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One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services

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HIGHLIGHTS

- Phosphate fertilization reduced arbuscular mycorrhizal colonization rates.
- Phosphate fertilization reduced the abundance of bacterial- and fungal-feeding nematodes.
- Phosphate fertilization shifted the bacterial, fungal and AM fungal community structures.
- Phosphate fertilization depressed abundance of *phoD* copy numbers.
- Rye grass dry matter yield did not significantly change with phosphate fertilization.

GRAPHICAL ABSTRACT

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ABSTRACT

This study evaluated the effect of one-time phosphate fertilization on the soil microbiota, its cycling of phosphorus (P) and grass growth. Soil columns were established in a greenhouse using a P-limited Irish soil (index 1), planted with *Lolium perenne* and fertilized with 0 (control), 5 (quarter), 10 (half) and 20 (full) kg P ha^{-1} as inorganic phosphate. Only traces of phosphate in soil solution were detected over the 14 week experiment, even after phosphate fertilization. Grass dry matter yield between treatments was not significantly different. Full phosphate fertilization significantly reduced the arbuscular mycorrhization (AM) rate, bacterial- and fungal-feeding nematode population, bacterial *phoD* gene abundance, but increased alkaline and acid phosphatase activities at the time of harvest. Full and half P treatments significantly shifted the bacterial, fungal and AM community structures compared to the control. Furthermore, the control had a significantly higher relative abundance of bacterial genera including *Bacillus*, *Bradyrhizobium*, *Paenibacillus*, *Nocardioides* and *Balneimonas*, that have been associated with P mobilization in the past, when compared to the full phosphate treatment. These results suggest that a positive effect of a single phosphate application on plant growth in a soil can be cancelled out by its negative effect on the soil microbiota and their ecosystem services.

1. Introduction

In order to feed the rising world population, the United Nations has predicted that food production needs to increase by 50% in 2020 (Nellemann et al., 2009). Grasslands play significant roles in food

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production as major source of feed for ruminants used for meat and milk production. About 70% of the world's agricultural land is occupied by grasslands making grass the world's most important crop (O'Mara, 2012). In Ireland, the main agricultural land use is grasslands with about 80% of the total land area devoted to permanent grasslands (Rath and Peel, 2005).

In conventional agriculture, high-yielding crop varieties, irrigation, pesticides and inorganic fertilizers are frequently used to attain higher crop yields (Matson et al., 1997) as well as to replace offtakes. However, decrease in agricultural soil quality and increased risks of environmental pollution are often associated with intensive use of inorganic fertilizer inputs (Tilman et al., 2002). Soil bacteria and fungi are important drivers of nutrient cycling in the soil. These microbes mediate the cycling of carbon (C), nitrogen (N), phosphorus (P), sulfur (S) and other elements thereby having great influence on crop productivity (Nannipieri et al., 2003; Kertesz and Mirleau, 2004). The development of sustainable agricultural systems is therefore premised on the understanding of the contribution of these microorganisms to plant nutrition and plant growth and their response to intensive application of inorganic fertilizers.

P is an essential macronutrient in plant nutrition and the second most important plant nutrient after N. It plays vital roles in most of the metabolic processes in plants including photosynthesis, energy transfer, signal transduction, biosynthesis of macromolecules and energy transduction (Khan et al., 2010). Unlike N with a large atmospheric pool, the world's source of high quality phosphate is finite making the development of more P efficient plant or agricultural systems essential (Cordell et al., 2009). Despite many soils containing a large amount of total P, only a small proportion (usually <1%; (George et al., 2011)) is available for plant uptake because most of the soil P is organically and inorganically bound. In order to provide plants with sufficient available P in agriculture, the principle of building up of P stocks via regular P fertilization is widely practiced (Voss, 1998). However, plant yield response to P fertilization can vary greatly. A recent study of Irish pastures has shown that in two out of eight soil series, no substantial increases in yield were identified within four years (Schulte and Herlihy, 2007). Microorganisms play important roles in the maintenance of plant available P pools because they mediate to a large extent the mineralization of organic P in soils (Macklon et al., 1997; Richardson et al., 2005). Soil microbes are important in the maintenance of pools of both inorganic and organic P in soil solution and biomass turnover represents an important potential source of plant P supply (Seeling and Zasoski, 1993; Oberson et al., 2005). The soil microbiota's ecosystem service in P cycling is an essential pathway for P movement and transport from various soil pools into plant available forms (Magid et al., 1996; Oberson et al., 2001). However, a paucity of studies have characterised the effects of inorganic P-based fertilizer management on soil microbial P cycling in P-limited soils until now.

