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Do earthworms affect phosphorus availability to grass? A pot experiment

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The largest part of phosphorus (P) in soil is bound by the soil solid phase; its release to the soil solution therefore often does not meet the demand of plants. Since global P fertilizer reserves are declining, it becomes increasingly important to better utilize soil P. We tested whether earthworm activity can increase P availability to grass (*Lolium perenne* L.) in a 75-day greenhouse pot experiment in a soil with low P availability. The full factorial design included two factors: P fertilization (control without P; phytate; and inorganic P) and earthworm population (control without earthworms; *Lumbricus rubellus* Hoffmeister, Lr; *Aporrectodea caliginosa* Savigny, Ac; and *Lumbricus terrestris* L., Lt). At four times during the experiment, aboveground plant growth and P uptake were determined. In a separate incubation experiment, earthworm casts and bulk soil were analyzed for inorganic and organic P in water extracts. We observed higher levels of dissolved P pools ($p < 0.001$) in the water extracts of earthworm casts compared to those of the bulk soil. The magnitude of the difference differed between earthworm species, with the largest levels for Lr: from <0.02 to 8.56 mg L^{-1} for inorganic P ($p = 0.007$) and from 0.18 to 1.30 mg L^{-1} for organic P ($p = 0.007$). After three harvests, presence of Lt significantly increased P uptake by grass to 44.1 mg per pot compared to 41.8 mg per pot for the control ($p = 0.010$). Plant growth increased from 15.68 to 16.85 g dry biomass per pot ($p < 0.001$). We conclude that earthworms casts contain higher levels of plant available P than the bulk soil, and that this might translate into increased plant P uptake. It is well-known that maintaining soil faunal biodiversity is important for a variety of ecosystem services; our results show that these ecosystem services may include improving the utilization of soil P in a world with rapidly declining P stocks.

1. Introduction

Phosphorus (P) is an essential nutrient for plant growth and it is taken up by plants from the soil solution in the form of orthophosphate (ortho-P) (Hawkesford et al., 2012). The quantity of ortho-P in soil solution usually represents <1% of annual P uptake by crops, while the remaining >99% is supplied by the soil solid phase over the growing season (Morel et al., 2000; Hesterberg et al., 2010). Desorption of ortho-P bound to reactive metal oxides within the soil solid phase to soil solution and subsequent diffusion to the surface of the plant roots are important processes in determining the rate by which ortho-P is taken up (Van Noordwijk et al., 1990; Jungk and Claassen, 1997; Morel et al., 2000; Koopmans et al., 2004). Additionally, the release of ortho-P to soil solution through mineralization of soil organic P can contribute to P nutrition of plants (Richardson, 2001). On the other hand, plants themselves have developed strategies to enhance P uptake, especially by adapting their root morphology, entering symbiosis with mycorrhizal fungi (Raghothama, 1999; Lynch and Brown, 2001), or by excretion of organic acids and phosphatases (Bieleski, 1973; Tarafdar and Jungk, 1987). Hence, P uptake by plants is the result of a complex interplay between physical, chemical, and biological processes.

Most agricultural soils are unable to supply sufficient amounts of P to plants to realize an optimal crop yield, due to their low native soil P contents and strong P binding to the soil solid phase (Hinsinger, 2001). In such soils, the low availability of P leads to a P-limited growth of plants and this is often ameliorated through application of inorganic P fertilizer or animal manure. The application of microbial inoculants (Richardson, 2001) or the use of new crop genotypes (Lynch and Brown, 2001) have been suggested as an alternative for inorganic P fertilizer application to alleviate low P availability.

A hitherto little studied topic is the role of soil macrofauna in the soil P cycle in relation to plant growth. Especially earthworms may

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play an important role in this respect, as they are considered "ecosystem engineers". They can to a large extent affect soil carbon (C) and nitrogen (N) cycles as well as physical–chemical properties of soils (e.g. soil aggregation and aeration) (Darwin, 1881; Jones et al., 1994).

Earthworms ingest their food selectively and especially feed on fresh organic matter or further decomposed organic matter associated with the soil solid phase (Doube et al., 1997; Curry and Schmidt, 2007). When earthworms burrow through the soil, they create macro-pores (Schrader et al., 2007), mix organic matter and soil particles, and disperse soil micro-organisms (Devliegher and Verstraete, 1997; Le Bayon and Milleret, 2009). Consequently, earthworms stimulate mineralization of soil organic matter (SOM) and increase soil fertility (Postma-Blaauw et al., 2006). Excretion of casts, enzymes, and mucus are other examples of how earthworms can modify microbial activity and SOM dynamics (Lopez-Hernandez et al., 1993; Tiunov and Scheu, 2000; Bernard et al., 2012).

The effects earthworms can have on soil properties differ between earthworm species. Observed differences are often attributed to variation in their feeding and burrowing behavior (Lattaud et al., 1997; Suárez et al., 2004). Earthworms are typically classified in three ecological categories: (i) epigeic (feed on surface litter and live in the litter layer and top centimeters of soil); (ii) endogeic (feed on soil and associated organic matter and live in non-permanent burrows deeper in the soil); and (iii) anecic species (feed on surface litter and make permanent vertical burrows) (Bouché, 1977). However, some species show characteristics belonging to multiple groups and can, therefore, not be fully classified within one functional group. For instance, *Lumbricus rubellus*, a common earthworm in temperate regions, is often classified as epi-endogeic, whereas the behavior of *Lumbricus terrestris*, an earthworm that used to be classified as anecic, is recently more often described as epi-anecic.

