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Inter-species and intra-annual variations of moss nitrogen utilization: Implications for nitrogen deposition assessment☆

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ABSTRACT

Moss nitrogen (N) concentrations and natural ^{15}N abundance (δ^{15}N values) have been widely employed to evaluate annual levels and major sources of atmospheric N deposition. However, different moss species and one-off sampling were often used among extant studies, it remains unclear whether moss N parameters differ with species and different samplings, which prevented more accurate assessment of N deposition via moss survey. Here concentrations, isotopic ratios of bulk carbon (C) and bulk N in natural epilithic mosses (*Bryum argenteum*, *Eurohypnum leptothallum*, *Haplocladium microphyllum* and *Hypnum plumaeforme*) were measured monthly from August 2006 to August 2007 at Guiyang, SW China. The *H. plumaeforme* had significantly ($P < 0.05$) lower bulk N concentrations and higher δ^{13}C values than other species. Moss N concentrations were significantly ($P < 0.05$) lower in warmer months than in cooler months, while moss δ^{13}C values exhibited an opposite pattern. The variance component analyses showed that different species contributed more variations of moss N concentrations and δ^{13}C values than different samplings. Differently, δ^{15}N values did not differ significantly between moss species, and its variance mainly reflected variations of assimilated N sources, with ammonium as the dominant contributor. These results unambiguously reveal the influence of inter-species and intra-annual variations of moss N utilization on N deposition assessment.

1. Introduction

Anthropogenic reactive nitrogen (N) (mainly as ammonia (NH_3) and nitrogen oxides (NO_x)) emissions have increased remarkably and continuously since the 1950s, especially in the densely-populated urban areas (Vitousek et al., 1997; Galloway et al., 2008). Consequently, increased atmospheric N deposition has been observed in many disturbed regions (Liu et al., 2013a; Harmens et al., 2014), which is considered as a main factor triggering changes in ecosystem structure and functions such as soil acidification, fresh water eutrophication and biodiversity losses (Arróniz-Crespo and Phoenix, 2008; van der Wal et al., 2008; Bobbink et al., 2010; Armitage et al., 2011; Sheppard et al., 2014). Thus, it is important to strengthen the understanding of levels, sources and biogeochemical processes of N deposition for better evaluating its ecological effects and environmental impacts (Bragazza et al., 2006; Elliott et al., 2007; Felix et al., 2012; Gundale et al., 2011).

However, atmospheric N deposition contains a variety of N compounds and occurs in different forms of dry and wet deposition (Vitousek et al., 1997), making the assessment of its levels and sources rather difficult (Zechmeister et al., 2008; Harmens et al., 2011, 2014). N is an essential macronutrient in mosses and is required for synthesis of amino acids, chlorophyll and hormones (Turetsky, 2003; Glime, 2007; Koranda et al., 2007; Arróniz-Crespo and Phoenix, 2008). Due to their unique specific leaf area and lack of cuticle barriers (Glime, 2007), most mosses obtain N nutrients from wet N deposition efficiently, with little N uptake from their substrates (Schröder et al., 2010; Harmens et al., 2011). Since the 1980s, moss N concentrations have been recognized as sensitive parameters for indicating N deposition levels and effects (e.g., Press and Lee, 1986; Christie, 1987). For mapping N deposition at regional scales, researchers have quantitatively examined relationships

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between N deposition levels and moss N concentrations for both single species and mixed moss samples, showing either linear (e.g., Pitcairn et al., 2006; Liu et al., 2008a; Harmens et al., 2011; Xiao and Liu, 2011) or logarithmic correlations (e.g., Pitcairn et al., 1998; Bragazza et al., 2005; Solga et al., 2005; Armitage et al., 2011; Limpens et al., 2012; Harmens et al., 2014; Schröder et al., 2014). However, the biotic factors and mechanisms regulating the relationships have not been clearly understood, which hinders more accurate estimation of N deposition using moss N concentrations (Zechmeister et al., 2008; Harmens et al., 2011, 2014). In fact, influences of N deposition on N concentrations are associated with the N utilization and metabolism mechanisms, which greatly depend on the levels and forms of N deposition, moss physiology difference among different species and among different growing periods (Soares and Pearson, 1997; Koranda et al., 2007; Liu et al., 2012). First, moss N concentrations are more sensitive to low N deposition when N cannot meet moss N demand than to high N deposition especially when N supply exceeds the N demand for moss growth (Pitcairn et al., 1998; Bragazza et al., 2005; Solga et al., 2005; Armitage et al., 2011; Limpens et al., 2012; Harmens et al., 2014; Schröder et al., 2014). Reduced N uptake rates of mosses have been reported as an ecophysiological adjustment to high N deposition (Wiedermann et al., 2009). Second, ammonium (NH_4^+) is more detrimental to some moss species than nitrate (NO_3^-) (Fangmeier et al., 1994; Krupa, 2003; Paulissen et al., 2004, 2005; Sheppard et al., 2014), while some moss species showed NH_4^+ preference over NO_3^- (Wiedermann et al., 2009; Liu et al., 2013a,b; Fritz et al., 2014; Varela et al., 2016). Third, differing N concentrations have been observed among some moss species (Soares and Pearson, 1997; Solga and Frahm, 2006; Schröder et al., 2010; Lequy et al., 2016), though its influences on estimating N deposition have not been well evaluated. Fourth, moss N concentrations can decrease due to the dilution effect of more rapid accumulation of moss biomass in the growing season (Malmer, 1990; Solga and Frahm, 2006). Accordingly, due to the temporal heterogeneities of environmental conditions (e.g., precipitation, temperature, N deposition), different mosses might express different strategies and efficiency of N utilization between growing and non-growing seasons, which would cause different moss N concentrations (Solga and Frahm, 2006). If this is common for different moss species, the one-off sampling that was widely adopted in moss monitoring studies of N deposition can only provide a 'snapshot' result thus potentially underestimate or overestimate the annual level of N deposition. Therefore, it is important to investigate the variations of moss N concentrations among different species and among different samplings.

