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.

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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

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

ideal and suitable place to carry out moss bio-monitoring studies of atmospheric deposition.

2.2. Sample sampling and chemical analyses

Four epilithic mosses (*B. argenteum*, *E. leptothallum*, *H. microphyllum*, *H. plumaeforme*) were selected in this study. The studied species belong to dominant epilithic mosses in SW China and have wide distributions in rocky places, thus basically meet the sample requirement of this investigation (Peng and Luo, 2003). These mosses are uniform pleurocarpous species, among which the *E. leptothallum* and *H. microphyllum* have been adopted in N deposition studies (e.g., Liu et al., 2007). The *H. plumaeforme* and *B. argenteum* may also have endurance of pollution due to their wide ranges of habitats in the urban settings. We set four plots on the upper part (1065 ± 10 m) of Mt. Guanfeng and selected 2—3 sampling sites in each plot, and each sampling of each species was conducted at 3—6 subsites (Fig. 1). An eligible sampling site must have an adequate distribution of any target moss species and avoid the influence from surface-water splashing. Epilithic mosses with soil substrates and sites possibly disturbed by domestic animals or pets were also avoided.

At the end of each month (24th—31st) from August 2006 to August 2007, we collected subsamples at sites where any eligible target moss species were available. Then we combined subsamples from all sampling sites into one representative composite sample of each species in each month for each site. Only green and healthy moss shoots (with a subjective assessment) were taken from a lump of moss clumps or mats with different cushion sizes, avoiding yellow and dark or senescent tissues, thus the sample amount is often very low especially in non-growing months.

Within 2 h of each sampling, moss samples were sonicated and washed with deionized water to thoroughly remove adsorbed pollutants. Washed mosses were dried in a vacuum oven connected with a vacuum pump at 70 °C and finely ground. Moss C and N concentrations (%, dry weight) were determined by an elemental analyzer (PE2400 II, USA), with an analytical precision of 0.02%.

Based on C and N concentrations, bulk $\delta^{13}C$ values of about 50 μg C and $\delta^{15}N$ values of about 100 μg N in each moss sample were determined separately by a Thermo MAT 253 isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with an elemental analyzer (Flash EA 2000). IAEA-C$_3$ ($\delta^{13}C = -24.97‰$, cellulose) was used as a standard for $\delta^{13}C$. IAEA-N-1 (Ammonium sulfate; $\delta^{15}N = 0.4‰$), USGS25 (Ammonium sulfate, $\delta^{15}N = -30.4‰$) and IAEA-NO-3 (Potassium nitrate; $\delta^{15}N = +4.7‰$) were used as standards for the calibration of $\delta^{15}N$ values. The average standard deviation for replicate analyses of an individual sample was ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$. The $\delta^{13}C$ or $\delta^{15}N$ was expressed in parts per mil (‰) by multiplying them by 1000:

$$\delta \,(^{13}C,^{15}N) = (R_{sample}/R_{standard} - 1) \times 1000, \,(1)$$

where $R = {}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$ in samples and standards (Vienna Pee Dee Belemnite and atmospheric N$_2$, respectively).

2.3. Statistical analysis and modelling

Statistical analyses were conducted using SPSS 12.0 software package for Windows (SPSS Science, Chicago, USA). Prior to analysis, raw data were tested for normality using the Shapiro-Wilk's W-test and for homogeneity of variance using Cochran's C-test. Transformations were performed when necessary to satisfy assumptions. Tukey HSD (Tukey Honest Significant Difference) and LSD (Least Significant Difference) tests were used to identify significantly different means among different moss species. For those whose variance remains non-homogeneity, Kruskal-Wallis test was used to identify significantly different means among different moss species. An independent-samples T-test was used to determine significant differences of moss C and N parameters between warmer and cooler months. All correlations were analyzed by one-way analysis of variance (ANOVA). We analyzed the relationship between moss N, sampling month and species variables.
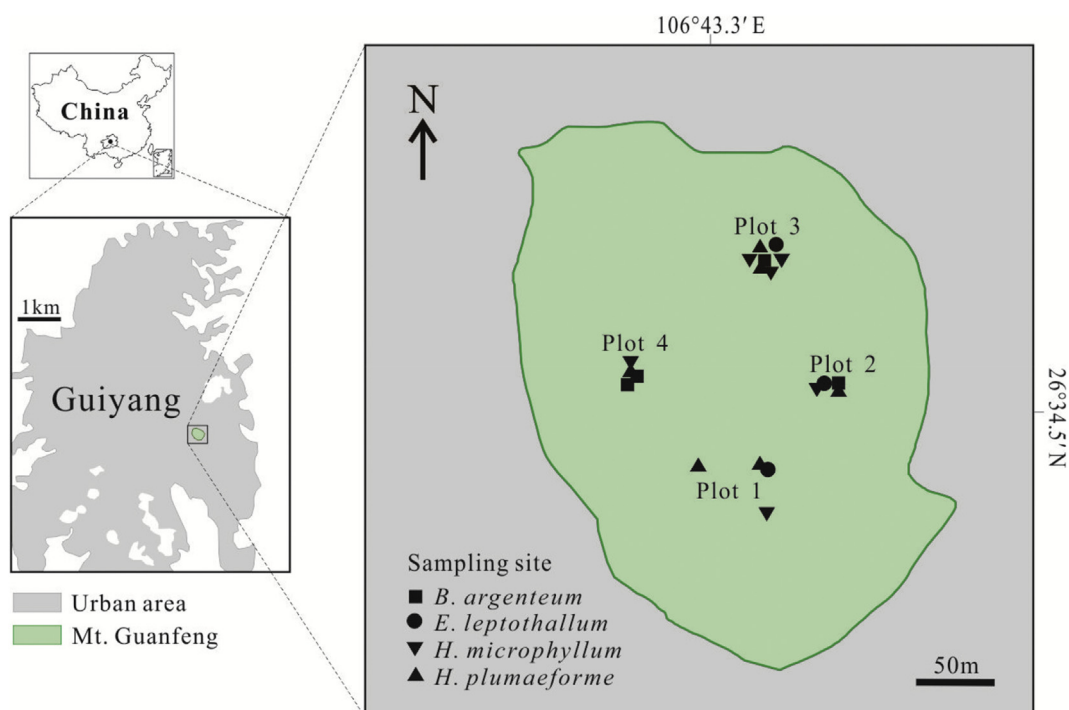

