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Phosphorus addition enhances loss of nitrogen in a phosphorus-poor soil

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ABSTRACT

Plants and microbes have limited stoichiometric flexibility to take up and store nitrogen (N) and phosphorus (P). Variation in the relative availability of N and P to plants and microbes may therefore affect how strongly N and P are held in terrestrial ecosystems with important implications for net primary productivity and carbon sequestration. We hypothesized that an increase in P availability in a P-poor soil would increase N uptake by plants and microbes thereby reducing N loss. We grew mixtures of the C3 grass *Phalaris aquatica* L. and the legume *Medicago sativa* L. in mesocosms with soils low in P availability and then used a novel technique by adding a ^{15}N tracer with and without 1 g P m^{-2} to soil with different moisture and available N conditions, and measured the ^{15}N recovery after 48 h in microbes, plants and soil. In contrast to our hypothesis, we found that P addition reduced ^{15}N in microbes without water stress by 80% and also reduced total ^{15}N recovery, particularly without water stress. Water stress in combination with N addition further showed low total ^{15}N recovery, possibly because of reduced plant uptake thereby leaving more ^{15}N in the soil available for nitrification and denitrification. Our results suggest that P addition can result in large gaseous N loss in P-poor soils, most likely by directly stimulating nitrification and denitrification.

1. Introduction

Nitrogen (N) and phosphorus (P) are important nutrients frequently limiting plant growth in terrestrial ecosystems (Elser et al., 2007; Harpole et al., 2011). Increased atmospheric N deposition caused by fuel combustion, agriculture and other human activities, has resulted in greater availability of N relative to P in many ecosystems worldwide affecting ecosystem structure, functioning and diversity (Vitousek et al., 1997; Galloway et al., 2008; Peñuelas et al., 2013). With dwindling supplies of phosphate rocks needed to manufacture P fertilizers, the imbalanced availability of N and P (i.e., reduced P availability compared to N) has become particularly critical in agricultural systems with important consequences for food security (Peñuelas et al., 2013; van der Velde et al., 2014). Excess of available N compared to P could enhance gaseous N loss through microbially-mediated processes of nitrification and denitrification (Vitousek et al., 1997; Hall and Matson, 1999; Stehfest and Bouwman, 2006) that are often limited by N (Bouwman et al., 2002). Because of N/P stoichiometric constraints of plants and microbes, excess of available N compared to P may enhance P limitation, which could affect N retention and loss in different ways than when plants and microbes are N limited.

Plant growth is frequently constrained by P availability (Vitousek et al., 2010), but soil microbial activity can also show P limitation. Microbes, more so than plants, have a high P requirement compared to N. Microbial N/P ratios were lower than plant N/P and soil N/P across a wide variety of ecosystems (Cleveland and Liptzin, 2007), suggesting that microbial activity may be more constrained by P than plant activity. The requirement for P may increase relative to N with increased microbial activity because of the greater need of P-rich RNA to synthesize proteins (Elser et al., 1996; Franklin et al., 2011). Microbial activity and growth may therefore particularly be constrained by P when activity is high. Microbial P limitation has mostly been observed in highly weathered tropical soils (Cleveland et al., 2002; Ehlers et al., 2010), but also in calcareous (Raiesi and Ghollarata, 2006), peat (Hill et al., 2014), and boreal forest soils (Giesler et al., 2002).

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The N and P cycles are intricately linked because of how these nutrients are taken up, stored and released by plants and microbes (Sterner and Elser, 2002; Finzi et al., 2011; Townsend et al., 2011). This coupled cycling of N and P by biological processes is enhanced with increased water availability (Delgado-Baquerizo et al., 2013). Because microbial activity in upland soils often increases with increased soil moisture (Ise and Moorcroft, 2006), microbial uptake of N and P increases as well (Matías et al., 2011; Sun et al., 2013). When microbial activity is limited by P, increased soil P availability may increase N inputs through biological N fixation (Houlton et al., 2008), and possibly N retention through microbial N immobilisation (Johannisson et al., 1999; Vesterdal and Raulund-Rasmussen, 2002). On the other hand, more recent studies have suggested that an increase in P availability can also enhance N mineralisation, nitrification and denitrification, thereby increasing N loss (Mori et al., 2010; Fisk et al., 2014; Zhang et al., 2014). Effects of P availability on N retention and loss could increase with increased soil moisture with important implications for plant productivity and carbon sequestration that remain poorly understood.

To assess short-term effects of P availability on plant and microbial N uptake we used a novel technique by adding a ^{15}N tracer with and without 1 g P m^{-2} to soil with different moisture and available N conditions, and measured the ^{15}N recovery after 48 h in microbes, plants and soil. We hypothesized that greater P availability increases N uptake by plants and microbes thereby reducing N loss. We further hypothesized that P availability effects on reducing N loss would particularly be important in soils without water stress and in soils with more N.

