



## Degree of short-term drying before rewetting regulates the bicarbonate-extractable and enzymatically hydrolyzable soil phosphorus fractions

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### ABSTRACT

Soil drying-rewetting can remarkably affect phosphorus (P) transformation, and thus, alter P distribution among P pools; however, little is known about the effect of the degree of drying before rewetting on labile P fractions and the bioavailability of organic P (P<sub>o</sub>). In this study, soils with distinct physico-chemical properties were allowed to desiccate to 5%, 10%, 20%, 30%, and 40% water holding capacity (WHC), and soils maintained at 50% WHC were used as controls, and the bicarbonate-extractable P and hydrolyzable P<sub>o</sub> fractions were analyzed after 5 h of rewetting. Bicarbonate-extractable P<sub>o</sub> accounted for 33.3–56.4% of extractable total P, and hydrolyzable P<sub>o</sub> constituted 34.4–79.7% of extractable P<sub>o</sub>. For extractable P<sub>o</sub>, 7.7–29.9% was labile monoester P, 6.5–14.9% was diester P, and 17.8–36.5% was phytate-like P. Bicarbonate-extractable inorganic P and P<sub>o</sub> contents were not affected by 20–40% WHC treatments, but increased by 26.3–48.1% and 5.7–52.9%, respectively, when the soils were dried to 5% WHC. Similarly, labile monoester, diester, and phytate-like P contents increased by 12.5–89.8%, 0–65.2%, and 24.6–65.6%, respectively, in 5% WHC soils. Pearson's correlation analyses showed that the relative increases in bicarbonate-extractable inorganic P and phytate-like P, following extreme drought, were positively correlated with soil organic carbon, oxalate-extractable aluminum (Al), and iron (Fe), suggesting regulatory roles of organic matter and Al/Fe oxides in P transformation during soil drying-rewetting. Taken together, our results suggest that extreme drought events before rainfall or irrigation facilitate an increase in the level of labile P, including considerable proportions of hydrolyzable P<sub>o</sub> fractions, potentially posing a substantial threat to water bodies in the context of climate change.

### 1. Introduction

Phosphorus (P) is often a limiting macronutrient in many territorial systems and its transformation is largely driven by fluctuations in soil moisture, such as drying-rewetting (DRW) (Blackwell et al., 2010). Soil DRW, a common form of abiotic perturbation, can remarkably affect microbial immobilization of P (Yevdokimov et al., 2016), alter soil P fractionation (Achat et al., 2012; Butterly et al., 2011; Styles and Coxon, 2006; Turner and Haygarth, 2003), and result in the loss of labile P via leaching and runoff (Blackwell et al., 2013; Pote et al., 1999; Schönbrunner et al., 2012; Turner et al., 2003a). The inorganic P (P<sub>i</sub>) fraction, solubilized as a result of DRW alternation, is predominantly available biologically. Soil organic P (P<sub>o</sub>) includes, but is not limited to, sugar phosphates, inositol phosphates, nucleic acids,

phospholipids, and condensed P; however, its bioavailability is still poorly understood. Numerous studies have suggested that a considerable fraction of dissolved P<sub>o</sub> in soils is amenable to hydrolysis, and thus, it might act as a potential source of P for soil microorganisms and plants (Hwang et al., 2015; Jones and Oburger, 2011) and microalgae in water bodies (Sanudo-Wilhelmy, 2006). Therefore, the characterization and determination of soil P<sub>o</sub> is of primary importance during DRW alternation.

In fact, numerous studies have quantified P<sub>o</sub> pools in soil extracts and leachates, primarily using conventional digestion (Blackwell et al., 2009; Butterly et al., 2011), <sup>31</sup>P nuclear magnetic resonance spectroscopy (Cade-Menun and Liu, 2014), or high-performance liquid chromatography (Wang et al., 2011). However, such techniques provide little information regarding the availability of the P<sub>o</sub> fraction.

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Alternatively, phosphatase hydrolysis has been used as a novel approach to characterize  $P_o$  compounds in various samples (DeLuca et al., 2015; Zhu et al., 2013). Because soil  $P_o$  must first be hydrolyzed to  $P_i$  for uptake by microorganisms and plants, enzymatic hydrolysis can provide an estimate of hydrolyzable and thus, potentially bioavailable  $P_o$  species in soils (Bünemann, 2008). Therefore, enzymatic hydrolysis is preferable for determining soil labile  $P_o$ , and more specifically, the hydrolyzable  $P_o$  fraction.