Fig. 1. Map showing the location of the Guiyang area and four plots distributed on Mt. Guanfeng.

The model selection was carried out using stepwise regression with Akaike's information criterion. Once the final regression models were selected using the step-wise procedure, the importance or explanatory power of each predictor variable was estimated as follows: the sum of squares (SS) of each predictor in the model was calculated, and then its explanatory power is calculated as the proportion of the SS of one predictor relative to the summary of SS across all the predictors in the model. The same procedure was carried out to moss C and N isotopic values. Statistically significant difference was set at P values < 0.05 unless otherwise stated.

A Bayesian isotope mixing model (SIAR, Stable Isotope Analysis in R) was used to calculate the contributions of precipitation NH_4^+, NO_3^-, DON to moss bulk N. The SIAR model adopts a Bayesian framework to establish a logical prior distribution based on Dirichlet distribution (Evans et al., 2000), and then to determine the distribution for the contributions of potential N sources to the observed N mixture (Wang et al., 2017). By defining a set of N mixture measurements on J isotopes with K source contributors, the applied SIAR model can be described as follows (Parnell et al., 2010):

$$X_{ij} = \sum_{k=1}^{K} F_k\left(S_{jk} + c_{jk}\right) + \varepsilon_{ij} \qquad (2)$$

$$S_{ij} \sim N\left(\mu_{jk}, \omega_{jk}^2\right)$$

$$c_{jk} \sim N\left(\lambda_{jk}, \tau_{jk}^2\right)$$

$$\varepsilon_{ij} \sim N\left(0, \sigma_j^2\right)$$

where all F values sum to 1 (unity), X_{ij} is the isotope value j of the mixture i, in which $i = 1, 2, 3, …, N$ and $j = 1, 2, 3, …, J$; S_{jk} is the source value k on isotope j ($k = 1, 2, 3, …, K$) and is normally distributed with mean μ_{jk} and standard deviation ω_{jk}; F_k is the proportion of source k estimated by the SIAR model; c_{jk} is the fractionation factor for isotope j on source k and is normally distributed with mean λ_{jk} and standard deviation τ_{jk}; and ε_{ij} is the residual error representing the additional unquantified variation between individual mixtures and is normally distributed with mean 0 and standard deviation σ_j. Detailed descriptions of the model can be found in Moore and Semmens (2008), Jackson et al. (2009) and Parnell et al. (2010).