In many studies, positive effects of earthworm presence on plant growth have been observed (Scheu, 2003), and different mechanisms have been suggested for this (Brown et al., 2004). However, relatively little attention has been paid to effects of earthworms on the soil P cycle (Le Bayon and Milleret, 2009). Higher levels of available P for plant uptake have been measured in earthworm casts compared to the bulk soil (Sharpley and Syers, 1976; Lopez-Hernandez et al., 1993; Kuczak et al., 2006) and increased plant growth as a result of a higher P availability has been documented before (Mackay et al., 1982). Hypothesized pathways for this effect included selective feeding behavior of earthworms, (thereby concentrating SOM, clay and nutrients), as well as processes taking place in the earthworm gut and cast (Brown et al., 2004). Furthermore, earthworms have recently been reported to be able to modify P dynamics and plant interactions in an intercropping system (Coulis et al., 2014). However, it is still largely unknown to what extent earthworms can beneficially affect P uptake and plant growth in agricultural systems, and through which mechanisms they influence the plant availability of P.

The objective of the present study was to examine whether earthworm activity can improve the utilization of soil P by grass, through increasing the fraction of ortho-P in the soil solution. Our hypotheses are: P availability in soil water extracts, which are used as a proxy for soil pore water (Torrent and Delgado, 2001; Koopmans et al., 2006) are elevated for earthworm casts compared to those for the bulk soil (H1); under P limiting conditions earthworm presence will increase P availability in water extracts of the bulk soil (H2); as well as P uptake and aboveground grass biomass production (H3). Furthermore, as we expect stimulation of the microbial community and elevated mineralization of organic P to be the main pathway through which earthworms affect P uptake by plants and plant growth, observed effects are expected to be largest for soil fertilized with myo-inositol hexa*kis*phosphate (phytate), which is a major form of organic P in soils (Turner et al., 2002) (H4). Finally, the effects on P uptake and aboveground grass biomass are hypothesized to be larger for epi-endogeic earthworms than for endogeic and anecic earthworms (H5), as epi-endogeic earthworms are active throughout the rooting zone.

2. Materials and methods

2.1. Soil characteristics

The topsoil (0–20 cm) of a Umbric Gleysol (FAO, 2006) was collected from an agricultural pasture in April 2013 in Joppe, the Netherlands (52°20′N, 6°23′E). For physical–chemical characterization, the soil was dried at 40 °C and sieved (2 mm). The particle size distribution was determined with a sieve and pipet and SOM was estimated from loss-on-ignition in a muffle furnace (550 °C). Loss of water from the crystalline structure of clay particles was taken into account (Houba et al., 1997). The pH was measured with a combined glass electrode in a 1:10 (w:v) suspension of soil in ultra-pure water (UPW). The availability of P for plant uptake was determined using an extraction with acetic acid and ammonium lactate (P-AL) (Egnér et al., 1960). In the Netherlands, P-AL is used as a soil test in agricultural practice for P fertilizer recommendation of grassland. The amount of P adsorbed to reactive metal oxides (P_{ox}) as well as the amounts of reactive aluminum and iron oxides (Al_{ox} and Fe_{ox}) were determined by the extraction of soil with acid ammonium oxalate (Schwertmann, 1964). Based on these results, the degree of P saturation of the soil with respect to its content of reactive metal oxides was calculated:

$$\alpha = \frac{P_{ox}}{[Al + Fe]_{ox}} \qquad (1)$$

where P_{ox} and $[Al + Fe]_{ox}$ are expressed in mmol kg^{-1} (Van der Zee and Van Riemsdijk, 1988). The soil organic P content was calculated as the difference between total inorganic P measured after combustion of organic matter in a muffle furnace (550 °C) and subsequent digestion of soil with 0.5 M H_2SO_4 and inorganic P measured after digestion of soil with 0.5 M H_2SO_4 (Kuo, 1996).

2.2. Experimental design

We set up a 75-day pot experiment consisting of 48 pots (*plant experiment*) in the greenhouse. The experiment was laid out as a fully factorial design with three P fertilization treatments and four earthworm treatments as independent factors. The replicates were distributed over four blocks, and both the location of the block and the setup within the block was randomized weekly. A separate experiment (*cast experiment*) was performed simultaneously. This consisted of six pots, with two replicates of the three earthworm species on the same non-P-fertilized soil as in the plant experiment. In both experiments, Mitscherlich pots with a diameter of 20 cm and a depth of 23 cm were used.

Each pot was filled with 6 kg of air-dried, sieved (5 mm), and homogenized soil. All pots were fertilized with 133 kg N ha^{-1} (with equal amounts of NH_4^+ and NO_3^-), 108 kg K ha^{-1}, 12 kg S ha^{-1}, 50 kg Ca ha^{-1}, and micro-nutrients. The P fertilization treatments included: (i) control without P fertilization; (ii) fertilization with phytate ($C_6H_{18}O_{24}P_6 \cdot xNa \cdot yH_2O$); and (iii) fertilization with inorganic P (KH_2PO_4). The P application rate in treatments (ii) and (iii) was 65 kg P ha^{-1}, based on the measured P-AL value of the initial soil and the Dutch P fertilization recommendation for grassland