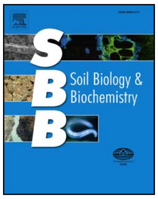
Soil Biology and Biochemistry 119 (2018) 50–58

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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



Changes in plant nitrogen acquisition strategies during the restoration of spruce plantations on the eastern Tibetan Plateau, China

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ARTICLE INFO

Keywords:
Nitrogen preference
Ammonium
Nitrate
Organic nitrogen
Plantation restoration
Stable nitrogen isotope

ABSTRACT

Despite the importance of plant nitrogen (N) acquisition strategies for plant growth and the structure and functioning of ecosystems, few studies have investigated the N acquisition preference of plants and their reliance on various N sources (nitrate (NO_3^-), ammonium (NH_4^+), and organic N (DON) in soil) during different stages of plantation restoration caused by land-use change. In this study, we used the isotopic mass-balance methods to quantitatively estimate the contributions of N sources (NO_3^- vs. NH_4^+ vs. DON) and plant N preferences in spruce (*Picea asperata* Mast.) plantations of different ages (i.e., 20, 30, 40, 50, and 70 years old) on the eastern Tibetan Plateau, China. Across all restoration stages, while plants in spruce plantations preferred soil inorganic N over soil DON, soil DON was a notable N contributor to plant N nutrition, with 23%–44% of plant N derived from DON. Moreover, with the development of plantation restoration, the N source preferences of plants switched from NO_3^- (i.e., 20- and 30-yr-old plantations) to NH_4^+ (age of plantations ≥ 40 years old). Our results suggest that soil DON can be an important N source in alpine coniferous forests. In addition, the shift in the dominant N source during plantation restoration may manifest a strategic adjustment of plant N acquisition in response to changes in soil N availability and/or physiological traits.

1. Introduction

Nitrogen (N) is an essential plant nutrient that is a principle limiting resource in most terrestrial ecosystems, and is available in different chemical forms (e.g., ammonium (NH_4^+-N), nitrate (NO_3^--N), and dissolved organic N (DON)) (Orwin et al., 2011; Hobbie and Högberg, 2012). Some plant species have been shown to accumulate greater quantities of nutrients or produce more biomass when grown in the presence of one N source compared with another, i.e., they appear to display a N preference (Britto and Kronzucker, 2013). Whether a given plant species can regulate its N preference and adjust to different N sources will determine its ability to adapt to environmental changes (Houlton et al., 2007). Additionally, the contributions of different N sources to plant N nutrition can mirror the degree of plants' reliance on specific N sources (Takebayashi et al., 2010; Liu et al., 2013). Thus, N utilization by plants for different forms of N among NH_4^+-N, NO_3^--N and DON exerts an important influence on ecosystem N cycling (Lovett and Mitchell, 2004; Liu et al., 2013), and is considered to be a significant determinant of plant productivity, competition, coexistence, and ecological succession (Burton et al., 2007b; Kahmen et al., 2008). Nevertheless, the ecological origins and significance of plant N acquisition strategies remain poorly understood, partly because of the complex interactions between plant N acquisition and multiple environmental variables (Hodge, 2004; Houlton et al., 2007).

Plant N acquisition strategies depend on a wide and dynamic range of biotic and abiotic factors that overlap simultaneously (Britto and Kronzucker, 2013), including vegetation coverage, plant physiological changes, environmental conditions, and soil nutrient status (Britto and Kronzucker, 2002; Tylova-Munzarova et al., 2005; Hofmockel et al., 2007; Liu et al., 2013). It has been found that plants switched N source from NO_3^- to NH_4^+ when going from drier to wetter environments (Houlton et al., 2007). By comparison, a shift of main source of plant N from NH_4^+ to NO_3^- was reported when the soil changed from acidic to alkaline (Hawkins and Robbins, 2010). In addition, changes in soil N availability are known to exert profound influences on N-acquisition characteristics and bring changes in apparent N source preferences

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https://doi.org/10.1016/j.soilbio.2018.01.002
Received 11 September 2017; Received in revised form 4 January 2018; Accepted 7 January 2018
0038-0717/

Table 1
Characteristics of the study areas and soil properties of the sampling sites (0–15 cm mineral soils).

Characteristics	Age of plantations (yr)					Natural forest
	20	30	40	50	70	
Location	31°35′22″N, 102°50′37″ E	31°48′57″N, 102°41′5″ E	31°47′48″N, 102°41′53″ E	31°47′5″N, 102°42′14″ E	31°37′18″N, 102°50′53″ E	31°37′15″N, 102°51′1″ E
Elevation (m a. s. l.)	2947	3300	3246	3190	3102	3175
Total C[a] (g kg^{-1})	58.02 (4.33)^{C}	60.84 (1.43)^{C}	84.30 (8.28)^{B}	70.28 (9.47)^{BC}	52.82 (7.33)^{C}	120.11 (5.72)^{A}
Total N[a] (g kg^{-1})	5.04 (0.30)^{B}	4.86 (0.11)^{B}	7.19 (0.51)^{A}	5.03 (0.47)^{B}	3.10 (0.33)^{C}	6.60 (0.11)^{A}
C: N ratio[a]	11.49 (0.21)^{C}	12.51 (0.19)^{B}	11.66 (0.37)^{BC}	13.82 (0.52)^{B}	16.91 (0.96)^{A}	18.19 (0.68)^{A}
Soil pH[b]	6.74 (0.24)^{A}	6.44 (0.11)^{A}	6.24 (0.10)^{AB}	6.13 (0.06)^{B}	6.18 (0.03)^{B}	5.91 (0.23)^{B}
Soil electrical conductivity[c] (μs cm^{-1})	129.25 (11.54)^{B}	152.25 (10.44)^{A}	186.25 (18.06)^{A}	129.00 (10.17)^{B}	118.33 (11.61)^{B}	149.50 (3.40)^{A}

Each observation is the ± 1 SE of the mean of samples from four replicate plots per site. Different upper case letters within a line denote significant differences among forest sites at $P < .05$.