In this study, the estimation using the SIAR model can substantially incorporate $\delta^{15}N$ variations of N sources (mean ± SD values were input into the model: $-10.6 ± 7.7$‰ for NH_4^+, $-1.9 ± 3.0$‰ for NO_3^- and $-0.7 ± 15.5$‰ for DON, respectively; Xiao et al., 2012; Liu et al., 2017) and $\delta^{15}N$ variations of mosses (replicate values of thirteen samplings for a given species (Fig. 2) or four moss species for a given sampling (Fig. 3) were input into the model to calculate the proportional contributions of each N form in precipitation to moss N). As there is negligible isotopic fractionation during moss N uptake processes (Liu et al., 2008a,b,c, 2013b; Xiao and Liu, 2011), we assumed no substantial difference in $\delta^{15}N$ values between moss bulk N and precipitation N sources (NH_4^+, NO_3^-, DON) used by mosses, i.e., $c_{jk} = 0$ in Eq. (2). Because moss $\delta^{15}N$ values were lower than those of precipitation NO_3^- and DON, the proportional contributions of these two N sources to moss bulk N could not be analyzed by the SIAR model if mosses were assumed to use precipitation NO_3^- and DON only (Fig. 2d), i.e., precipitation NH_4^+ should be considered as an N source for all mosses in our study. Then the proportional contributions of precipitation N sources (NH_4^+, NO_3^-, DON) to moss N were analyzed by the SIAR model under three scenarios (Scenario 1: mosses assimilated NH_4^+
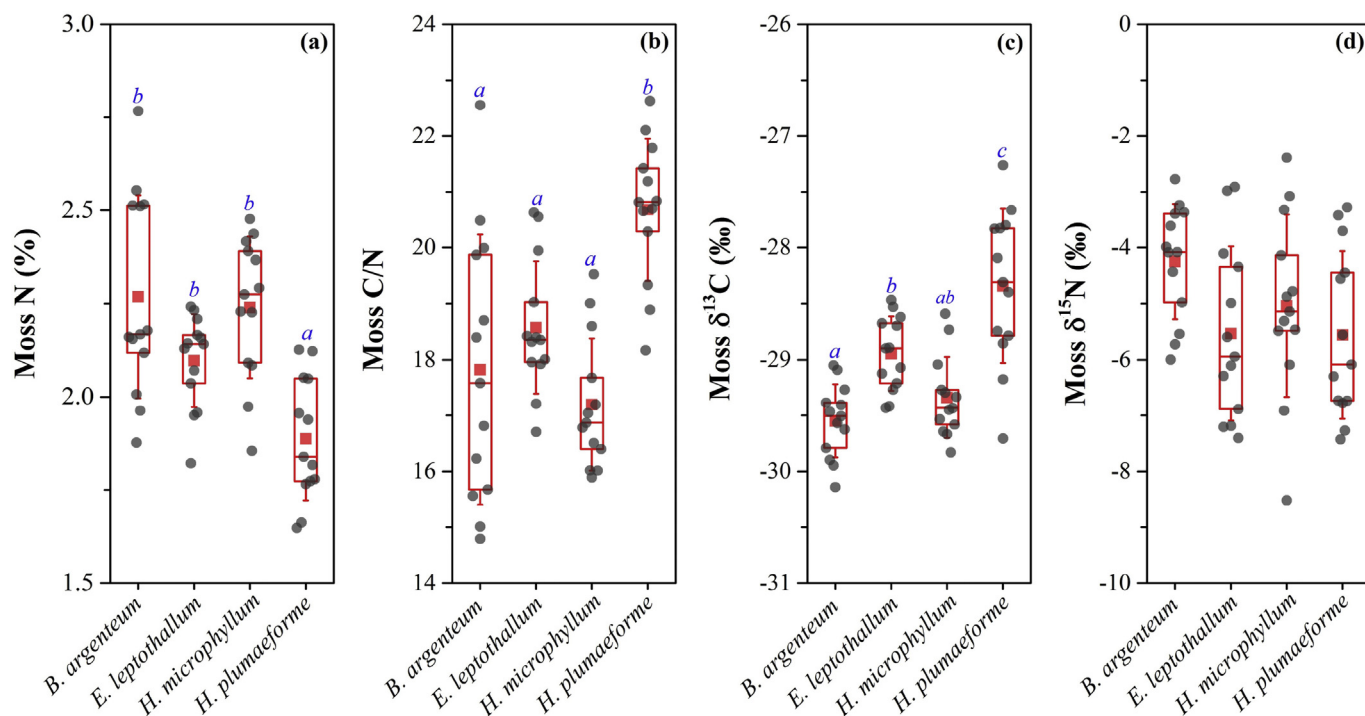

Fig. 2. Concentrations (panels a & b) and isotopic signatures (panels c & d) of bulk C and bulk N in four epilithic mosses at Guiyang, SW China. The boxes encompass the 25th − 75th percentiles and the whiskers show SD values. The line and square (in red) in each box mark the median and arithmetic mean values ($n = 13$), respectively. The jittered data (gray-filled) of each species were monthly values from August 2006 to August 2007. Completely different letters above the boxes indicate a significant statistical difference between values (means ± SD) of different moss species ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and NO_3^- in precipitation, Fig. S3a; Scenario 2: mosses assimilated NH_4^+, NO_3^- and DON in precipitation, Fig. S3b; Scenario 3: mosses assimilated NH_4^+ and DON in precipitation, Fig. 6). The percentage data ($n = 10\ 000$ for each N source in each analysis) output from the SIAR model were calculated for mean and SD values.

3. Results

3.1. Inter-species differences of moss C, N concentrations, $\delta^{13}C$ and $\delta^{15}N$

Moss C concentrations (39.0 ± 1.8%) ranged between 35.6% and 43.9%, and no significant difference was found among four moss species (data not shown). Moss N concentrations (2.1 ± 0.2%) ranged between 1.6% and 2.8%, among which the *H. plumaeforme* had significantly ($P < 0.05$) lower N concentrations (1.9 ± 0.17%) than other three species (2.2 ± 0.2%) (Fig. 2a). Moss C/N values ranged between 14.8 and 22.6, with no significant difference among moss species (Fig. 1b). Moss $\delta^{13}C$ values ranged between −30.1‰ and −27.3‰, among which the *H. plumaeforme* had significantly higher $\delta^{13}C$ values (−28.3 ± 0.7‰) than other three species (−29.3 ± 0.4‰) (Fig. 2c). Moss $\delta^{15}N$ values ranged between −8.5‰ and −2.4‰ (−5.1 ± 1.5‰) (Fig. 2d), with no significant difference among moss species.

3.2. Intra-annual variations of moss C, N concentrations, $\delta^{13}C$ and $\delta^{15}N$

Moss C concentrations showed no significant difference between in cooler months and in warmer months (data not shown). Moss N concentrations, $\delta^{13}C$ and $\delta^{15}N$ values fluctuated distinctly across all thirteen samplings (Fig. 3). In general, moss N concentrations were higher in cooler months (November to April) than in warmer months (May to October) ($P < 0.05$) (Fig. 3a), whereas moss $\delta^{13}C$ and $\delta^{15}N$ values were higher in warmer months than in cooler months ($P < 0.05$) (Fig. 3bc).