2. Materials and methods

2.1. Experimental design

In September 2013, we collected surface soil (0–20 cm) from a grassland, located at Westwood farm of the University of Sydney, near Camden, NSW, Australia (latitude 34.01°S, longitude 150.65°E). The soil is a sandy loam red Kurosol (Australian soil classification, Isbell, 2002), with a pH of 5.3, 5.6% C, 0.4% N and 0.14% P, and showed a high capacity to fix P (Dijkstra et al., in review). The soil was crushed and sieved through a 4 mm sieve and visible roots were removed. We packed the soil (2.5 kg, air-dry basis) into 32 pots (diam. 10 cm, height 25 cm) that were made of polyvinyl chloride (PVC) and sealed with caps at the bottom that prevented leaching. The pots were placed in a growth chamber at the Centre for Carbon, Water and Food in Camden, NSW. Temperature was kept at 25/15 °C during the day/night with 12 h of light of ~500 μmol m^{-2} s^{-1} using 1000 W metal halide lamps. Relative humidity during the experiment was approximately 75%. In each pot, 20 seeds of *Medicago sativa* L.(legume) and 10 seeds of *Phalaris aquatic* L.(C3 grass) were sown and thinned to 5 seedlings of each plant at 7 days after sowing (DAS). These species are the two most widely used perennial species for improved pastures in New South Wales, Australia (Pearson et al., 1997). We added a basal nutrient solution at 10 DAS to all pots with the following nutrients (g m^{-2}): K (19.3); S (2.95); Mg (2.15); Ca (1.27); Cu (0.006); Mn (0.083); Zn (0.045); B (0.014); Fe (0.09). We included a N addition treatment to examine if P addition effects depended on soil N availability. We applied potassium nitrate [KNO_3] (5 g N m^{-2} or 39.3 mg pot^{-1}) to half of the pots (N addition treatment: 0 vs. 5) together with the basal nutrient solution. We kept the soil water content of the pots at 60% field capacity (180 g kg^{-1}) by watering each pot daily to a specific target weight. At 51 DAS we stopped watering to half of the pots, while watering was maintained to the other half of the pots (water stress treatment: constant vs. drought). At 56 DAS, plants in the water stress treatment showed signs of water stress and we started with the ^{15}N labelling and P treatment. We injected 0.5 ± 0.02 g ^{15}N m^{-2} as $^{15}NH_4^{15}NO_3$ (98 atom%, 3.93 mg pot^{-1}) to each of the 32 pots. We injected a total of 12 ml ^{15}N tracer into each pot using 18-gauge Quincke spinal needles (6 injections of 2 ml each in each pot; 3 injections at 10 cm soil depth and 3 injections at 5 cm soil depth). In half of the pots the ^{15}N tracer was combined with 1 g P m^{-2} as KH_2PO_4 (7.85 mg pot^{-1}, P addition treatment: 0 vs. 1). The watering, N and P addition treatments were conducted in a full factorial design with 4 replicates of each treatment combination. We also packed four extra pots that we used to obtain background values of N and ^{15}N content in plant and soil.

2.2. Processing samples and analyses

Pots were harvested at 58 DAS, two days after the ^{15}N and P additions. We decided against a longer period, because this would increase the likelihood for ^{15}N to be recycled into the source pool thereby complicating the interpretation of microbial ^{15}N immobilisation (Stark, 2000). We separated soil from roots of *M. sativa* and *P. aquatica* by hand. Soil samples were dried (65 °C) and ground with mortar and pestle. Shoots and washed roots of the two plant species were dried (65 °C), weighed, and ground in a Wiley mill. Soil and plant samples were analysed for total N and ^{15}N using an isotope ratio mass spectrometer (IRMS, Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany).

Plant samples were ashed at 500 °C and then analysed for P colorimetrically using the ammonium paramolybdate/vanadate reagent (Jackson, 1958) at 400 nm on a UV-VIS Spectrophotometer (UVmini-1240, Shimadzu Scientific Instruments, Sydney, NSW, Australia). Soils were analysed for microbial P using the fumigation-extraction method (Brookes et al., 1982). Fumigated (24 h of fumigation) and non-fumigated soil samples were extracted with 0.03 M NH_4F-0.025 M HCl and analysed for P colorimetrically using the ammonium paramolybdate/stannous chloride reagent (Olsen and Sommers, 1982) at 660 nm on a spectrophotometer. Microbial P was calculated as the difference in P concentration between fumigated and non-fumigated samples, divided by an extraction efficiency of 0.4 (Brookes et al., 1982).

We also measured microbial biomass C, N and ^{15}N using the fumigation-extraction method (Bruulsema and Duxbury, 1996) with a 72 h fumigation period and extraction with 0.05 M K_2SO_4. Extracts were analysed for total C and N using a TOC-VCPN analyser (Shimadzu Scientific Instruments, Sydney, NSW, Australia) and microbial C and N were calculated using an extraction efficiency of 0.45 and 0.54 respectively (Brookes et al., 1985; Beck et al., 1997). We measured inorganic N (NH_4^+ and NO_3^-) in the non-fumigated extracts using a flow injection analyser (Lachat Instruments, Loveland, CO, USA). The ^{15}N in the fumigated and non-fumigated extracts was measured on an IRMS, after drying the extracts in a ventilated oven at 60 °C (Dijkstra et al., 2006). The ^{15}N atom% in microbial biomass ($^{15}N_{mic}$) was calculated as:

$$^{15}N_{mic} = (^{15}N_{fum} \times N_{fum} - {}^{15}N_{non-fum} \times N_{non-fum})/(N_{fum} - N_{non-fum}) \quad (1)$$