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Stimulation of nitrogen-hydrolyzing enzymes in soil aggregates mitigates nitrogen constraint for carbon sequestration following afforestation in subtropical China

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ABSTRACT

Soil nitrogen (N) availability is a major constraint for plant growth and consequently impacts soil carbon (C) sequestration following afforestation. The transformation of soil organic N to plant available form is predominantly catalyzed by N-hydrolyzing enzymes. Yet how N-hydrolyzing enzymes affect N availability for soil C sequestration under afforestation remains unclear. Here, we examined N-hydrolyzing enzyme activities, N masses (N contents in equivalent soil mass) and the $\delta^{15}N$ values of total N (TN) pool and stable N pool (SN, NaOCl–resistant) in soil aggregates following 30 years of afforestation in subtropical China. The relationships of soil N mass and supply via enzymes with those of C were also developed. Afforestation increased TN masses and N-hydrolyzing enzyme activities, but declined the percentages of SN in TN and the $\delta^{15}N$ values in soil aggregates. Soil TN mass was positively correlated with soil organic C mass in aggregates across land use types. Similarly, soil enzymes for N acquisition scaled isometrically with C acquisition with a slope of ~1.0. Our results indicate that N constraint for soil C sequestration can be alleviated by increasing soil N-hydrolyzing enzyme activities combined with reducing SN:TN ratios and homeostatic ecoenzymatic C:N ratios following afforestation, which lead to tight coupling of soil N and C cycling.

1. Introduction

As a key factor limiting plant growth and primary productivity, soil nitrogen (N) availability is crucial for carbon (C) budgets in terrestrial ecosystems (Yang et al., 2011; Thomas et al., 2015; Zhang et al., 2018). Changes in land use, especially the conversion of cultivated or uncultivated land to forest plantation (i.e., afforestation) can potentially increase plant productivity and sequestrate C in soil (Knops and Tilman, 2000; Cheng et al., 2013). Thus, afforestation has been widely proposed as an effective approach for the mitigation of the anthropogenic climate change (Gelfand et al., 2012; Li et al., 2012). Previous studies have indicated that soil N constraint for plant growth becomes even more aggravated in afforested soils likely due to stimulation of plant growth and increase in N transformation from the mineral soil to plant biomass and soil organic matter (SOM) (Luo et al., 2006; Gelfand et al., 2012). As over 90% of N is incorporated into soil as organic forms (Bremner, 1965), available N released from soil organic N (SON) predominantly determines soil N availability and ecosystem N cycling (Nannipieri and Eldor, 2009), which in turn potentially impacts terrestrial C balance under global land use change (Mendham et al., 2004; Yang et al., 2011; Gelfand et al., 2012).

The release of plant available N from SON is predominantly catalyzed by a sequence of soil enzymes, among which the mostly measured are those targeting proteins (e.g., leucine aminopeptidase, LAP) and chitin hydrolyzing (e.g., N-acetyl-β-glucosaminidase, NAG) (Sinsabaugh et al., 2009; Mooshammer et al., 2012). Although extensive researches have reported sensitive responses of soil N-hydrolyzing enzyme activities to afforestation, responses in different studies are commonly inconsistent (Trasar-Cepeda et al., 2008; Singh et al., 2012; Raiesi and Beheshti, 2014). These inconsistent results can be related to differences in land use history (Trasar-Cepeda et al., 2008), change in litter quality (Knops and Tilman, 2000; Singh et al., 2011) and microbial activity (Singh et al., 2011; Wu et al., 2016) following afforestation. Variations of soil physical structure following afforestation could also contribute to these inconsistent responses of soil enzymes, as SON compounds protected in different aggregates have

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various decomposition potentials and intrinsic decay rates (Zeller and Dambrine, 2011; Dungait et al., 2012). However, how soil enzyme activities in different soil aggregates respond to afforestation remains elusive (Mendham et al., 2004; Zeller and Dambrine, 2011), which is imperative for our understanding of soil N-mineralization in the whole soil during afforestation. Meanwhile, information on the chemical characteristics of N protected in different aggregates can help further elucidate SON mineralization process via enzymes (Mendham et al., 2004; Mikutta et al., 2006). In addition, natural abundance of N isotope (^{15}N) analysis is an excellent indicator of systematic changes in ecosystem N turnover processes and efficiencies (Amundson et al., 2003; Pardo et al., 2004). The overall N dynamics in soil aggregates following afforestation thus can be further clarified if the ^{15}N analyses are conducted simultaneously.

Whether progressive N limitation will occur as a consequence of additional C sequestration following afforestation remains a controversial issue (Luo et al., 2006; Gelfand et al., 2012; Li et al., 2012). For instance, although soil N contents increased linearly with the increase of soil C contents following afforestation (Yang et al., 2011; Li et al., 2012), soil C mineralization:N mineralization rates significantly varied following afforestation (Mendham et al., 2004) with different changes in various soil aggregates (Bimüller et al., 2014). Recent studies have suggested that the relative abundance of enzymes for C, N and P acquisition, i.e., ecoenzymatic C:N:P stoiochiometry has the potential to indicate the relative C and nutrient demand of microorganisms in relation to soil nutrient availability (Sinsabaugh et al., 2009; Mooshammer et al., 2012). These researches have advanced our understanding and the assessment of balanced nutrient supply, but how soil ecoenzymatic stoichiometric C:N ratio varies following afforestation remains unclear, particularly when soils are fractionated into more functional pools.