The maximum differences of moss N among different samplings were 0.9% for *B. argenteum*, 0.4% for *E. leptothallum*, 0.6% for *H. microphyllum*, and 0.5% for *H. plumaeforme* (Table 1). When N concentrations averaged for four species of the same sampling, moss N differences among different samplings ranged from 0.0% to 0.5%, showing an SD value of ±0.2% annually (Table 1). The maximum differences of moss $\delta^{13}C$ among different samplings were 1.0‰ for *B. argenteum*, 0.9‰ for *E. leptothallum*, 1.2‰ for *H. microphyllum*, and 2.4‰ for *H. plumaeforme*, respectively (Fig. 3b). The maximum differences of moss $\delta^{15}N$ among different samplings were 3.2‰ for *B. argenteum*, 4.5‰ for *E. leptothallum*, 6.1‰ for *H. microphyllum*, and 4.1‰ for *H. plumaeforme* (Fig. 3c).

3.3. Proportional contributions of precipitation N sources to moss N

Proportional contributions of precipitation NH_4^+ and NO_3^- were 36.8 ± 17.5% and 63.2 ± 17.5% for four analyses of moss species, 30.1 ± 9.4% and 69.9 ± 9.4% for thirteen analyses of moss sampling when assuming precipitation NH_4^+ and NO_3^- as moss N sources (Scenario 1; Fig. S3a). Proportional contributions of precipitation NH_4^+, NO_3^- and DON were 30.9 ± 9.5%, 61.2 ± 11.5% and 7.9 ± 5.9% for four analyses of moss species, 34.7 ± 15.0%, 44.6 ± 17.9% and 20.7 ± 14.0% for thirteen analyses of moss sampling when assuming precipitation NH_4^+, NO_3^- and DON as moss N sources (Scenario 2; Fig. S3b). Proportional contributions of precipitation NH_4^+ and DON were 66.3 ± 11.6% and 33.7 ± 11.6% for four analyses of moss species, 59.0 ± 19.1% and 41.0 ± 19.1% for thirteen analyses of moss sampling when assuming precipitation NH_4^+ and DON as moss N sources (Scenario 3; Fig. 6).

4. Discussion

4.1. Mosses N and $\delta^{13}C$ variations

The mean N concentration of *H. microphyllum* (2.2 ± 0.2%) was similar to that of this species (2.3 ± 0.1%) measured previously at Guiyang urban area (Liu et al., 2008a). However, N concentrations of *H. plumaeforme* were found significantly lower than those of other three species in this study (Fig. 2a), which indicated that inter-species differences of moss N concentrations can be a pitfall in estimating N deposition (Delgado et al., 2013). According to the relationships between N deposition levels and moss N concentrations integrated in southern China (Liu et al., 2008a; Xiao et al., 2010) and European countries (Harmens et al., 2011) (Table 1), the estimated N deposition at our sampling sites differed significantly among four moss species. The maximum inter-species difference of N concentrations was observed in December (up to 0.7%, Fig. 3a), resulting in a variation of estimated N deposition as 13.5 kg N ha^{-1} yr^{-1} and 9.1 kg N ha^{-1} yr^{-1}, respectively (Table 1). The precipitation N deposition at Guiyang urban was measured as 30.7 kg N ha^{-1} yr^{-1} (Liu et al., 2012), which corresponds to a moss N concentration of 2.3% according to the relationship integrated in southern China (Liu et al., 2008a; Xiao et al., 2010). Clearly, N fixation ability could differ among non-vascular mosses, even at the same site with very similar habitats and N supply (Gordon et al., 2002; Mitchell et al., 2002; Gundale et al., 2011), which informed a necessity to select moss species with N concentrations better fitting to the response relationships established in previous studies.

Moreover, intra-annual variations of moss N concentrations among different samplings (Fig. 3a) would also bias the estimating results of N deposition levels. The maximum intra-annual difference of N concentrations in *B. argenteum* (up to 0.9%, 1.9–2.8%; Table 1) resulted in differences in estimated N deposition as 17.1 kg N ha^{-1} yr^{-1} and 11.5 kg N ha^{-1} yr^{-1} according to relationships integrated in southern China (Liu et al., 2008a; Xiao et al., 2010) and European countries (Harmens et al., 2011) (Table 1), respectively. Clearly, one-off sampling of mosses in a random date has an even higher risk in evaluating N deposition than the inter-species differences. Therefore, without careful examination of moss N variations, there is a risk of over-estimating or under-estimating N deposition levels using N concentrations of mosses to map site-specific and regional N deposition.

Further variance component analyses showed that species differences play an important role in determining moss N concentration (accounting for nearly 42.3% of the variance) and moss $\delta^{13}C$ variations (accounting for nearly 52.8% of the variance), much more so than different samplings (which accounts for only 31.0% and 29.6% of the moss N concentration and $\delta^{13}C$ variances, respectively) (Fig. 4). Plant $\delta^{13}C$ signatures could integrate ^{13}C discriminations of photosynthetic activities throughout the growing period (Rice and Giles, 1996), which for vascular plants generally include ^{13}C discriminations during the diffusion of atmospheric CO_2 into moss cells and during the photosynthetic assimilation of CO_2 (Farquhar et al., 1989). Differently, mosses are non-vascular plants and have no stomata (Glime, 2007). Thus, no substantial inter-species $\delta^{13}C$ differences was often assumed for mosses under the same growing conditions (Dawson et al., 2002), which also supported that moss $\delta^{13}C$ could respond to environmental factors influencing photosynthetic performance more sensitively than vascular plants (Rice and Giles, 1996; 2000; Fletcher et al., 2006; Loisel et al., 2009). However, there were actually very few studies on inter-species ^{13}C discriminations among moss species (e.g., Ménot and Burns et al., 2001; Skrzypek et al., 2007; Delgado et al., 2013). In this study, significantly lower N concentrations but higher $\delta^{13}C$ values of *H. plumaeforme* than other three species across thirteen samplings

