangement with six replications in Delaware and Maryland (55 m<sup>2</sup> per plot), and four replications in Iowa (400 m<sup>2</sup> per plot). In Delaware and Maryland, three different treatments have been compared since 2000: 1) no P fertilizer application (Control), 2) IF (30 kg P ha<sup>-1</sup> year<sup>-1</sup>, as triple super phosphate) and 3) PL (30 kg P ha<sup>-1</sup> year<sup>-1</sup>, as PL). In Iowa, the evaluated treatments were: 1) Control, 2) inorganic N fertilization without P (Control + N) (90 kg N ha<sup>-1</sup>, as 32-0-0), and 3) PL (90 kg P ha<sup>-1</sup> year<sup>-1</sup>), all maintained since 1998. Since their establishment, Delaware and Maryland field trials were managed under corn monoculture while the Iowa study was managed under a corn/soybean (*Glycine max* L.) rotation.

All soil samples were taken eleven months after the last fertilizer application. Three out of the four-six field replications were sampled (one composite soil sample per field replicate), resulting in three samples per treatment and site. After sampling, soils were sieved (4 mm), mixed, and kept at 4 °C for up to 3 months until the laboratory experiments were performed. A portion of the soil was air-dried right after sampling, sieved through a 2-mm sieve and used for soil analysis (Section 2.2). Replicates from each soil and treatment combination were analyzed separately, except for the isotopic dilution experiment (Section 2.4), for which equal portions of all replicates of a given soil and treatment combination were mixed into a composite soil sample.

### 2.2. Soil analysis

Total C ( $C_t$ ) and N ( $N_t$ ) concentrations in soil were determined by dry-combustion. Soil  $P_o$  was determined by the ignition method (Saunders and Williams, 1955),  $P_t$  by wet digestion with H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub> (Anderson and Ingram, 1989), and  $P_i$  was derived from  $P_t$  minus  $P_o$ . Soil pH was measured in a 1:2.5 soil-to-water suspension, and particle size distribution was determined by the pipette method (Robinson, 1922). All soil analyses were performed with three analytical replicates per plot.

Additionally, the soil was physically separated into two fractions: the CF and the fine fraction (FF), following a modification of the procedure described by Cambardella and Elliott (1992). Briefly, 10 g of dry soil sample was shaken overnight with 30 ml of 1 M NaCl instead of 0.05 M (NaPO<sub>3</sub>)<sub>6</sub> to avoid the addition of P (Salas et al., 2003). After this, the suspension was wet-sieved through a 53- $\mu\text{m}$  sieve. The fraction that remained on top of the sieve was designated the CF, while the one that passed through the sieve was called the FF. After drying and milling both fractions, the concentrations of  $P_i$ ,  $P_o$ ,  $P_t$  and  $C_t$  were measured in CF and FF as detailed

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