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Stable carbon and nitrogen isotopes of the moss *Haplocladium microphyllum* in an urban and a background area (SW China): The role of environmental conditions and atmospheric nitrogen deposition

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Abstract

The C and N concentrations and stable isotopes in new and old tissues of the moss *Haplocladium microphyllum* were investigated at Guiyang and Gongga Mountain in SW China, aiming at revealing responses of these parameters to different environmental conditions and N deposition, elucidating the effect of N deposition on C fixation and signal variations during senescence. Atmospheric N deposition could be quantified by N in new tissues, showing a level of 30.18 $\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$ at Guiyang and 8.46 $\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$ at Mt. Gongga, old tissues presented lower C and N concentrations than new tissues, but there was no significant difference between N of new and old tissues at background area with lower N deposition, and the positive effect of N supply on C fixation was observed only for urban mosses under higher N deposition. More negative δ^{13}C mainly indicated the influences of anthropogenic CO_2 sources on urban mosses, and higher δ^{13}C of mosses at background area was also related to higher altitude and lower temperature. More negative δ^{15}N of mosses mainly indicated N deposition at Guiyang was dominated by NH_x–N from city wastes and sewage, while δ^{15}N of mosses at Mt. Gongga mainly indicated little anthropogenic N pollution in background area. Besides, no significant isotopic difference was found between new and old tissues in both areas, suggesting no isotopic effect occurred during the senescence of *H. microphyllum*.

Keywords: Atmospheric nitrogen deposition; Biomonitoring; Stable isotope; Moss; Environment

1. Introduction

Both elemental concentrations and isotopic signatures have been extensively employed for understanding the interrelations between plants and environment (Dawson et al., 2002). Foliar carbon (C) concentration directly reflects the C fixation

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ability of plants in varying environments, and $\delta^{13}C$ signal is a key indicator of photosynthetic performance and of effects on C assimilation imposed by environmental stresses (Farquhar et al., 1989; Schulze et al., 1996; Fletcher et al., 2006). As a biologically important element, plant nitrogen (N) level can show the status of N nutrition in ecosystem, and in the past two decades, great interest in measuring plants $\delta^{15}N$ has produced a considerable literature on the environmental and ecological significance of isotopically different N sources, e.g. previous studies by Skinner et al. (2004, 2006) indicated a direct link between N deposition and %N/$\delta^{15}N$ in *Calluna vulgaris*, and these two parameters can be used to quantify N deposition.

Due to the special biological and morphologic characteristics of nonvascular plants, mosses are very sensitive to environmental change, pollution, nutrient condition and ecosystem health (Merritt, 2003). Some species (e.g. *Pleurozium schreberi* and *Sphagnum*) have been identified as reliable indicators of atmosphere components ranging from highly localized to regional or even global (Pitcairn et al., 2001; Ménot and Burns, 2001). However, the majority of related works have focused on the accumulation of heavy metals or trace elements (e.g. Berg and Steinnes, 1997), less has been done around C and N topics, and even much less on stable isotopes. Earlier studies on mosses $\delta^{13}C$ by Rundel et al. (1979) and Teeri (1981) opened our understanding of mosses photosynthesis and function of environmental indication, and recent works further showed that mosses $\delta^{13}C$ could sensitively reflect the variations of environmental factors, such as temperature (Skrzypek et al., 2007), altitude (Ménot and Burns, 2001), water limitation and microhabitats (Rice, 2000). Therefore, it is necessary to further explore the potential of mosses $\delta^{13}C$ in the field of bioindication, and more studies on the response and adaptation of mosses to environmental and atmospheric variations should be conducted.