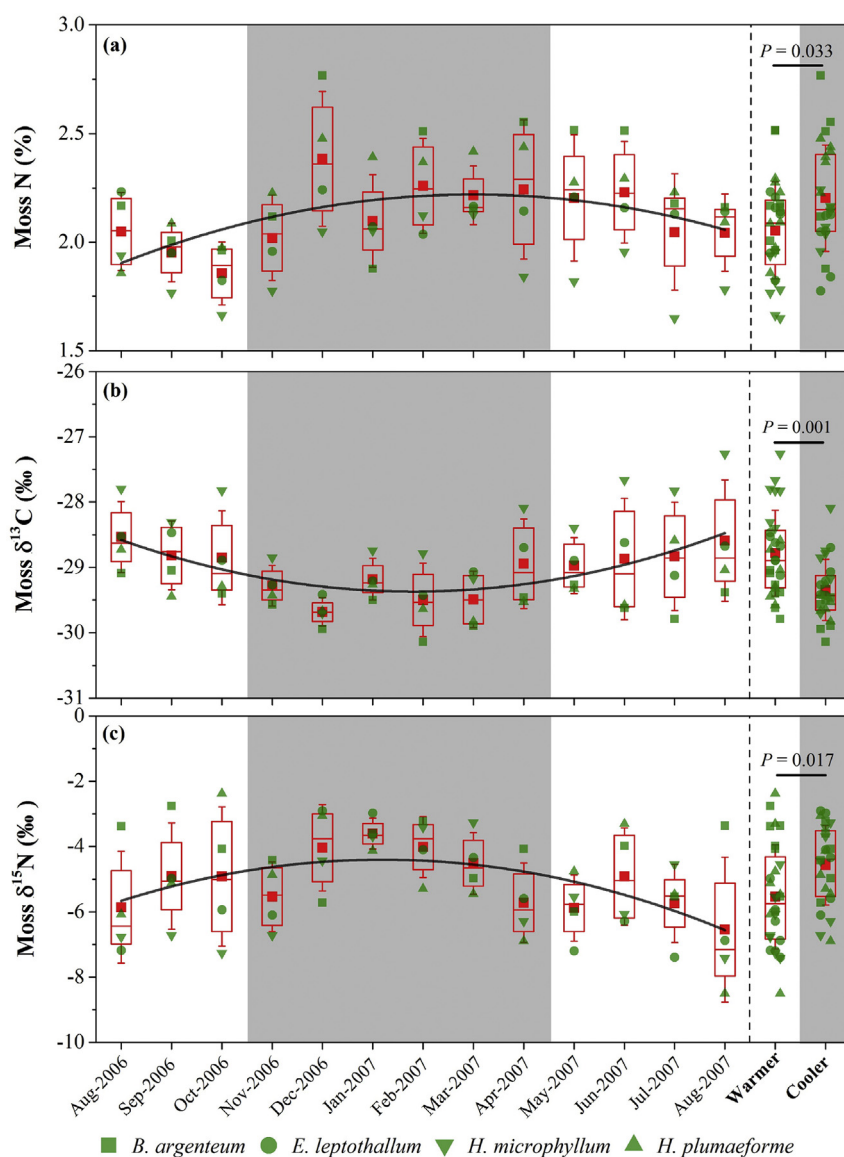**Fig. 3.** Nitrogen concentrations (a), $\delta^{13}C$ values (b) and $\delta^{15}N$ values (c) in four epilithic mosses sampled monthly from August 2006 to August 2007 at Guiyang, SW China. The boxes encompass the 25th – 75th percentiles and the whiskers show SD values. The red line and square (red-filled) in each box mark the median and arithmetic mean values ($n = 4$), respectively. The black solid line represents the fitting line of mean monthly parameters of four moss species. The jittered data (green-filled) of each month were values of four moss species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2bc) clearly show that mosses indeed differed in photosynthetic ^{13}C discriminations and N fixation among different species. As well as significantly lower N concentrations and higher $\delta^{13}C$ values in mosses collected in warmer months than in cooler months ($P < 0.05$) (Fig. 2bc) clearly show the influence of environmental conditions on ^{13}C discriminations during photosynthesis process.

It is intriguing to understand the physiological mechanisms behind moss N variations among different moss species and different samplings (Poikolainen et al., 2009; Armitage et al., 2011). Guiyang has a subtropical monsoon climate, the major period of plant growth (May to October) couples with the warm and rainy seasons, during which N deposition via precipitation was higher than that in non-growing seasons (November to April) (Fig. S1). Moss N concentrations, however, were lower in warmer months than in cooler months ($P < 0.05$) (Fig. 3a). This indicates that N concentrations in studied epilithic mosses were not elevated by higher N deposition in the growing season, though total N uptake

should be elevated. Instead, more rapid biomass accumulation relative to N uptake might be stimulated by elevated N supply (Solga and Frahm, 2006; Solga, 2007), which consequently resulted in relatively lower N concentrations in growing season than in non-growing season (Fig. 3a). Correspondingly, lower N concentrations in *H. plumaeforme* reflected more rapid biomass accumulation than its N uptake (Solga and Frahm, 2006; Delgado et al., 2013).