[a] Measured by direct combustion on an elemental analyser (Multi N/C 2100; Analytic Jena, Jena, Germany).

[b] Measured in 1:2.5 soil:water suspensions with a pH electrode.

[c] Measured in 1:5 soil:water suspensions using a DD-307 conductivity apparatus.

(Munzarova et al., 2006; Song et al., 2015). These factors are likely to be influenced by changes in land use or forest types. For instance, land-use changes such as clear-cut harvesting and site preparation can cause disturbance to the soil ecosystem, which may have an impact on soil microbial environments and subsequent N availability (Tan et al., 2005; Burton et al., 2007a). Meanwhile, a shift in plant physiological traits and the chemical composition of dominant N forms in soil may occur at various stages of plant development due to changed forest types or habitat restoration (Gessler et al., 1998; Beyschlag et al., 2009). These consequences caused by changes in land use or forest types may potentially lead to changes in the N-acquisition strategies of plants (Kronzucker et al., 1997). However, to date, there is little empirical evidence related to the variation of plant N acquisition strategies during these processes, especially during forest restoration.

Planted forests (plantations) have been a significant element of land-use change, and are widely accepted as an effective approach to restore and rehabilitate degraded or abandoned habitats (Behera and Sahani, 2003; Cusack and Montagnini, 2004; Wang et al., 2009). A critical aspect of forest restoration is to understand soil N dynamics and the status of plant N utilization in the process of recovery (Wang et al., 2010). The availability of soil N has been found to affect species composition and may determine the long-term sustainability and productivity of forest plantations (Burton et al., 2007b; Orwin et al., 2011). Nevertheless, most previous studies of plantation restoration have mainly focused on the species diversity and forest structure (de Souza and Batista, 2004; Wang et al., 2009), and thus, the extent to which plant N nutrition relies on different N source and how plants modify their N-acquisition preferences during different stages of plantation restoration remain open questions. Such knowledge is of global importance for promoting plantation management and may improve current biogeochemical models. During the last century, natural coniferous forests in southwestern China suffered from substantial deforestation to meet the increasing demands of the timber market for fuel materials and other forest products. After deforestation, a monoculture of dragon spruce (*Picea asperata* Mast.) was immediately planted in the cut area with the goal of restoration (Zhang et al., 2017). Currently, over one million hectares of dragon spruce plantations exist in Western Sichuan (Xu et al., 2010) accompanied by a relatively integrated restoration sequence of spruce plantations of different stand ages. These plantations provide a natural platform for assessing the changes in plant N acquisition strategies during the restoration process.

Hence, in this study, by measurement of the natural abundance of ^{15}N in foliage and soil extractable N in spruce plantations of different ages, we quantitatively estimated the source proportions of foliar N (NO_3^- vs. NH_4^+ vs. DON) using mass balance equations. In addition, plant N preference was determined by comparing the contributions of N sources in plants with the respective N availabilities in the soil. The natural abundance of N isotope (δ^{15}N) has been widely used to gain insight into available N sources and physiological processes of plant species (Houlton et al., 2007; Craine et al., 2009; Hobbie and Högberg, 2012). Compared with the commonly used isotopic labelling technique, the analysis of natural abundance of δ^{15}N can avoid the potential disturbance of the soil N pool, microbial turnover, and plant N-uptake kinetics caused by artificial N addition (Peri et al., 2012; Liu et al., 2013). Foliar δ^{15}N values have been demonstrated as being close to the δ^{15}N of their N sources, and can be used to integrate N availability in forest ecosystems (Högberg, 1997; Houlton et al., 2007; Takebayashi et al., 2010). Given that N-mineralization limitation is previously reported in many ecosystems, especially in cold climates (Weigelt et al., 2005; Kielland et al., 2007; Näsholm et al., 2009), we hypothesized that soil DON can be a notable N contributor to plant N nutrition in alpine coniferous forests. Another hypothesis we tested was that the N preference of plants could change with variation in soil N availability and physiological traits of plants during the process of plantation restoration.

2. Materials and methods

2.1. Study sites

This study was conducted at six forest sites located in the Miyaluo Experimental Forest of Lixian County, eastern Tibetan Plateau, in Sichuan, China (31° 35′N ~ 31° 49′N, 102° 40′E ~ 102° 51′E, and 2800–3400 m a.s.l.). The mean annual temperature is 8.9 °C with a maximum monthly mean air temperature of 12.6 °C in July and a minimum of −8 °C in January. Annual precipitation ranges from 600 to 1100 mm. Soils are classified as Cambic Umbrisols according to the IUSS Working Group (2007). The dominant type of mycorrhizae in soils of alpine coniferous forests in western Sichuan, China, is ectomycorrhizae (Li et al., 2015). Basic location information and soil properties of the six forests are shown in Table 1. Based on the principle of space-for-time substitution, five plantations dominated by dragon spruce were selected in July of 2016, representing the five different restoration stages of spruce plantations after the clear cutting from natural forests (NFs); since then no management practices such as irrigation or artificial fertilization were performed in this region. A limited understory is present, with *Festuca ovina*, *Deyeuxia arundinacea*, and *Carex capilliformis* mainly growing under these plantations. A spruce NF, within approximately 300 m of the 70-yr-old plantation, was chosen to represent the original status for the transition from NF to plantation as a control. The understory of the spruce NF is dominated by mosses and grasses (e.g., *Anemone rivularis*, *Carex capilliformis*) (Zhang et al., 2017).