The N concentration and $\delta^{15}N$ signature in mosses have been recognized as more sensitive and reliable tools to answer questions about the level and sources of N deposition. Some recent studies have observed that the N content of various mosses species was proportional to atmospheric N inputs (e.g. Pitcairn et al., 2003), which could contribute to the estimation of N deposition level in remote areas with scarce monitoring (Solga et al., 2005). Moreover, studies by Pearson et al. (2000) and Gerdol et al. (2002) have found that mosses $\delta^{15}N$ could effectively identify the atmospheric N sources of urban traffic NO_x (relatively positive) and rural animal NH_3 (relatively negative). Solga et al. (2005) and Bragazza et al. (2005) further established the negative correlation between mosses $\delta^{15}N$ and the ratio of NH_x–N/NO_x–N in atmospheric deposition, and studies by Harrison et al. (1999), Kosior et al. (2008) have shown that mosses $\delta^{15}N$ could respond to both temporal and spatial variations of NH_x–N in atmospheric deposition.

However, most of former works on mosses isotopes were mainly carried out in Europe, we could find much less work in China/Asia region except for abundant studies on taxology. In addition, it is known that there are different elemental concentrations and isotopic signals between new tissues and old tissues for many tracheophytes (Robinson et al., 1998; Evans, 2001), but it is still unclear in mosses, which is important for sampling strategies in applying mosses for biomonitoring purposes and helpful for understanding the elemental utilization, storage and translocation in moss tissues.

Therefore, in this study, we measured the C and N concentrations, isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) in new tissues and old tissues of the same moss (*Haplocladium microphyllum*) in an urban area and a background area of southwest China, specifically attempts to:

1. Indicate the level and sources of regional N deposition based on mosses N concentrations and $\delta^{15}N$ signatures.
2. Compare the differences of environmental conditions and effects of N deposition according to tissue C and $\delta^{13}C$ signals.
3. Reveal the elemental and isotopic variations (C and N) during the senescence of *H. microphyllum* based on comparison between new and old tissues.

2. Materials and methods

2.1. Descriptions of study areas

This study was conducted in Guiyang urban area and Gongga Mountain (Mt. Gongga), southwestern China (Fig. 1). The city of Guiyang has a subtropical monsoon climate with an annual

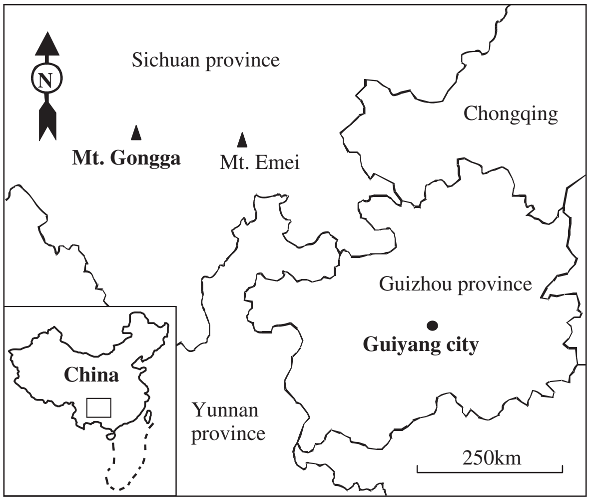

Fig. 1. Map showing the locations of Guiyang city and Gongga Mountain in southwestern China.

average temperature of 15.3 °C, annual rainfall of 1174 mm (900–1500 mm), and the relative humidity (RH) averages about 86%. Sampling sites in Guiyang were chosen around a woodland in the southeast of Guiyang downtown (26°34′N, 106°43′E), they concentrated in a small area of 0.2 km^2 with an average altitude of 990 ± 3 m, so there is no difference of environmental conditions between sampling sites, and six eligible sampling sites were chosen within the studying area.

Sampling sites in the background area were located around Hailuogou glacier with an altitude of 3276 ± 16 m (29°33′N, 101°58′E), the east slope of Mt. Gongga (the peak of 7556 m). The glacier is the only existing low-altitude glacier in present world, and it represents one of the atmospheric background monitoring locations in China. The climate type is mountainous cold temperature zone with annual average temperature of 4.29 °C, annual precipitation of 1980 mm and annual average RH of 90%. Considering the uniform species and atmospheric condition, only seven sampling sites were found with *H. microphyllum* layers in the background area.