Previously, moss $\delta^{13}C$ variations were interpreted as influences from environmental factors (e.g., temperature, water and nutrient availability; Körner et al., 1991; Rice and Giles, 1996, Rice, 2000; Ménot and Burns, 2001; Fletcher et al., 2006; Loisel et al., 2009) regulating ^{13}C discriminations of CO_2 diffusion into moss cells (Williams and Flanagan, 1996; Liu et al., 2010). In this study, $\delta^{13}C$ values in mosses collected in warmer months were higher than that collected in cooler months at the same habitat conditions ($P < 0.05$) (Fig. 3b). The result indicates that concentrations and isotopic signatures of atmosphere CO_2 might be lower in warmer months than in cooler months at Guiyang urban area, as photosynthetic uptake
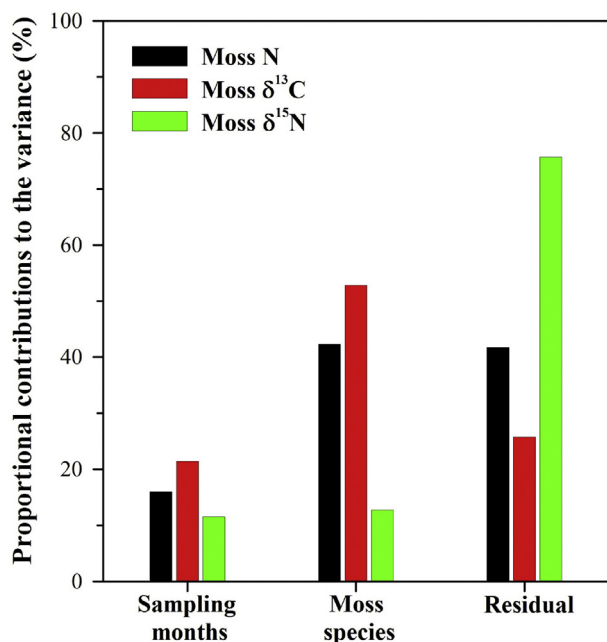
Fig. 4. Results of the variance component analyses performed for influences of different moss species and samplings on variations of moss N, $\delta^{13}C$ and $\delta^{15}N$ at Guiyang, SW China. Residual represents factors other than moss species and sampling months potentially influencing the variations of moss N, $\delta^{13}C$ and $\delta^{15}N$ (e.g., different micro habitats and the physiological adaptation of mosses to the environments).

becomes weaker and more anthropogenic depleted $^{13}CO_2$ emissions from fossil fuel burning in cooler months (Pataki et al., 2003). A positive relationship between moss $\delta^{13}C$ and mean monthly temperature (MMT, which represents the mean value of mean daily temperature in each of our sampling months) was established (Fig. S2), this pattern was similar to the results in previous studies (Yasuda et al., 2015). As the surface water film on epilithic mosses

might become thinner as the higher water evaporation rate in warmer months due to the higher temperature, which would reduce barriers for carbon assimilation due to the much lower CO_2 diffusivity in water than in air (Rice and Giles, 1996). Recently, studies further revealed that mosses with thicker cell walls would prevent the diffusion of ambient CO_2 into cells smoothly, during which ^{13}C discriminations became stronger and $\delta^{13}C$ values of mosses would be more negative (Waite and Sack, 2010; Bramley-Alves et al., 2015). In this study, significantly higher $\delta^{13}C$ signatures of *H. plumaeforme* than other three species (Fig. 2c) suggests lower ^{13}C discrimination due to an easier diffusion of CO_2 through thinner cell walls into cells. This result indicates that leaf anatomy structure of *H. plumaeforme* is significantly different from other three moss species. As Rice and Giles (1996) found that without surrounded by water-filled hyaline cells, photosynthetic cells are exposed to the atmosphere directly at the leaf surface, and that is a morphology expected to lead to high diffusional resistance to CO_2 uptake. At the same time, mosses in growing months showed low N concentrations. All these factors lead to a negative correlation between $\delta^{13}C$ and N concentrations in mosses at Guiyang (Fig. 5).

