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Tree species effects on asymbiotic N_2 fixation in subtropical karst and non-karst forests

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ABSTRACT

Asymbiotic dinitrogen (N_2) fixation (ANF) plays an important role in determining forest nitrogen (N) availability. Yet, the controls on ANF variation within or between forests remain poorly understood. In the present study, ANF in bulk leaf litter (or litter ANF hereafter) and soil (or soil ANF hereafter) within the crown radius of the dominant tree species was investigated in a karst forest over limestone and a nearby non-karst forest over clasolite, southwest China. Lithology exerted significant ($P < 0.05$) influences on ANF in litter but not in soil. ANF in litter was 4.9 times higher in the karst forest than in the non-karst forest. Tree species had significant effects on litter ANF in the karst and non-karst forests with the rates varying 23-fold and 71-fold, respectively. Significant effect of tree species on soil ANF was only encountered in the non-karst forest. The strongest explanatory variables were substrate and forest specific, but generally included litter moisture, total nitrogen, total phosphorus, calcium and soil organic carbon. The findings suggest that tree species and lithology may provide new mechanisms explaining the great variation of ANF within and between forests, respectively.

1. Introduction

Nitrogen (N) is usually the major limiting nutrient of net primary productivity in terrestrial ecosystems at a global scale (LeBauer and Treseder, 2008). Cumulative evidences show that N availability determines the capacity of carbon (C) sequestration in many terrestrial ecosystems, and hence plays a key role in mediating the climate - C cycle feedbacks (Thomas et al., 2013; Zaehle, 2013). In order to better predict ecosystem C dynamics under climate change, it is undoubtedly crucial to investigate the external N inputs (which are directly related to N availability) and their controls. For most unmanaged terrestrial ecosystems, biological dinitrogen (N_2) fixation (or N_2 fixation hereafter) is the primary pathway of external N inputs (Houlton and Morford, 2015). Symbiotic N_2 fixation (i.e., N_2 fixation conducted by N_2-fixing microbial symbionts in plant root nodules) may be dominant in ecosystems with abundant N_2-fixing plants (Reed et al., 2011). Nevertheless, asymbiotic N_2 fixation (ANF hereafter, which is carried out by bryophyte-cyanobacteria associations or by other free-living diazotrophs in soil, litter or other ecosystem compartments), which accounts for 0.01–60 kg N ha^{-1} yr^{-1}, may be the primary pathway of external N inputs for those ecosystems lacking (or with a low abundance of) N_2-fixing plants (Reed et al., 2011). Yet, the controls on both symbiotic and asymbiotic N_2 fixation are poorly understood (Reed et al., 2011; Thomas et al., 2015; William et al., 2015). Consequently, a mechanistic representation of N_2 fixation is regarded as one of the biggest challenges in earth system models, which are the primary tool used for predicting climate - C cycle feedbacks (Thomas et al., 2015).

Though a mechanistic understanding is currently absent, a few biotic and abiotic factors have been found to affect asymbiotic N_2 fixation, including climate change factors (Gundale et al., 2012; Rousk et al., 2017b), tree species (Reed et al., 2008), and the availability of key nutrients, including molybdenum (Mo) (Barron et al., 2009; Rousk et al., 2017a), iron (Fe) (Winbourne et al., 2017), vanadium (V) (Darnajoux et al., 2017), phosphorus (P) (Reed et al., 2013), calcium (Ca) (Rodríguez et al., 1990), N (Barron et al., 2009), and C (Pérez et al., 2017). Among the factors, tree species have great influences on both litter and soil physicochemical properties due to tree species-specific differences in litter quantity and quality, root exudate quantity and chemistry (Snell et al., 2016). Accordingly, tree species affect the composition and structure of free-living microbial communities in soil and litter/forest floor (Ushio et al., 2010; Prescott and Grayston, 2013; Snell et al., 2016). Due to their roles in influencing litter and soil

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properties, tree species were found to significantly affect ANF in litter (or litter ANF hereafter) and soil (or soil ANF hereafter) in a tropical rain forest (Reed et al., 2013). However, since only one study is available, whether tree species controls on ANF are widespread and mediated by other factors is yet unknown.