2.2. Sample collection and treatment

Sampling was carried out in August 2005 at Guiyang and in July 2007 at Mt. Gongga, respectively. Samples were collected from natural moss layers (5–6 cm thickness at Guiyang and 8–10 cm at Mt. Gongga), they were weft-building without weeds and other plants mixed. Eligible sampling sites should be in open fields without influences of tree canopy or overhanging vegetation, and samples must be above ground level to avoid surface water splashes, and sites possibly disturbed by domestic animals or pets were also given up.

H. microphyllum was chosen based on its higher presence, which has made this study possible. Besides, characterized with regular pinnate branches, quicker growth rates and higher pollution endurance, this species has been applied it for evaluating heavy metals pollution and 'moss desert' of some cities in China (e.g. An et al., 2006).

New tissues (green sections) and old tissues (yellow segments) were divided in the field, and fresh mosses were stored in cleaned plastic bags enroute to the laboratory. After identification, all samples were gently rinsed with 1.5 mol L^{-1} HCl solution, then sonicated and washed with deionized water several times to remove adsorbed pollutants on mosses thoroughly. All samples were dried (about 1 day) in a vacuum oven connected with a vacuum pump at 70 °C and re-dried after ground.

2.3. Element analysis and isotopic determination

Tissue C and N contents (%, dry weight) were determined by elemental analyzer (PE2400 II, USA) with an analytical precision of 0.1%. The stable C and N isotopic values were measured on a Finnigan MAT 252 gas isotope ratio mass spectrometer after purification with liquid N, and δ^{15}N measurement of mosses was conducted following the method of Kendall and Grim (1990). From three to five replicated measurements per sample were carried out, and values are presented as the average of these measurements. IAEA-C_3 ($\delta^{13}C = -24.97‰$, cellulose) was used as a standard for δ^{13}C and the analytical precision ($n = 5$) was $\pm 0.1‰$. Analysis of potassium nitrate standard (MOR2386-01, 1.92‰) provided by Shoko Co., Ltd., Tokyo, Japan, gave a mean (±S.D.) $\delta^{15}N_{air}$ value of $1.9 \pm 0.2‰$ ($n = 5$).

The natural abundance of ^{13}C and ^{15}N were calculated as δ^{13}C and δ^{15}N values in per mil (‰):

$$\delta^{13}\mathrm{C}(‰ \text{ vs V-PDB}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}} - 1}\right) \times 1000,$$

$$\delta^{15}\mathrm{N}(‰ \text{ vs at-air}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}} - 1}\right) \times 1000,$$

where R is the ratio of mass 45/mass 44(carbon) or mass 29/mass 28 (nitrogen). All experimental analyses were performed in the State Key Laboratory of Environmental Geochemistry, Chinese Academy of Sciences.

2.4. Statistical analysis

Statistical analysis was conducted by using SPSS 11.5, graphs were created with SigmaPlot2000 software (both SPSS Science, Chicago, USA). A multiple comparison test (Tukey HSD, LSD) was used to determine significant differences between mean values, and correlations were analyzed by one-way analysis of variance (ANOVA).

3. Results

3.1. Carbon and nitrogen concentrations

Average C concentration in new and old tissues of mosses at Guiyang are 41.6±0.6% and 39.8±1.4%, respectively, which is higher than values (37.8±2.5% and 31.7±5.2%) at Mt. Gongga ($P<0.05$) (Fig. 2a), and average N concentrations in new and old tissues at Guiyang (2.3±0.1% and 1.9±0.1%, respectively) are also significantly higher than those (1.6±1.2% and 1.4±1.1%) at Mt. Gongga ($P<0.05$) (Fig. 2b). However, there is no significant difference between N of new tissue and old tissue for mosses at Mt. Gongga, except for that, C and N concentrations of new tissues are higher than those of old tissues for both areas ($P<0.05$) (Fig. 2).

It is strongly correlated between tissue N and tissue C for mosses in Guiyang area ($y = 5.2333x + 29.863$, $R^2 = 0.8143$), but it does exist for mosses at Mt. Gongga ($y = -0.1476x + 34.946$, $R^2 = 0.0001$). Besides, for both areas, there are good correlations between the elemental concentrations (C and N) of new tissues and old tissues (Fig. 3).