In the last decade, few studies reported remarkable changes in the hydrolyzable  $P_o$  species when moist soils or animal manures were air-dried (He et al., 2007; Turner et al., 2002a; Turner, 2005). Similarly, other studies that focused on the effects of DRW on P transformation primarily exposed soils to be basically air-dried (Achat et al., 2012; Butterly et al., 2011; Turner et al., 2002a). However, few studies considered that, in semi-arid regions, rainfall distribution is highly uneven among seasons, implying that soils would be dried to different extents before the following rainfall events, or that even within individual sites and areas, the water potential of soils would differ based on the presence and extent of vegetation. Moreover, in nature, soils are rarely dried to extremely low water potentials. Our previous study revealed that labile P pulses in a grassland soil largely depended on the degree of drying during a DRW alternation (Sun et al., 2017). Some studies have suggested that microbes make an important contribution to P pulses following rewetting (Turner and Haygarth, 2001; Turner et al., 2003c); however, our findings suggested that P pulses were primarily of non-microbial origin (Sun et al., 2017). These contrasting findings raise unanswered questions regarding the factors that play a key role in labile P transformation caused by soil DRW and whether changes in levels of labile P are related to soil physico-chemical properties, such as soil organic matter content and soil texture. Accordingly, the goal of this study was to investigate short-term P transformation in soils exposed to various intensities of drying before rewetting and to characterize the  $P_o$  fractions by phosphatase hydrolysis. We also evaluated the factors that affected P transformation during soil DRW alternation.

## 2. Materials and methods

### 2.1. Soil collection and soil characteristics

The experiment was conducted using five soil samples (0–5 cm), which were obtained from Shandong Province, eastern China. Four samples were aquic cinnamon soils (FAO classification: Luvisol) obtained from long-term wheat-corn rotation croplands located in Shouguang City (36°56' N, 118°55' E), where the mean annual precipitation and temperature were 650 mm and 12.7 °C, respectively. The remaining sample was brown soil (FAO classification: Luvisol) acquired from a natural secondary forest in Zhaoyuan City (37°27'12" N, 120°26'48" E), where the mean annual precipitation and temperature were 630 mm and 11.6 °C, respectively. These sampling sites were characterized by a similar temperate continental monsoon climate and rainfall that primarily occurred in the summer and early autumn. The total annual evapotranspiration was approximately 2.5 times that of total precipitation and therefore, the soils often experienced rapid and severe drought events. All soils were obtained in late September 2015, when the corn had been harvested and winter wheat had not yet been sowed. Plant litter and roots were carefully removed from the fresh soil samples before they were homogenized, sieved (< 2 mm), and stored at 4 °C.

The sample soils were selected based on their distinct physico-chemical properties, particularly that of soil organic carbon (SOC), iron (Fe)/aluminum (Al) oxides, and available P contents (Table 1). The soils were ranked in an ascending order according to organic C content, which ranged between 4.18 g kg<sup>-1</sup> (soil 1) and 39.09 g kg<sup>-1</sup> (soil 5). In addition, soil 5 had the highest clay, total N, ammonium oxalate-extractable Al and Fe ( $Al_{ox}$  and  $Fe_{ox}$ ) content, and soil 3 contained the highest total P, bicarbonate-extractable  $P_i$ , and  $P_o$  content. Soil 5 was