4.2. Mosses $\delta^{15}N$ for indicating dominant forms of precipitation N

Epilithic mosses receive N mainly from N deposition (Liu et al., 2008a,b,c), but it remains unclear whether their $\delta^{15}N$ records reflect all or only part of dissolved N species they received (Liu et al., 2012). In particular, no study has examined explicit N utilization among moss species and among different samplings. According to the results of the variance component analyses, moss $\delta^{15}N$ variations were less influenced by different species (12.8%) than by different samplings (32.6%) (Fig. 4). This elucidates that moss $\delta^{15}N$ was mainly determined by $\delta^{15}N$ values of N sources they really assimilated during the whole growing period (Bragazza et al., 2005; Zechmeister et al., 2008; Xiao et al., 2010, Xiao and Liu, 2011; Liu et al., 2012, 2013b; Skudnik et al., 2015, 2016; Felix et al., 2016), which differed from the patterns and mechanisms of physiological
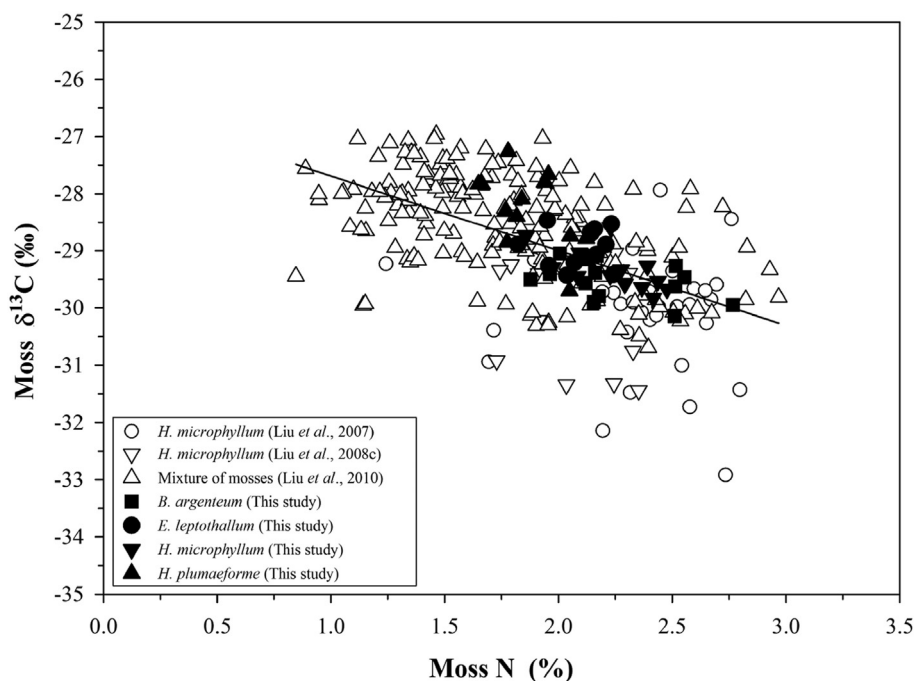

Fig. 5. Correlations between moss N concentrations and $\delta^{13}C$ values at Guiyang, SW China. (Δ represents mixture of *H. microphyllum*, *Haplocladium angustifolium*, *Brachythecium salebrosum* and *E. leptothallum*).
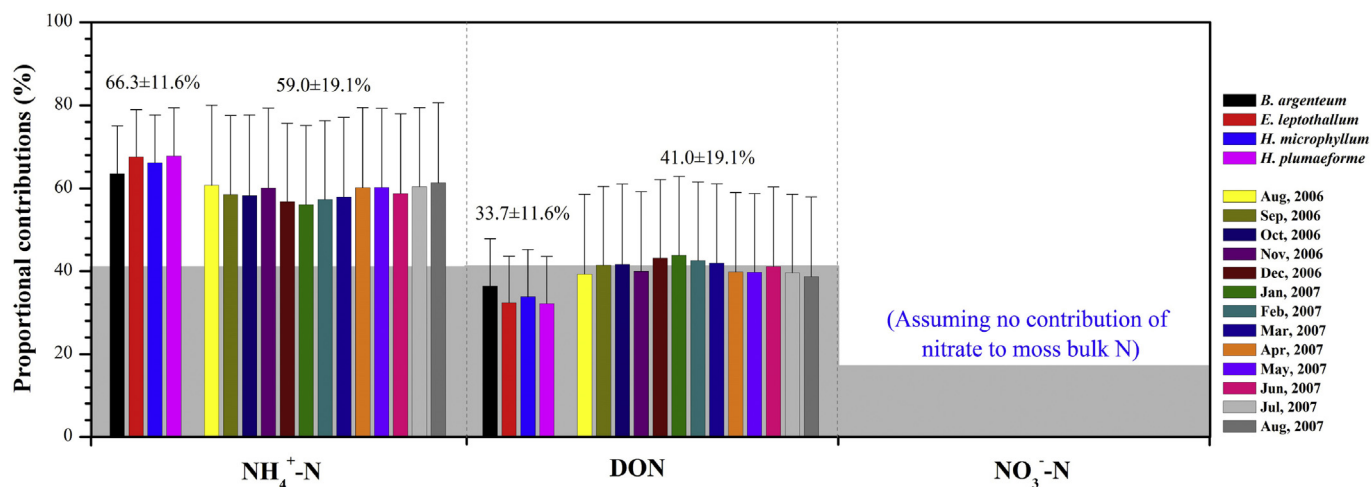
Fig. 6. Proportional contributions (*F*, %) of NH_4^+-N and DON in precipitation TDN deposition (gray areas in panels a & b; cited from Liu et al., 2017) and in bulk N of four epilithic mosses (color-filled bars; whiskers for SD values; *n* = 10000) at Guiyang, SW China. Values above the bars are averages of proportional contributions analyzed by SIAR model for four moss species (*n* = 40000) and thirteen different samplings (*n* = 130000), respectively. The *F* values for mosses were estimated by the SIAR model by assuming the use of NH_4^+ and DON only. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Bulk N concentrations in epilithic mosses and levels of atmospheric N deposition at Guiyang urban estimated by relationships between moss N concentrations (*y*) and N deposition levels (*x*). Values are mean ± SD (min − max). Different mean values show inter-species differences, while SD values (min − max) show variations among different samplings across the study period.