3.2. Carbon and nitrogen isotopic signatures

Average δ^{13}C in new and old tissues of urban mosses were −30.2±1.1‰ and −30.1±0.9‰, respectively, which is more negative than values (−26.5±1.3‰ and −26.5±1.5‰) at Mt. Gongga ($P<0.05$) (Fig. 4a), and average δ^{15}N values in new and old tissues at Guiyang (−6.5±1.1‰ and −6.8±1.5‰, respectively) are also significantly lower than those (−1.3±1.8‰ and −1.0±2.2‰) at Mt. Gongga ($P<0.05$) (Fig. 4b).

However, unlike C and N concentrations, there are no significant differences between mean isotopic values of new tissues and old tissues ($P<0.05$) (Fig. 4). But good correlations are observed between the isotopic signatures (δ^{13}C and δ^{15}N) of new tissues and old tissues in both studying areas (Fig. 5).

4. Discussions

4.1. Mosses N and regional atmospheric N deposition

As higher mosses N concentrations indicate higher atmospheric N inputs, tissue N of mosses could be applied for describing the spatial variation of regional N deposition and assessing its level (e.g. Pitcairn et al., 1995; Skinner et al., 2006). However, due to the lack of site-based N deposition data, only a few studies have established the quantitative relations between N concentration in natural growing mosses (y) and the corresponding atmospheric N deposition (x). We integrated these data as a pattern ($y = 0.052x + 0.7305$) for calculating N

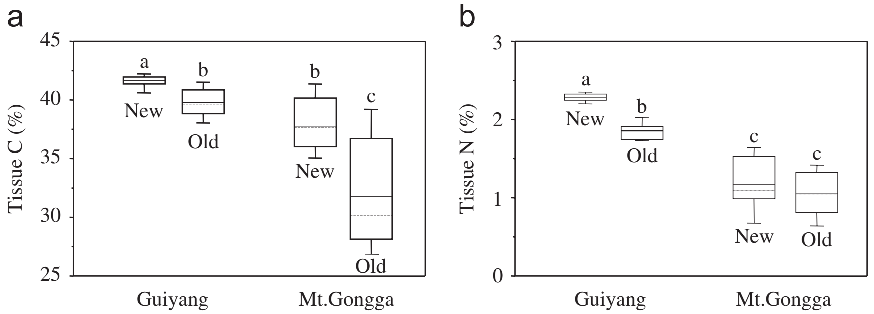

Fig. 2. Carbon (a) and nitrogen (b) concentrations in new and old tissues of *H. microphyllum* at Guiyang and Mt. Gongga. The boundary of the box indicates the 25th and 75th percentile, the solid and dash lines within the box mark the mean and the median, respectively, values with the same letter are not significantly different at level of $P<0.05$ and error bars are expressed as 1 S.E.M.

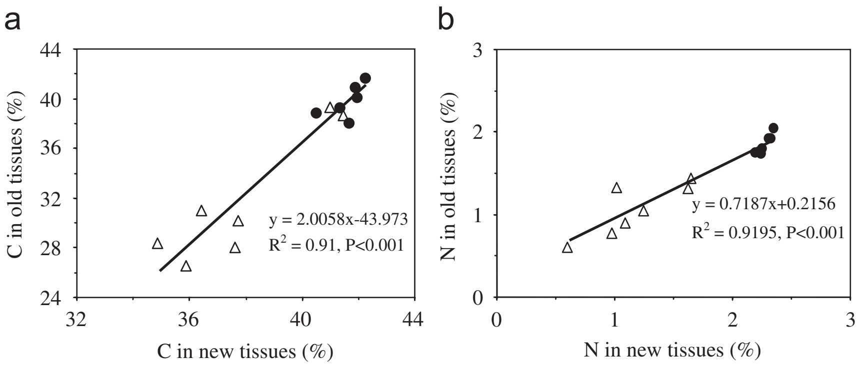

Fig. 3. Correlation of carbon concentrations (a) and nitrogen concentrations (b) between new tissues and old tissues of mosses at Guiyang (●) and Mt. Gongga (△).