Surficial lithology, which determines the geochemical, mineralogical, and physical properties of rocks (Dürr et al., 2005; Hartmann and Moosdorf, 2012; Gray et al., 2014), is among the neglected factors which have the potential to impact N_2 fixation variation. The lithology can significantly affect both the chemical and physical properties of soils (Anderson, 1988; Kosmas et al., 2000; Leuschner et al., 2006; Neff et al., 2006; Gray et al., 2014). Among the aforementioned elements affecting N_2 fixation, Mo, P, V, Ca and Fe are all mostly derived from bedrocks in unmanaged terrestrial ecosystems. Theoretically, lithology has the potential to greatly affect N_2 fixation variation from site to site, and may largely determine which element(s) would limit N_2 fixation via its role in determining the availability of the above elements. The effects of lithology on ANF have been demonstrated by a recent study, which shows that Fe and Mo are the limiting elements, respectively, in adjacent tropical forests over limestone and volcanic rock (Winbourne et al., 2017). The ANF rate was found to be significantly higher in the forest over limestone than over volcanic rock during the wet season (Winbourne et al., 2017). Additionally, lithology has been found to affect soil organic C (SOC) level and stability (Li et al., 2017b, 2017c; Wen et al., 2017), and the composition and function of plant and microbial communities (Reith et al., 2012). Since all these factors are related to the variation of ANF, lithology thus has the potential to influence the variation of ANF from site to site.

In the present study, the effects of tree species on ANF in leaf litter and soil were assessed in a karst forest over limestone and a nearby non-karst forest over clasolite, southwest China. Our previous studies found that exchangeable cations, soil pH, contents and stability of SOC, total N (TN), total P (TP), and soil inorganic N concentrations were all greater in the karst forest than in the non-karst forest (Li et al., 2017c, 2017d). We hypothesized that ANF in leaf litter and soil would be greater in the karst forest (**Hypothesis I**), as P has often been proposed as the major constraint of ANF (Reed et al., 2008, 2011, 2013; Rousk et al., 2017a). Since tree species exerted significant influences on both biotic and abiotic properties in litter and soil, we hypothesized that significant tree species effects on ANF in leaf litter and soil would occur in both the karst and non-karst forests (**Hypothesis II**). Accordingly, the main objectives of the present study were to address: (i) How does lithology affect ANF in litter and soil? (ii) Are tree species effects on ANF in litter and soil mediated by lithology?

2. Materials and methods

2.1. Site description

This study was conducted in Huanjiang County of Guangxi Zhuang Autonomous Region, southwest China. This region is located in the subtropical humid forest life zone with a monsoon climate. The mean annual air temperature is 17.8–22.2 °C, with the lowest monthly mean in January (7.8–13.0 °C) and the highest in July (25.8–29.4 °C). The mean annual precipitation ranges from 1346 to 1640 mm, with a wet season from April to August and a dry season from September to March, accounting for about 71% and 29% of annual precipitation, respectively. Total atmospheric N deposition is about 37 kg N ha^{-1} yr^{-1} (Zhu et al., 2015). The region is mountainous and interwoven with karst areas and non-karst areas. Both types of areas are characterized by gentle valleys flanked by hills. The bedrock in the karst areas is mostly limestone, dolomite and their mixtures, while is clasolite in the non-karst areas. The soil is Calcareous lithosols (limestone soil) over karst areas, and is Haplic acrisol (red soil) over clasolite according to the FAO/UNESCO classification system (Li et al., 2017d).

