Geoderma 322 (2018) 12–18

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Responses of soil phosphorus availability to nitrogen addition in a legume and a non-legume plantation

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ARTICLE INFO

Handling Editor: Junhong Bai

Keywords:
Nitrogen deposition
Phosphorus availability
Legume plantation
Tropical or subtropical

ABSTRACT

Elevated nitrogen (N) deposition can alter the composition and availability of soil phosphorus (P) and thus affect long-term plant growth. However, it remains elusive whether this effect differs between legume and non-legume forest ecosystems, which have distinctly different abilities to use N and P. In this study, soil P fractions were measured in a legume (*Acacia auriculiformis*) and a non-legume (*Eucalyptus urophylla*) plantation in subtropical China, after four-years of N addition. Results showed that the concentrations of soil total P, total Po, and NaOH-Pi (Po and Pi are organic and inorganic P, respectively) were significantly higher, but soil $NaHCO_3$-Po was significantly lower, in the legume plantation compared to the non-legume plantation. Nitrogen addition significantly decreased soil labile P fractions (i.e. $NaHCO_3$-Pi and $NaHCO_3$-Po) in the legume plantation, but they did not change in the non-legume plantation. In contrast, intermediate P fractions (i.e. NaOH-Pi and NaOH-Po) significantly increased with N addition in the legume plantation, but only NaOH-Po experienced a small increase in the non-legume plantation. The recalcitrant P fractions were not significantly influenced by N addition in either plantation. Due to the greater decrease in labile P in the legume plantation, our results suggest that N deposition may lead to greater P limitation in legume plantations compared to non-legume plantations.

1. Introduction

Nitrogen (N) and phosphorus (P) are important limiting nutrients for plant growth (Elser et al., 2007). Their dynamics can largely influence ecosystem processes, especially the carbon (C) cycle, which can regulate the global climate (de Vries et al., 2014; Wang et al., 2017). Over the past few decades, atmospheric N deposition has substantially increased globally due to increased fossil fuel combustion and widespread use of chemical N fertilizers (Galloway et al., 2008). Since biogeochemical cycling of N and P are tightly coupled (Elser et al., 2007), elevated N deposition is expected to have a substantial influence on terrestrial P cycling. Recently, one of the most interesting topics is whether N input induces ecosystem P limitation broadly (Crowley et al., 2012; Finzi, 2009; Phuyal et al., 2008), but much uncertainty remains. Part of the uncertainty lies in the fact that soil P includes various chemical forms which differ markedly in their behavior, mobility, and bioavailability in the soils (Hedley et al., 1982). Thus, to assess whether N deposition causes P limitation and identify the underlying mechanisms, it is essential to study the effects of N inputs on the different forms soil P availability.

Soil P includes inorganic and organic forms, each of which can be divided into labile, intermediate and recalcitrant fractions (Hu et al., 2016; Redel et al., 2008). Nitrogen addition may affect soil P fractions by different mechanisms. For example, N addition may alter soil microbial activities and community structure thereby affecting the mineralization of organic P to inorganic P (Kritzler and Johnson, 2010). Nitrogen addition may enhance the formation of organic P by stimulating primary production and thus stimulating the demand of inorganic P by microbes or plants (Vitousek et al., 2010). Nitrogen addition to soil can also influence the transformations among labile, intermediate and recalcitrant P fractions. For example, labile inorganic or organic P can be transformed to intermediate or recalcitrant P due to N-addition-induced decreases in soil pH, which mobilizes soil aluminum and iron and therefore causes P sorption to soil (Carreira et al., 2000). Conversely, if soil pH increases, the intermediate P can be desorbed as the labile P.

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https://doi.org/10.1016/j.geoderma.2018.02.017
Received 30 June 2017; Received in revised form 5 February 2018; Accepted 9 February 2018

Thus far, few studies have addressed the effects of N addition on soil P fractions, and the results from existing studies lack consistency. For example, Yang et al. (2014) reported that N additions to a larch (*Larix gmelinii*) plantation in northeastern China over nine-years decreased soil labile inorganic P (i.e. $NaHCO_3$-Pi), but increased intermediate inorganic P (i.e. NaOH-Pi). However, other studies found that N addition increased labile inorganic P (Block et al., 2012; Zhang et al., 2014a) and caused only small changes in intermediate inorganic P (Huang et al., 2014). Some studies noted no changes in soil P fractions associated with N additions (Mirabello et al., 2013; Weand et al., 2010). These inconsistent results can be attributed to a number of factors, but vegetation composition has been found to be a particularly important factor (Cross and Schlesinger, 2001).

Legume species and non-legume species have different profiles in using N and P (Gei and Powers, 2013). Compared to non-legume species, legume species are rich in N due to their ability to fix atmospheric N_2 (Vitousek et al., 2002). However, high rates of N fixation may require relatively high rates of soil P supply, therefore, soil P cycling is often faster in the legume species with higher phosphatase activity (Houlton et al., 2008; Venterink, 2011) or higher rates of P uptake by plants (Compton and Cole, 1998) compared to in non-legume species. Due to these differences, previous studies have suggested that P cycling in legume-dominated and non-legume dominated ecosystems may respond differently to N addition. For example, organic matter decomposition rates and soil phosphatase production under N addition has been found to differ in legume-dominated and non-legume dominated ecosystems (Venterink, 2011; Zhu et al., 2016). Moreover, N additions to legume-dominated ecosystems can aggravate imbalances in the C and N cycles resulting in soil acidification (Tang et al., 1999; Zhang et al., 2014b). This may lead to increased inorganic P sorption to soil surfaces in legume ecosystems (Carreira et al., 2000). Since these changes are strongly linked to the composition and fractions of different P forms in soil, it is reasonable to think that soil P fractions in response to N addition should be different between these two kinds of ecosystems. However, few studies that have added N to soil to observe changes in P have occurred in pure legume and non-legume ecosystems within the same region and under similar environmental and climatic conditions. Thus, more research is needed to determine the response of soil P composition and availability to N deposition, and how these responses differ between legume and non-legume ecosystems.