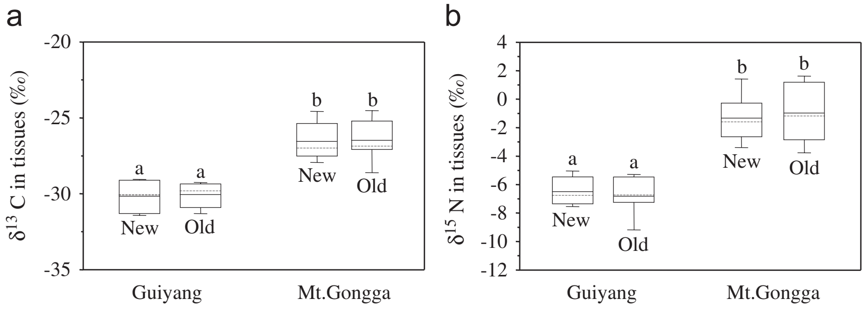

Fig. 4. δ^{13}C values (a) and δ^{15}N values (b) in new and old tissues of *H. microphyllum* at Guiyang and Mt. Gongga. The boundary of the box indicates the 25th and 75th percentile, the solid and dash lines within the box mark the mean and the median, respectively, values with the same letter are not significantly different at level of $P<0.05$ and error bars are expressed as 1 S.E.M.

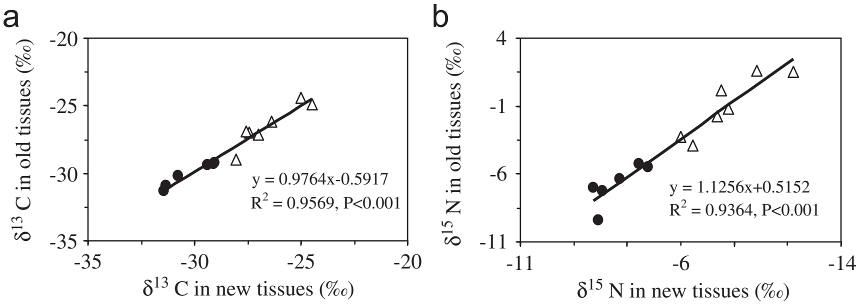

Fig. 5. Correlation of δ^{13}C signatures (a) and δ^{15}N signatures (b) between new tissues and old tissues of mosses at Guiyang (●) and Mt. Gongga (△).

deposition in this study (Fig. 6). According to the tissue N concentrations, we found that atmospheric N deposition could be quantified by N in new tissues more exactly. The level of N deposition in the studying area of Guiyang is about 30.18 kg N ha^{-1} yr^{-1}, which approximates to the mean value (31 kg N ha^{-1} yr^{-1}, Xiao et al., unpublished data) calculated according to NH$_x$ and NO$_x$ in dry and wet deposition.

Mt. Gongga has been taken as a station of atmosphere background observation in southwest China since May 2004, but there is still no report of atmospheric N deposition. According to moss N biomonitoring, the level of atmospheric N deposition is about 8.46 kg N ha^{-1} yr^{-1}, which is similar to the background value of atmospheric N inputs (8 kg N ha^{-1} yr^{-1}) at Whim Moss, a semi-natural ecosystem in the Scottish borders (Leith et al., 2004;

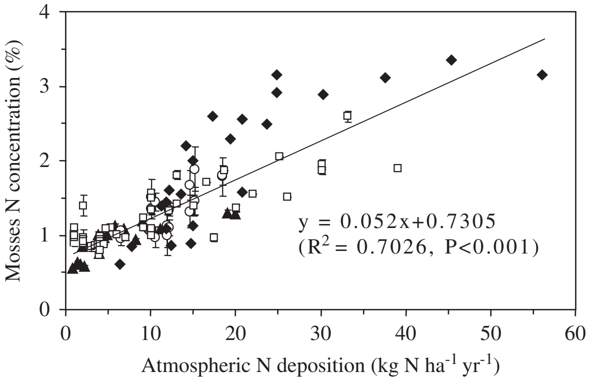

Fig. 6. Quantitative relation between atmospheric N deposition and mosses N concentration integrated from different studies: (▲) Bragazza et al (2005); (○) Solga et al (2005); (◆) Pitcairn et al (1995, 2002); (□) Pitairn et al (2001).