**Table 1**  
Chemical and physical properties of the five soils.

|  | Soil 1     | Soil 2   | Soil 3   | Soil 4    | Soil 5          |
|--|------------|----------|----------|-----------|-----------------|
| pH <sup>#</sup>  | 8.40a      | 8.18b    | 7.33d    | 8.01c     | 6.10e           |
| Water holding capacity (%) <sup>*†</sup>                         | 34d        | 50c      | 52c      | 58b       | 69a             |
| Soil texture (%) <sup>†</sup>                                    | Sandy loam | Loam     | Loam     | Silt loam | Silty clay loam |
| Sand (2000–50 μm)  | 65.8a      | 50.8b    | 31.0c    | 14.5d     | 4.2e            |
| Silt (50–2 μm)   | 30.3e      | 40.0d    | 49.0c    | 67.2a     | 56.5b           |
| Clay (< 2 μm)  | 3.9d       | 9.2c     | 20.0b    | 18.3b     | 39.3a           |
| $Fe_{ox}$ (g·kg <sup>-1</sup> ) <sup>§</sup>                     | 1.20b      | 0.80c    | 0.87c    | 0.99bc    | 9.14a           |
| $Al_{ox}$ (g·kg <sup>-1</sup> ) <sup>§</sup>                     | 0.47d      | 0.55d    | 0.76c    | 0.86b     | 1.13a           |
| Soil organic carbon (g·kg <sup>-1</sup> ) <sup>¶</sup>           | 4.18d      | 9.17c    | 12.84b   | 13.29b    | 39.09a          |
| Total N (g·kg <sup>-1</sup> ) <sup>¶</sup>                       | 0.54d      | 0.92c    | 1.17b    | 1.09b     | 2.55a           |
| C:N ratio <sup>¶</sup>   | 7.80e      | 9.92d    | 10.94c   | 12.24b    | 15.31a          |
| Total P (mg·kg <sup>-1</sup> ) <sup>*</sup>                      | 685.72 cd  | 723.16c  | 1141.84a | 1080.81b  | 658.06d         |
| $NaHCO_3$ -extractable $P_i$ (mg·kg <sup>-1</sup> ) <sup>*</sup> | 11.86d     | 14.55c   | 74.49a   | 39.40b    | 10.46d          |
| $NaHCO_3$ -extractable $P_o$ (mg·kg <sup>-1</sup> ) <sup>*</sup> | 11.33d     | 14.97c   | 37.25a   | 27.46b    | 13.53c          |
| Land use   | Cropland   | Cropland | Cropland | Cropland  | Forest          |

Data represent mean (n = 3). Significant differences among the five soils are indicated by different letters based on one-way ANOVA ( $P < 0.05$ ).

<sup>#</sup> 1:2.5 soil:water ratio.

<sup>\*</sup> Water contents of soils saturated and allowed to drain overnight in a Buchner funnel (Miller et al., 2005).

<sup>†</sup> Sedimentation method based on Stokes Law (Pansu and Gautheyrou, 2007).

<sup>§</sup> Ammonium oxalate-extractable Al and Fe ( $Fe_{ox}$  and  $Al_{ox}$ ), analyzed using ICP-Mass (Uusitalo and Tuhkanen, 2000).

<sup>¶</sup> Wet-oxidized and titrated with the 0.2 mol·L<sup>-1</sup>  $Fe^{2+}$  solution (Pansu and Gautheyrou, 2007).

<sup>\*</sup> Tested in moist soils (see Section 2.3).

<sup>\*</sup> Spectrocolorimetry after digestion (Pansu and Gautheyrou, 2007).

weakly acidic, whereas the others were neutral to weakly alkaline.

### 2.2. Experimental setup

Soil samples (2 g oven-dry base) were pre-incubated in 50-mL centrifuge tubes at 50% water holding capacity (WHC) and 25 °C for 7 d. Next, a water-stress event was imposed by drying the samples using nylon bags filled with self-indicating silica desiccant, and the target water content was achieved by periodically weighing the centrifuge tubes and replacing the nylon bags until the soils reached 5%, 10%, 20%, 30%, 40%, or 50% WHC within 3 d (n = 3 per treatment). After the water-stress event, the soils were maintained at their respective water contents for another 7 d because the inter-rainfall intervals ranged between 7 d and 14 d in the summer and autumn when seasonal evapotranspiration rates were the greatest.

Subsequently, the soils were rewetted to 50% WHC for 5 h and extracted with 0.5 mol L<sup>-1</sup>  $NaHCO_3$  (pH 8.5, 1:20 soil: solution) on an end-over-end shaker for 30 min (Olsen et al., 1954). Next, the extracts were centrifuged at 3000 × g for 10 min and the supernatant was filtered through a 0.45-μm membrane. The filtrates were immediately analyzed for P fractions, or stored at -20 °C and thawed at 4 °C before analysis.

### 2.3. Soil bicarbonate-extractable $P_i$ and $P_o$ determination

The  $NaHCO_3$ -extractable  $P_i$  was determined colorimetrically using the malachite-green method (Ohno and Zibilske, 1991), with absorbance measurement in 4-mL cuvettes at 630 nm. Reagents for the assay were prepared as described by Ohno and Zibilske (1991). Reagent A was 14.2 mmol L<sup>-1</sup> ammonium molybdate tetrahydrate prepared in 3.15 mol L<sup>-1</sup>  $H_2SO_4$ . Reagent B was 3.5 g L<sup>-1</sup> polyvinyl alcohol

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