This study aimed to provide a qualitative and quantitative understanding of how the soil microbiota and plants are affected by phosphate fertilization. The specific objectives were to: assess the microbial community (bacteria, fungi, AM fungi, nematodes) size, abundance, composition, and function in P cycling alongside the uptake of phosphate by the grassland plant *Lolium perenne* as affected by one-time application of different rates of phosphate fertilizer. We hypothesized that reduced phosphate application will benefit the abundance and affect the structure of soil microbiota that can contribute to P availability in grasslands.

2. Material and methods

2.1. Experimental setup, column harvest, plant and soil analyses

Soil columns were setup in a greenhouse using a P-limited soil (Irish soil P index 1) collected from Moorestown Cahir (County Tipperary, Ireland). In Ireland, Morgan's extractable P is used as the agronomic soil test for P and this has been classified as indices: 1 (deficient), 2 (low), 3 (optimum) and 4 (excessive) (Lalor and Coulter, 2008). The site had not been under cultivation for over 20 years (Cornelius Traas, personal communication, 2015). The soil type is a grey brown podzolic soil, with a sandy silt loam topsoil (10% clay) with a pH of 6.8 (FW; Lancrop Laboratories Ltd., York, UK). Soils were sampled from 0 to 20 cm and 20–40 cm of the profile, sieved through a 3.35 mm mesh to remove stones, mixed and repacked into the columns in layers of 0–20 and 20–40 cm as in the field. The columns consisted of 16 cm × 40 cm pipes and were planted with rye grass (*Lolium perenne* variety Trend). They were fertilized with 0, 5, 10 and 20 kg P ha^{-1} inorganic phosphate P treatments (potassiumphosphate in water, pH 7, applied on column surface) representing control, quarter, half and full rates respectively (full P and half P represents the recommended amount of P application for build-up rates at indices 1 and 2 respectively) alongside a full complement of other nutrients (125 kg ha^{-1} N, 150 kg ha^{-1} K, 20 kg ha^{-1} S and micronutrients). Each treatment was replicated six times and managed for 14 weeks. The columns were watered with 200 ml of rain water, three times a week. Weekly soil solution samples were collected in 10 cm height intervals using Rhizons (Rhizosphere Research Products, Wageningen, Netherlands) and analysed for phosphate, sulfate and other anions via ion chromatography using a Dionex ICS1100 with an AS23 column and a carbonate mobile phase following the manufacturer's recommendation (Dionex, Sunnyvale, CA).

Grasses were cut back to 5 cm height after seven weeks of growth and entire grass shoots were harvested at the end of the experiment for dry matter determination. At the end of 14 weeks of grass growth, columns were deconstructed and grass shoot and root biomass were determined. Rhizosphere soil samples (soil shaken off roots) were collected for phosphatase activity determination, nematode analyses and other soil properties, while root samples were collected for mycorrhizal colonization analysis (see below).

For both cuts, grass shoots were dried at 55 °C for 72 h in a fan oven and dry weights were recorded. The elemental compositions of the dried shoot biomass were determined at Lancrop Laboratories Ltd. (employing atomic absorption spectroscopy, inductively coupled plasma spectrometry, titrations, and spectrophotometry, accredited to ISO/IEC 17025:2005).

Prior to P determination, soil samples were dried overnight at 40 °C and sieved using a 2 mm mesh. Morgan's extractable P (surrogate for plant available P) was determined by extracting soil with sodium hydroxide-acetic acid solution (pH 4.8) in a 1:5 soil to solution ratio (Peech and English, 1944). The extracts were filtered through Whatman No. 2 filter paper. P concentrations in the extracts were determined by colorimetry at 880 nm using a Camspec M500 UV–Visible Spectrophotometer (Camspec, UK) following the molybdate-ascorbic acid method (Murphy and Riley, 1962). The pH of the soil was measured potentiometrically using deionised water in a 1:2 soil solution ratio (McCormack, 2002).

2.2. Cultivation-dependent analysis of P mobilizing bacteria, potential phosphatase activity, mycorrhizal root colonization and nematode abundance determination

Bacteria were extracted from the rhizosphere soil in the top 10 cm of the column as follows. After the deconstruction of the columns, 3 g of roots with attached soils were added to 50 ml tubes containing 20 ml sterile saline (0.85% [w/v] NaCl) solution and rotated at 75 rpm on an Elmi Intelli-Mixer RM-2 (Elmi Tech Ltd., Latvia) for 30 min at 4 °C. Serial dilution was made by using 0.1 ml of the resultant suspension. The total heterotrophic bacteria (cultivated in Reasoner 2 media) and cultivable bacteria capable of utilizing P from phytate (phosphate-esters, MM2Phy) and phosphonoacetic acid (MM2PAA) as sole source of P, were quantified via a most probable number (MPN) approach in microtiter plates (Fox et al., 2014). Colony forming units (CFU) were established to determine the cultivable bacteria solubilizing P from