Two secondary forests, i.e., one karst forest over limestone and one non-karst forest over clasolite were selected. The distance is about 26 km between the two forests. Both forests were about 50 years old, and naturally regenerated after clear-cut in the end of 1950s. The karst forest is located in Mulun National Nature Reserve (25°08′ N, 108°01′ E, with an elevation of 420 m), while the non-karst forest is located in Huashan State Forest Farm (25°06′ N, 108°16′ E, with an elevation of 310 m). The soil texture is silt clay loam in the karst forest and is silt loam in the non-karst forest. The five most dominant tree species in the karst forest were *Liquidambar formosana* (LIQ), *Eurycorymbus cavaleriei* (EUR), *Cryptocarya chinensis* (CRY), *Cladrastis platycarpa* (CLA), and *Itoa orientalis* (ITO). The five most dominant tree species in the non-karst forest were *Castanopsis chinensis* (CAS), *Brassaiopsis glomerulata* (BRA), *Liquidambar formosana* (LIQ), *Cinnamomum bodinieri* (CIN), and *Sinoadina racemosa* (SIN).

2.2. Sample collection and biological N_2 fixation measurement

The measurements of ANF were conducted in August 2016. At each forest, a representative area of 100 m × 100 m was chosen in the valley or low slope. The five most dominant tree species were selected in each forest. Each species included five trees (i.e., replicates). Therefore, 25 individual trees were included in each forest. Bulk leaf litter for individual trees were collected within the crown radius of the selected trees. Similarly, three soil cores (0–2 cm in depth and 5 cm in diameter) were collected using clear stainless steel auger boring after removal of the organic layer within the crown radius of the selected trees. Both the leaf litter and soil samples were mixed well by each tree.

The measurements were conducted in the field using acetylene reduction assay (Hardy et al., 1968). Each fresh sample (~15 g soil or ~ 7 g litter) was sealed into 330 ml glass flask with rubber stoppers. 10% of the headspace of each flask was replaced with acetylene (cylinder acetylene with 99.99% purity) using a gas-tight syringe. The acetylene was purified by passing through 98% H_2SO_4 and 5 M NaOH solution before use (Keuter et al., 2014). After acetylene injection, the flasks were put on the forest floor. In addition, flasks with acetylene only and samples only were used as references. After incubation of 16 h (leaf litter samples) or 21 h (soil samples), 30 ml gas sample was collected from the headspace of each flask and transferred into a 12 ml pre-evacuated glass vial (Labco Exetainer, Labco Limited, UK). Ethylene in the gas samples was analyzed using a gas chromatograph (Agilent GC 7890A, Agilent, USA) equipped with a flame ionization detector (FID). Ethylene production rates or acetylene reduction activity (ARA, nmol C_2H_4 g^{-1} dry mass d^{-1}) were calculated from the slope of the regression line between ethylene concentrations and time.

2.3. Analyses of physicochemical properties

Leaf litter was oven-dried at 70 °C for 48 h, and then ground to fine powder using a mortar and pestle. Soil samples were air dried and sieved to 1 mm (for the measurements of soil pH and exchangeable cations) or 0.15 mm (for the measurements of other variables) for physicochemical analyses (Carter and Gregorich, 2006). Sub-samples were used to determine water content of litter (LWC, %) and soil (SWC, %) by drying litter and soil at 70 °C for 48 h and 105 °C for 24 h, respectively, and then weighed. Litter C and N, and soil TN were analyzed using an elemental analyzer (EA 3000; EuroVector, Italy). SOC was measured by wet oxidation with dichromate redox colorimetric method. Litter and soil total P was determined colorimetrically using ascorbic acid molybdate method after being digested with H_2SO_4 + $HClO_4$ solution. Soil pH (1: 2.5 soil/water ratio) was measured with a pH meter (FE20K, Mettler- Toledo, Switzerland). Exchangeable Ca and magnesium (Mg) were displaced via compulsive exchange in 1 M ammonium acetate at pH 7.0 and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Soil labile P was extracted with 0.5 M $NaHCO_3$, and measured colorimetrically using ascorbic acid molybdate method. All the chemical