Skinner et al., 2006), suggesting little anthropogenic N addition in Mt. Gongga area. Therefore, the level of N deposition (8.46 kg N ha^{-1} yr^{-1}) obtained in this study could be an important supplement or a reference for the atmosphere monitoring in background area of southwest China.

4.2. Nitrogen supply and moss carbon fixation

There is huge interest in better understanding the link between N nutrition and C gain in plants (Merritt, 2003). In this study, higher tissue C for urban mosses indicated that they have higher C fixation ability than mosses in unpolluted area (Fig. 2a). For urban mosses, higher atmospheric N supply may have enhanced the CO_2 absorption (photosynthesis), which can be explained by the correlation between tissue N and C concentration in both new and old tissues ($y = 5.2333x + 29.863$, $R^2 = 0.8143$). This mechanism lies to that C fixation and N requirement are strongly correlated in plants, because N is needed to produce chlorophyll and Rubisco, and construct proteins and nucleic acid during photosynthetic process. Koranda et al. (2007) showed that N addition will cause a significant increase in amino acid in mosses, enhanced C pool turnover and significant growth reduction. Therefore, the growth of mosses at Guiyang seems not influenced by urban pollution, instead higher atmospheric N supply has an effect to promote tissue C fixation.

However, there was a lack of correlation between tissue C and N for mosses at Mt. Gongga. The main reason is that the effect of N supply on moss growth can occur only under higher atmospheric N inputs, which will disappear at locations with low atmospheric N deposition (Aerts et al., 1992; Kooijman and Kanne, 1993). Thus, good correlation between tissue N and tissue C can only observed for urban mosses. Besides, the other important reason for relatively lower mosses C at Mt. Gongga was possibly the long-term restrain of low temperature (4.29 °C) on CO_2 incorporation.

4.3. Variations of C and N between new and old tissues

As is shown in Fig. 2, tissue C decreased in the stage of senescence for mosses at both urban and clean areas, but N decrease only occurred for urban mosses. There are two main reasons for these differences. Firstly, protein (mainly Rubisco), RNA/DNA and free N will decrease resulting from the decline of photosynthesis and metabolism in the final stage of life activities (Aerts, 1996). Earlier study by Pakarinen and Vitt (1974) also reported that greater amounts of soluble proteins and carbohydrates are associated with higher metabolic activities in new-growing tissues.

Secondly, nutrient translocation from older senescent tissues to metabolically active ones is a key mechanism for reducing nutrient loss in plants (Aerts, 1996). For mosses, although they do not have vascular conducting structure, they do not lack the ability to transport substances within tissues, i.e. translocation (Aldous, 2002). Many moss species can transport substances through their leptoids (phloem-like cells) and hydroids (xylem-like cells) (e.g. *Polytrichum*, Reinhart and Thomas, 1981), or through capillary action (e.g. *Racomitrium lanuginosum*, Jónsdóttir et al., 1995), plasmodesmata (Wells and Brown, 1996). Thus, we think the translocation should be partly responsible for the C and N decrease in old tissues of *H. microphyllum*, because C and N in senescent tissues would partly transport to supply new growing apical tissues after physiological malfunction (e.g. ebb of photosynthesis and metabolism) (Skre et al., 1983). As can be seen in Fig. 3, C or N concentrations between new and old tissues are strongly correlated in mosses at both areas, showing the evidence of stable physiological variation of C and N in the same species. Similar consequence also has been found in other species. Rydin and Clymo (1989) demonstrated that *Sphagnum* is able to move both phosphorus and carbon upward through 7 cm of stem length.

Gerdol (1990) found that nitrogen, phosphorus and potassium in *Sphagnum* moved from ageing tissues to the growing capitulum. And Eckstein and Karlsson (1999) observed N recycling from older segments to current year's growth in *Hylocomium splendens* and *Polytrichum commune*, and older tissues turned brown or dark during this N reallocation.