To address this research gap, a four-year experiment was conducted whereby N was added to a legume (*Acacia auriculiformis*) and a non-legume (*Eucalyptus urophylla*) plantation in subtropical China. Soil P fractions, i.e. labile, intermediate and recalcitrant organic or inorganic P, were measured in two plantations. The aim of this study was to compare the different responses of soil P fractions to N addition between legume and non-legume plantations. We hypothesized that (i) soil total P concentration would be lower in the legume plantation compared to the non-legume plantation due to higher P uptake by legume trees; (ii) N addition will result in a greater decrease in soil labile P under the legume plantation compared to the non-legume plantation due to greater labile P uptake by plants or microbes, and more soil P sorption in the legume plantation after N addition; and (iii) as a result of soil P sorption, intermediate P will be higher in the legume plantation compared to the non-legume plantation.

2. Material and methods

2.1. Study sites

The experiment was conducted in a subtropical region located in the center of Guangdong Province, south China (112°50′E, 22°34′N). The region is dominated by a tropical monsoon climate with distinct wet and dry seasons. Mean annual precipitation is 1543 mm, with the wet season extending from April to September. Annual mean temperature is 22.5 °C, with a coldest month in January (average temperature is 10.9 °C) and a hottest month in July (average temperature is 28.0 °C) (Wu et al., 2011). Atmospheric N deposition in precipitation is 43.1 ± 3.9 kg N ha^{-1} yr^{-1}, which is almost five times higher than the atmospheric N deposition that was measured in 1995 (Huang et al., 2015).

We established research sites in two different plantations (a legume plantation, dominated by *Acacia auriculiformis* trees; and a non-legume plantation, dominated by *Eucalyptus urophylla* trees) located 500 m apart. These plantations were established on degraded grassland sites in 1984, and each has an area of approximately 5–8 ha. The soil is acrisol (FAOUN, 2006) in both plantations. A pretreatment survey of the general soil properties was shown in the Zhang et al. (2012).

2.2. Experimental design

The experiment was a complete randomized block design (Zhang et al., 2012). Three N addition treatments were established within each plantation: control (CT, without N addition), medium-N (MN, 50 kg N ha^{-1} yr^{-1}) and high-N (HN, 100 kg N ha^{-1} yr^{-1}). Each treatment was randomly located in three blocks. In total, there were 9 plots (10 m × 10 m) per plantation type. Each plot was surrounded by a 10 m wide buffer strip. The N additions were added as 10 l of ammonium nitrate (NH_4NO_3) solution, which was sprayed monthly on the forest floor with a backpack sprayer starting in August 2010. Each control plot received 10 l water simultaneously.

2.3. Soil and litter sampling

Rhizosphere soil was sampled by shaking roots at the depth of 0–15 cm in June 2014. In each plot, five rhizosphere soil samples were taken randomly under the trees and mixed into a composite sample. Roots and stones were removed using forceps. Soil was sieved to 2-mm and divided into two portions for further processes. One portion was stored at 4 °C for measurement of microbial biomass P and acid phosphatase activity, and the other portion was air-dried for the analysis of soil P fractions, exchangeable cations (Al^{3+}, Fe^{3+}, and Mn^{2+}) and soil pH. Fresh litter samples were collected using litterfall traps. Litter samples were dried at 45 °C, and then passed through a 0.1 mm mesh sieve prior to measuring the litter P concentration.

Soil pH was measured with a glass electrode using a 1:2.5 soil-water suspension. Soil extractable cations (Al^{3+}, Fe^{3+}, and Mn^{2+}) were extracted with 0.1 M $BaCl_2$ (50:1, solution:soil) and determined using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Lu et al., 2014). Soil microbial biomass P was determined using the chloroform fumigation extraction method (Vance et al., 1987). Litter P concentrations were determined using Kjeldahl digestion followed by the Mo-Sb colorimetric method on a UV-8000 spectrophotometer (Metash Instruments Corp., Shanghai, China) (Hobbie and Vitousek, 2000).

Soil acid phosphatase activity was determined according to the method of Tabatabai (1994). Briefly, soil samples were incubated with *p*-nitrophenyl phosphate (*p*-NPP) added as a substrate for 1 h. The reaction was terminated by adding 0.5 M NaOH and 0.5 M $CaCl_2$, and the absorbance was determined spectrophotometrically at 400 nm. Controls without enzymes were processed in parallel to determine non-enzymic hydrolysis of the substrate and to correct for background coloration. Enzyme activities are expressed as μmol *p*-NPP g^{-1} soil h^{-1}.

2.4. Measurement of soil P fractions

Soil P was sequentially fractionated following a modified version of the fractionation scheme proposed by Hedley et al. (1982) (Fig. 1). We did not use anion exchange resin for the first extraction. We assume that resin-extractable P was included in the $NaHCO_3$ extract. Briefly, 3 g of soil was successively extracted with 30 ml 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH, and 1 M dilution HCl (D. HCl) for 17 h each to extract the $NaHCO_3$-, NaOH-, and D. HCl-extractable P fractions. Then the residue