	B. argenteum	*E. leptothallum*	*H. microphyllum*	*H. plumaeforme*	References
Moss N concentration (%; *n* = 13)	2.3 ± 0.3 (1.9−2.8)	2.1 ± 0.1 (1.8−2.2)	2.2 ± 0.2 (1.9−2.5)	1.9 ± 0.2 (1.6−2.1)	This study
Estimated N deposition based on $y = 0.73 + 0.052x$ (kg N ha^{-1} yr^{-1})	29.6 ± 5.2 (22.1−39.2)	26.3 ± 2.4 (21.0−29.1)	29.0 ± 3.7 (21.6−33.6)	22.3 ± 3.2 (17.7−26.9)	Liu et al., 2008a; Xiao et al., 2010
Estimated N deposition based on $y = 0.54 + 0.077x$ (kg N ha^{-1} yr^{-1})	22.4 ± 3.5 (17.4−28.9)	20.2 ± 1.6 (16.6−22.1)	22.1 ± 2.5 (17.1−25.2)	17.5 ± 2.1 (14.4−20.6)	Harmens et al., 2011

parameters such as N concentrations and δ^{13}C variations (Fig. 4).

In principal, proportional contributions of precipitation N forms (NH_4^+, NO_3^- and DON) to moss bulk N (estimated by the SIAR model) should assemble proportional contributions of corresponding N forms in total dissolved N (TDN) in precipitation (40.7% for NH_4^+, 18.6% for NO_3^- and 40.7% for DON; Xiao et al., 2012; Liu et al., 2017) (Fig. S3 & Fig. 6). However, according to analyzing results of scenarios 1 and 2 (Fig. S3), the proportional contributions of precipitation NO_3^- to moss bulk N were much higher than those of NO_3^- in TDN of precipitation. These two scenarios show a preferring utilization of precipitation NO_3^- over NH_4^+ and DON in mosses, which should be not applicable to interpret moss δ^{15}N signatures in our study site. The main reasons include that 1) NO_3^- is not a dominant N form in TDN compared with reduced N forms in precipitation at Guiyang, SW China (Xiao et al., 2010; Liu et al., 2012); 2) most studies have shown NH_4^+ or reduced N preference over NO_3^- even they are equally available, instead very little evidences on moss NO_3^- preference (Wiedermann et al., 2009; Liu et al., 2013b). Differently, when considering precipitation NH_4^+ and DON as moss N sources (Scenario 3; Fig. 6), the proportional contributions of precipitation NH_4^+ to moss N (56.1−67.8%) were higher than those of NH_4^+ in TDN of precipitation (40.7% on average), and the proportional contributions of precipitation DON to moss N (32.2−43.9%) assembled those of DON in TDN of precipitation (40.7% on average) (Fig. 6). This scenario shows a preferring utilization of precipitation NH_4^+ over NO_3^-, which is more applicable for interpreting moss δ^{15}N signatures in our study site. The main reasons include that 1) NH_4^+ is a dominant N form in TDN compared with NO_3^- in precipitation at Guiyang, SW China (Xiao et al., 2010; Liu et al., 2012); 2) mosses showed a substantial NH_4^+ preference

over NO_3^- (Liu et al., 2017) and δ^{15}N of tissue NO_3^- in mosses were found assembling that of NO_3^- in precipitation, suggesting negligible NO_3^- assimilation occurred (Liu et al., 2012). More detailed mechanisms related to moss NH_4^+ preference over NO_3^- or inhibition of NO_3^- reduction can be found in previous works (Soares and Pearson, 1997; Wiedermann et al., 2009; Varela et al., 2013, 2016; Fritz et al., 2014). The use of DON, by mosses, has been widely reported (Wanek and Pörtl, 2008; Wiedermann et al., 2009; Liu et al., 2013b). In this study, moss δ^{15}N records were higher in cooler months than in warmer months (*P* < 0.05) (Fig. 3c), confirming the hypothesis that epilithic mosses might use DON, whose isotopic value was higher in cooler months than in warmer months (Liu et al., 2017). Under high N supply relative to moss N demand and for energetic economy, the preferential uptake of NH_4^+ and amino acids over NO_3^- have been evidenced in both experimental and *in situ* studies (Woodin et al., 1985; Limpens and Berendse, 2003; Wiedermann et al., 2009; Liu et al., 2013b). All these results support the observed responsiveness of moss ^{15}N depletion to N deposition elevated and/or dominated by NH_4^+ in southern China and European countries (e.g., Bragazza et al., 2005; Liu et al., 2008a,b,c, 2012; Xiao et al., 2010). The contributions of precipitation NH_4^+ and DON in moss bulk N did not differ significantly among species and samplings (Fig. 6), which also support little influences of species traits on moss δ^{15}N and moss δ^{15}N as an integrator of both growing and non-growing N inputs.

5. Conclusions

This study presented moss C, N concentrations, δ^{13}C and δ^{15}N signatures among moss species and different samplings. Moss N

concentrations differed among species, which would bias the estimation of N deposition levels. Moss $\delta^{15}N$ values did not significantly differ among moss species and different samplings, but was mainly controlled by N sources they really assimilated. Species selection and multiple sampling comparisons, instead of single species and one-off sampling, are important steps for accurate biomonitoring works of atmospheric N deposition in China. This work contributes to the underlying mechanisms of N use in moss bioindicators and furthered the isotope application for biogeochemical processes of atmospheric N pollutants.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.06.058.

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