However, it seems that physiological variations or N translocation in mosses at Mt. Gongga was not enough to change the N concentrations between new and old tissues, because no significant N decrease was observed for mosses at Mt. Gongga (Fig. 2b). Most possibly, this was attributed to the lower tissue N connected with lower atmospheric N deposition at Mt. Gongga. For example, Aldous (2002) demonstrated the similar mechanism that translocation of N in *Sphagnum* in a relatively clean site (11–32%) was much lower than that in an N-polluted site (64–83%).

4.4. Isotopic variations between new and old tissues

As mentioned above, mosses $\delta^{13}C$ and $\delta^{15}N$ has been identified as reliable tools to indicate changing environment and N sources, but isotopic deviation or fractionation between new and old tissues may confuse our explanation of sources and lead to wrong indication.

In this study, no significant difference of isotopic values ($\delta^{13}C$ and $\delta^{15}N$) was observed between new tissues and old tissues of *H. microphyllum* (Fig. 4), but strong isotopic correlations between new and old tissues were observed at both urban and unpolluted area (Fig. 5). Firstly, this demonstrated that the regional environment and atmospheric N sources did not substantially change during recent years, at least within the lifecycle of mosses. Secondly, it is indicated that the intra-plant isotopic signals were not significantly changed by physiological senescence and translocation in *H. microphyllum*, thus the whole moss tissues (new and old) could be applied in isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) for bioindication purposes.

In contrast, different $\delta^{13}C$ or $\delta^{15}N$ variations have been observed between different aging tissues in tracheophytes resulting from internal translocation and redistribution (e.g. Yoneyama et al., 1997; Schmidt and Gleixner, 1998). An isotopic fractionation theory has been proposed for $\delta^{15}N$ by Robinson et al. (1998) despite being restricted to NO_3^- grown plants, and Evans (2001) also comprehensively reviewed the physiological mechanisms influencing N isotope fractionation in plants. For bryophytes, we suggest more works on $\delta^{13}C$ and $\delta^{15}N$ variations between tissues of other species should be conducted.

4.5. Implications of mosses $\delta^{13}C$ signals

$\delta^{13}C$ values of *H. microphyllum* (−31.4‰ to −29.0‰ at Guiyang and −29.0‰ to −24.4‰ at Mt. Gongga) were within the $\delta^{13}C$ range of C_3 plants (i.e. −20‰ to −35‰). As the differences of photosynthetic and genetic effects on mosses isotopes could be eliminated in this study, mosses $\delta^{13}C$ was mainly regulated by their local environmental conditions, especially by atmospheric parameters.

More negative $\delta^{13}C$ of urban mosses (Fig. 4a) was mainly influenced by intensive anthropogenic CO_2 injections in city area, because they were isotopically more ^{13}C-depleted CO_2 sources than natural atmospheric CO_2 (−7‰) and caused relatively higher CO_2 concentration (CO_2 partial pressure) in city atmosphere. Widory and Javoy (2003) reported in Paris that the carbon isotope characterization of CO_2 emitted by the different urban sources varied widely from −40.5‰ to −24.6‰ (depending on different combustion processes), even the average $\delta^{13}C$ of human respiration CO_2 was very negative (−24.5 ± 0.5‰). Secondly, in agreement with vascular species, the fractionation against the heavy stable isotope of carbon ($\delta^{13}C$) by mosses is also dependent on atmospheric CO_2 concentration, the higher the CO_2 concentration, the lower the $\delta^{13}C$ of plants foliage (Fletcher et al., 2006). Although there was no report of atmospheric CO_2 concentration in Guiyang city, but value at Mt. Gongga (about 375 μmol mol^{-1} in 2005–2006, Li et al., 2005) was slightly higher than the global background value in 2000 (368 μmol mol^{-1}), which was significantly lower than that in city area (e.g. 422–441 μmol mol^{-1} for Beijing in winter from 1993 to 2000, Wang et al., 2003). Based on the above two mechanisms, more negative $\delta^{13}C$ signals of urban mosses than those at background sites showed the fact that moss (*H. microphyllum*) $\delta^{13}C$ could respond to atmospheric CO_2 pollutant from city anthropogenic activities.