The Danjiangkou Reservoir area is a crucial water source for the Middle Route of China's South-to-North Water Transfer Project (Zhang, 2009). Following the re-organization of lands by the government in the 1980s, afforestation has been conducted to reduce soil erosion, water pollution and C loss in the reservoir area (Zhu et al., 2010). Our previous results in this area have indicated that afforestation leads to increases in C and N contents, C:N ratios and microbial activities (Cheng et al., 2013; Wu et al., 2016). Here, we build on our previous findings and investigate the dynamics of SON turnover in soil aggregates following afforestation, based on the determination of N-hydrolyzing enzyme activities, N contents and $\delta^{15}N$ values in soil aggregates. We also aimed to explore whether soil N availability will constrain additional soil C sequestration following afforestation by the measurements of soil ecoenzymatic C:N stoichiometric ratios in soil aggregates. We hypothesized that: (1) the accumulation of N in soil aggregates would increase following increasing plant residues inputs under afforestation because plant residues are the major source for soil nutrients (Kogel-Knabner, 2002); (2) soil N-hydrolyzing enzyme activities in soil aggregates would increase following afforestation, reflecting the increases in organic substrates and soil microbial activities (Trasar-Cepeda et al., 2008); (3) different soil enzymes could be optimally allocated to acquire the most limiting resource (Allison et al., 2011) and consequently enzyme activities for N acquisition would be higher relative to C acquisition due to the higher C:N ratios in soil substrate under afforestation.

2. Materials and methods

2.1. Study area and experimental design

The research site was located at the Wulongchi Experimental Station (32°45′N, 111°13′E, 280–400 m a.s.l.) in the Danjiangkou Reservoir region (Cheng et al., 2013; Wu et al., 2016). The climate of the study region belongs to the subtropical humid monsoon, with hot–humid summers and cold–dry winters. Mean annual precipitation is 749.3 mm, with approximately 80% occur during the wet season, from April to October. Mean annual temperature is approximately 15.7 °C, with average minimum and maximum temperature of 4.2 °C in January and 27.3 °C in July, respectively. The soil is of limestone and red sandstone origin, and classified as Haplic Luvisols according to Food and Agriculture Organization (1993). The soil is a loamy clay, which contains 11% sand, 41% silt and 48% clay (Zhu et al., 2010). Soil pH ranges from 8.17 to 8.53 with strong carbonate reaction (Zhang et al., 2016). Human activities, such as deforestation and tillage have resulted in soil erosion, soil C and N depletion and water pollution in this region (Zhu et al., 2010). In the 1980s, large areas of croplands (predominantly wheat and maize rotation) were converted to afforested plantations, which subsequently received no fertilization. *Robinia pseudoacacia* and *Amorpha fruticose* in shrubland and *Platycladus orientalis (Linn.) Franco* in woodland were planted to reforest the area (Zhu et al., 2010). N Mineral fertilizations, including urea (375 kg ha^{-1} yr^{-1}) and urea-ammonium mixed N fertilizer (200 kg ha^{-1} yr^{-1}) were applied in cropland. In addition, superphosphate (63 kg ha^{-1} yr^{-1}) and potassium chloride (83 kg ha^{-1} yr^{-1}) were also applied to maintain soil nutrient equilibrium in cropland. The aboveground biomass of wheat and maize in cropland was removed through harvesting.

The study was a randomized complete block design containing four blocks/sites. Each block was about 3 ha (600 m × 50 m) with 100 m buffer rows between blocks. Four adjacent land types i.e., open area (control), cropland, shrubland and woodland were included at each block. A comprehensive survey of vegetation and soils were conducted in April, 2017 in order to ensure the comparability (such as similar topographies, soil types etc.) of the sampling blocks.

2.2. Sampling and soil aggregate fractionation

In April 2017, five sub-plots (2 m × 2 m) were selected randomly in each land use type from four blocks. Bulk density (0–10 cm and 10–30 cm) was sampled from each sub-plot using a 5 cm diameter soil core. Then three soil cores (10 cm depth, 5 cm diameter) were sampled at random and homogenized within each sub-plot. A total of twenty soil samples were harvested to represent each land use type. Soil samples were stored at 4 °C for aggregate fractionation and the subsequent determination of chemical and biochemical analyses.

Soil aggregate fractionation was conducted using a wet-sieving method for biological analyses (Smith et al., 2014). Four aggregate sizes were separated through a sequence of sieves (2000, 250, and 53 μm). In brief, a 100 g (equivalent dry weight) field-moist soil samples were submerged in de-ionized water (1 cm) on top of the 2000 μm sieve, for 5 min before performing the wet-sieving process. Soils were sieved by gently moving the sieve in and out of water for 50 repetitions with an amplitude of 3 cm over a period of 2 min. The > 2000 μm floating organic material was aspirated away. Soils remaining on the sieve were transferred to an aluminum cane and recovered as > 2000 μm soil aggregates. The slurry that passed through the sieve was transferred to the next finer sieve, and the sieving process was repeated following a similar procedure. The final slurry that passed through 53 μm sieve was centrifuged at 2500 rpm for 2 min, and then decanted to collect < 53 μm silt and clay particles (Smith et al., 2014). One complete fractionation procedure took approximately 3 h. An aliquot of each fraction was taken to measure the moisture content. A subsample of soil fraction was freeze-dried for the determinations of N concentration and isotopic value. The remained moist soil fractions were stored at 4 °C for enzyme analyses within 3 d.

2.3. Stable organic N fractionation

Stable N (SN) was obtained by an oxidation method using NaOCl (Mikutta et al., 2006). In brief, 5 g of ground freeze-dried bulk soil sample or aggregate fraction was treated with 50 mL of 6 wt% NaOCl (adjusted to pH 8.0 using 32% HCl). Three oxidation cycles (6 h each)