Natural carbon (C) and N isotopes (expressed as $\delta^{13}C$ and $\delta^{15}N$ values, respectively) in plants are unique and effective tools to imprint the environmental influences on photosynthetic C fixation and plant N utilization, respectively (Evans, 2001; Dawson et al., 2002; Craine et al., 2015). So far, all observed moss $\delta^{13}C$ values (−35‰ to −22‰; unpublished data compiled by Dong and Liu) were distributed within the $\delta^{13}C$ range of C_3 plants (−35‰ to −20‰; Dawson et al., 2002), but photosynthetic pathways of mosses and associated ^{13}C discriminating mechanisms are far from being fully understood due to the lack of specific studies. As mosses are poikilohydric and non-vascular plants (Glime, 2007), their $\delta^{13}C$ values are found to be more sensitive to changes in temperature, precipitation and N deposition that potentially influence moss photosynthetic performance (Skrzypek et al., 2007; Liu et al., 2010; Bramley-Alves et al., 2015). However, it is unknown whether moss $\delta^{13}C$ differs significantly among species due to the heterogeneities of genetic and morphological traits, which would potentially bias the interpretation of moss $\delta^{13}C$ signals for changes in environmental conditions (Rice and Giles, 1996; Delgado et al., 2013; Bramley-Alves et al., 2015). Recently, $\delta^{13}C$ values of mixed moss samples were found to correlate with both N concentrations and atmosphere CO_2 concentrations negatively in southwest (SW) China (Liu et al., 2010). Nevertheless, it remains unclear whether environmental conditions or moss species are more important in determining variations of moss $\delta^{13}C$ values. Accordingly, it is necessary to investigate variations of moss $\delta^{13}C$ values among different moss species and among different growing periods.

Due to the reliance of mosses on N deposition as the dominant N supply, moss $\delta^{15}N$ can provide a quick screening of dominant N sources and species in deposition (Pearson et al., 2000; Bragazza et al., 2005; Solga et al., 2005). In the last decade, moss $\delta^{15}N$ analyses have substantially contributed to the understanding of sources and compositions of regional N deposition (e.g., Bragazza et al., 2005; Zechmeister et al., 2008; Xiao et al., 2010, Xiao and Liu, 2011; Liu et al., 2012, 2013b; Varela et al., 2013; Skudnik et al., 2015, 2016; Felix et al., 2016). Because of lower $\delta^{15}N$ values of NH_4^+ than NO_3^- in precipitation (Heaton, 1987; Kendall et al., 2007) and NH_4^+ preference over NO_3^- during moss N utilization (Soares and Pearson, 1997; Wiedermann et al., 2009; Liu et al., 2013b; Varela et al., 2013, 2016; Fritz et al., 2014), moss $\delta^{15}N$ exhibited decreasing values with the increase of NH_4^+/NO_3^- ratios in wet deposition (Bragazza et al., 2005; Liu et al., 2008b,c, 2012; Xiao and Liu, 2011). Recent studies on tissue NO_3^- isotopes of mosses also suggest the physiological preference for NH_4^+ over NO_3^- and the inhibition of NO_3^- reduction activities by reduced dissolved nitrogen (RDN, mainly as NH_4^+ and DON) in mosses under N deposition with high RDN/NO_3^- ratios (Liu et al., 2012, 2014). These studies highlight the importance of moss N utilization in regulating moss $\delta^{15}N$ recording of N deposition. However, these insights have been mostly based on mixed samples of multiple moss species and/or one-off sampling. It is unanswered whether moss N utilization differs among species, and among different growing periods, which might influence reconstructing the compositions of N deposition using moss $\delta^{15}N$ (Liu et al., 2012). Therefore, it is necessary to investigate inter-species and intra-annual variations of moss $\delta^{15}N$ values in order to enrich the knowledge of moss N-use mechanisms.

To explore above questions, we measured the concentrations and isotopic ratios of C and N in four moss species (*Bryum argenteum* Hedw. *Eurohypnum leptothallum* (C. Muell.) Ando, *Haplocladium microphyllum* (Hedw.) Broth, *Hypnum plumaeforme* Wils.) monthly from August 2006 to August 2007 at an urban site of Guiyang, SW China. The objectives of this paper are to uncover moss N and $\delta^{15}N$ variations among different moss species and among different samplings, and to explore potential mechanisms regulating these variations.

2. Materials and methods

2.1. Study site

This study was conducted at Mt. Guanfeng in the southeast of Guiyang downtown (26°34.5′N, 106°43.3′E). Guiyang is the capital city of Guizhou province, SW China (Fig. 1). Guiyang has a typical subtropical monsoon climate, and most of the landforms have an altitude of 1000–1500 m. The annual rainfall at Guiyang is 1174 mm, which has a distinct seasonal pattern, with about 70% falling during the warmer and rainy months (May to October). The mean annual relative humidity is 86% and the mean annual temperature is 15.3 °C (Guiyang Environmental Protection Bureau, 2006). With the mild climate and widespread naked carbonate rocks in the Karst rocky desertification region, there are abundant epilithic moss resources in Guiyang area. Due to the dominance of naked rocks and few shrubberies, there are no big canopies or overhanging vegetation on the upper Mt. Guanfeng, thus it is an