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Geochemical and microbial controls of the effect of citrate on phosphorus availability in a ferralsol



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ABSTRACT

The secretion of organic anions, particularly citrate, by plant roots may be an effective process to mobilize phosphate (PO_4) from solid constituents. However, microbial growth and corresponding phosphorus (P) immobilization can act adversely. This experimental study was aimed at studying the relative influence of geochemistry and microbiology on the influence of citrate on P availability in a tropical soil. We carried out water extractions with different citrate concentrations (0–500 μ M). A first set of experiment was performed at low temperature (T = 2 °C) in order to have only geochemical effects. We performed two other set of experiments at room temperature (T = 25 °C), without and with addition of glucose and nitrate, in order to investigate the influence of an increase of microbial activity. We measured an overall increase of P availability with citrate concentration in all the treatments. Results obtained at low temperature were consistent with the lack of significant P immobilization by microbial activity. The concomitant increase of dissolved Si, Al and Fe suggested that citrate enhanced the dissolution of PO₄-adsorbing minerals. This geochemical process should release PO₄ and increase P availability. The overall increase of P availability with temperature further suggested that the dissolution of Al- and Fe-PO₄ minerals was also responsible of the effect of citrate. Lastly, results obtained at 25 °C without addition of glucose and nitrate exhibited a weak effect of microbial activity, as a decrease of P availability was only observed at the lowest citrate concentration (2 µM). The addition of glucose and nitrate enhanced microbial activity as a net immobilization of PO₄ was observed up to 20 µM of citrate. Beyond this threshold, microbial growth appeared limited by other factors as time. Our study revealed that the effect of citrate on P availability was mainly controlled by complex geochemical interactions in a range of temperature conditions. The adverse influence of microbial growth increased with temperature and nutrient supply but revealed limited. The nature and intensity of controlling geochemical processes depend on soil type and fertilization.

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1. Introduction

Phosphorus (P) is an important nutrient for plant growth as it is an essential element required for cellular function and it cannot be replaced in most of the plants biological functions. This resource is very scarce as phosphate (PO₄) concentration in the soil solution is extremely low (e.g. Hinsinger, 2001). The high adsorption affinity and capacity of Fe/Al oxides and clay minerals is probably responsible for this (e.g. Gérard, 2016; Shen et al., 2011). In addition, the solubility of variscite (AlPO₄), strengite (FePO₄), and apatite (Ca-P mineral) may also control dissolved PO₄ under acid and alkaline conditions respectively (Cui and Weng, 2013; Iuliano et al., 2007; Lindsay et al., 1959; Tunesi et al., 1999). Such low phosphorus availability in soil represents a major constraint to crop production (e.g. Runge-Metzger, 1995). The use of

* Corresponding author. *E-mail address:* gerard@supagro.inra.fr (F. Gerard). mineral P fertilizer increases soil P availability and crop yield (e.g. Tilman et al., 2002), but P overloads from manures and fertilizers, which enrich the surface layers of soil, contribute to pollution and eutrophication of surface waters (e.g. Gerber et al., 2005). Moreover, due to increasing demand of mineral P fertilizer and declining stocks, it is predicted that current global reserves of phosphate rock may be depleted within the next century (Cordell et al., 2009). Then, P use must therefore become much more efficient in the future.

Management practices for a better crop P use efficiency of P derived from soil or from fertilizer will be based on a better understanding of rhizosphere processes. Many plant species have evolved to respond to P limited environments. As a result, they exhibit adaptive features that can enhance P acquisition from soil (e.g. Raghothama, 1999; Richardson et al., 2007; Vance et al., 2003). These adaptive functions include the secretion of phosphatase enzymes and low molecular weight organic anions by roots into the soil to mobilize P from inorganic and organic P sources and bound P (George and Richardson, 2008; Hinsinger,



2001; Jones and Oburger, 2011; Marschner, 1995). Generally, P deficiency conditions stimulate an increasing of organic anion efflux from roots (Hoffland et al., 1989; Kirk et al., 1999; Lipton et al., 1987). The presence of organic anions can alter P-controlling processes so that an increase of dissolved PO₄ can occur in the root zone (e.g. Gang et al., 2012; Ryan et al., 2001). Among the variety of exuded organic anions, citrate is recognized as the most effective to increase P availability in soil (Hu et al., 2001; Oburger et al., 2011; Ryan et al., 2001; Ström et al., 2005). The competition between adsorbed organic ligands and PO₄ for the same binding sites may be responsible for the increase of available P in soil (Duputel et al., 2013a; Geelhoed et al., 1999; Oburger et al., 2011). Ligand-promoted dissolution of PO₄-adsorbing minerals such as illite can also contribute to the release of PO₄ from soil after citrate addition (Duputel et al., 2013b). The presence of organic ligands in the rhizosphere can also enhance the dissolution rate of soil minerals including those containing PO₄ (e.g. Qin et al., 2013).

However, citrate as many other forms of dissolved carbon (e.g. glucose) is also an important source of energy for most microorganisms. Accordingly, when soluble carbon is available for microbial respiration and growth, P immobilization by microbes can directly affect P availability by removing PO₄ from the soil solution (Bünemann et al., 2004a, Olander and Vitousek, 2004). Such an increase in microbial P is usually linked with a rapid decrease of soil solution P concentration (Oehl et al., 2001). Microorganisms therefore can compete with plants for available P (e.g. Olander and Vitousek, 2004). Soluble carbon is not the only nutrient resource that can affect microbial activity. Nitrogen (N) is also an important factor for microbial growth. For example, Bünemann et al. (2004b) and Ehlers et al. (2010) showed that microbial growth was colimited by C and N in soils with low P availability. These effects are accentuated by abiotic factors, such as temperature, one of the most important environmental factors known to stimulate microorganism growth and biological activities of soils (e.g. Biederbeck and Camplbell, 1973; Qiu et al., 2005). In brief, the presence of nutrients resources for microorganisms and temperature should have significant effects on the citrate-induced release of PO4 from soil. The increase of dissolved PO₄ induced by geochemical interactions between organic ligands and soil minerals (i.e. desorption and/or dissolution) can be offset by microbial immobilization of dissolved PO₄. The influence of microbial activity resulted in Duputel et al. (2013a) to set incubations at low temperature (T = 2 $^{\circ}$ C) in order to investigate the nature of geochemical processes controlling P availability after addition of citrate in soil. These authors used a mechanistic approach of the modeling of adsorption/desorption (i.e. sorption process) to ascribe the observed decreases of P availability after addition of relatively low citrate concentrations (2-50 µM) to the effect of electrostatic interactions involving 2:1 clay minerals and adsorbed calcium. The geochemical model used in this former study also predicted that citrate adsorption should increase P availability in ferralsols at any citrate concentration (Duputel et al., 2013b).

The objectives of the present investigation are two-fold: (i) to study effect of different citrate concentrations on P availability in a fertilized ferralsol under low temperature conditions (T = 2 °C), and (ii) to investigate the influence of an increase of microbial activity as induced by an increase of temperature and C and N supply.

2. Material and methods

2.1. Soil origin and properties

The soil studied was collected in the Malagasy Highlands at Lazaina (18°46′ S, 47°32′ E). It is a sandy clay loam quite typical of the *tanety* (hills) of the crystalline basement of the island. It is classified as a Ferralsol (IUSS Working Group WRB, 2014). The area is characterized by long-term grassland fallows dominated by *Aristida* species. Selected properties of the uncropped ferralsol were presented by Chapuis-Lardy et al. (2009).

An experimental design was established on the site in 2006. It included traditional hand tillage and plots received various amounts of phosphorus. We collected soil samples from the 0–20 cm horizon of four plots that received 50 kg P ha⁻¹ year⁻¹ as triple superphosphate (TSP) during five years of cultivation. We studied a composite soil sample made with the four samples. The composite soil sample was airdried and sieved at 2 mm. We also ground a part of it at 0.2 mm to perform specific analyzes.

The physico-chemical properties and mineralogy of our soil are shown in Table 1. Total carbon and nitrogen were determined by dry combustion in a CHN Analyzer (FISONS Instruments, NA 2000, N Protein) using 20 mg of ground soil. Total P was determined by the molybdenum blue method using a UV/VIS Spectrophotometer (Metertech SP 8001), after digestion with concentrated perchloric acid (Kuo, 1996). The cation exchange capacity (CEC) was determined by the cobaltihexamine chloride method using sieved soil with a soil:solution ratio of 1:10. The amounts of gibbsite and kaolinite were obtained by thermogravimetric analysis (TGA) with a ATD/TG analyzer (STA 409, NETZSCH). This method involves measuring the variation of mass of a sample by using thermobalance depending on the temperature in an inert environment. Analyses were conducted from 20 to 1000 °C under argon atmosphere using 200 mg of finely crushed soil. The amount of gibbsite and kaolinite were calculated using theoretical water losses of 31.2% and 14.0%, respectively. The amount and type of iron oxides in the soil were determined after extraction with sodium citrate-bicarbonate-dithionite (Fe CBD) and oxalate (Fe oxalate). These measures show that most of the iron oxides of the soil are well-crystallized, as Fe CBD largely exceeded Fe oxalate (see Table 1), and certainly correspond to goethite.

2.2. Experimental setup

We performed soil extractions using sub-samples with a ratio 1:5 using ultrapure water (for control) and water containing different dissolved concentrations in trisodium citrate dihydrate, ranging from 2 to 500 µM of citrate (i.e. 2, 10, 20, 50, 100, 250 and 500 µM). These values cover the range of dissolved citrate concentrations present in soil and rhizosphere (Jones, 1998). Note that we used the same concentrations and went slightly beyond the maximum considered in the early experimental study of Duputel et al. (2013a): i.e. 100 µM. All the soil extractions were replicated five times and extractions lasted 30 min at 75 rpm with a continuous stirring using rotating mixer (Intelli-Mixer RM2, EMLI). Then, samples were centrifuged at 3000 rpm during 10 min (JOUAN GR4i Centrifuge, Thermo Electron Corporation) and supernatants were filtered through a 0.2 µm cellulose acetate membrane (Legallais).

We performed three sets of extractions corresponding to different experimental conditions. The first set was performed at low temperature ($T = 2 \ ^{\circ}C$) in an attempt to minimize microbial activity and thus to better reveal the effects of geochemistry. Microbial activity was promoted in the two other treatments, corresponding to soil extractions performed (i) at room temperature ($T = 25 \ ^{\circ}C$) using the same conditions as at low temperature and (ii) with addition of 1 mM of glucose (C source) and 1 mM of sodium nitrate (N source) in the extracting

 Table 1

 Physicochemical properties and mineralogy of the ferralsol studied.

Total carbon (g kg ⁻¹)	16.4
Total nitrogen (g kg ⁻¹)	0.91
Total phosphorus (mg kg $^{-1}$)	607
C/N	18.1
$CEC (cmol_c kg^{-1})$	1.6
Gibbsite (g kg ⁻¹)	247
Kaolinite (g kg $^{-1}$)	244
Fe CBD (g kg ^{-1})	25
Fe oxalate (g kg^{-1})	1.5

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