Except for little influence from anthropogenic CO_2 source, higher mosses $\delta^{13}C$ at Mt. Gongga (Fig. 4a) are also related to the conditions of higher altitude (3276 m a.s.l.) and lower annual temperature (4.29 °C). The primary cause of mosses $\delta^{13}C$

increase with altitude is thought to be decreased atmospheric CO_2 partial pressure (Ménot and Burns, 2001). Secondly, it is known that the $\delta^{13}C$ values of plants may get less negative with decreasing temperature (Fletcher et al., 2006), for example, Ménot and Burns (2001) found the change of $\delta^{13}C$ varies from −0.2 to −0.4‰ $°C^{-1}$ in peat-forming *Sphagnum*, and recent study by Skrzypek et al. (2007) showed that a 1 °C increase in air temperature results in a −1.6‰ (*Sphagnum*) and a −1.5‰ (*Polytrichum*) decrease in $\delta^{13}C$. Therefore, differences of environmental conditions (e.g. altitude and temperature) could be indicated by mosses $\delta^{13}C$ signals.

4.6. Implications of mosses $\delta^{15}N$ signatures

Mosses $\delta^{15}N$ signatures varied from −9.4‰ to −5.0‰ at Guiyang and from −3.9‰ to +1.8‰ at Mt. Gongga, respectively, indicating there are distinctly different atmospheric N sources for each studying area.

More negative $\delta^{15}N$ of mosses at Guiyang (Fig. 4b) was more close to $\delta^{15}NH_4^+$ (−12.2±6.7‰) in rainwater sampled at the same site, rather than $\delta^{15}NO_3^-$ (+2.0±4.4‰) (Xiao and Liu, 2002), indicating that mosses were mainly influenced by NH_x–N in atmospheric N deposition. According to $\delta^{15}N$ inventories of atmospheric NH_3, the sources of atmospheric NH_x at Guiyang were mainly released from extensive city excretory wastes ($\delta^{15}NH_3 =$ −15.2‰ to −8.9‰) and sewage ($\delta^{15}NH_3 = -15$‰ to −4‰) (Freyer, 1978; Heaton, 1986). The average atmospheric NH_3 concentration determined in vicinity of a waste water drainage around our studying area in 2003 was 7.6 $\mu g\,m^{-3}$ (7–8 $\mu g\,m^{-3}$, Xiao et al., unpublished data), which was close to the critical load of atmospheric NH_3 for natural ecosystem (8 $\mu g\,m^{-3}$) (Pitcairn et al., 2001). However, the annual average atmospheric NO_2 concentration was only 20 $\mu g\,m^{-3}$ in 2005 at Guiyang (Guiyang Environmental Protection Bureau, 2006), slightly higher than that of urban background in London (mean = 17.3 $\mu g\,m^{-3}$) (Carslaw and Carslaw, 2007). Therefore, more negative $\delta^{15}N$ of urban mosses in this study suggested that NH_x–N was the prominent N form in local N deposition, which was in agreement with higher reduced form N (NH_x) than oxidized N (NO_x) in atmospheric deposition at the sampling site (Table 1).

Similarly, previous studies also found that mosses would express negative $\delta^{15}N$ signals at sites where NH_x–N was higher in N deposition, the higher NH_x–N/NO_x–N ratio is, the more negative mosses $\delta^{15}N$ will be (Solga et al., 2005; Bragazza et al., 2005; Kosior et al., 2008). Besides, source-transect studies by Harrison et al. (1999) and Skinner et al. (2006) at Whim Moss have found that the amount of dry NH_3 deposition had greater influences on mosses $\delta^{15}N$ values, thus the diffusion of NH_3 with distance and transport under wind could be indicated by mosses $\delta^{15}N$ signals.

Differently, study by Pearson et al. (2000) in London showed distinctly positive moss $\delta^{15}N$ (+3.66‰, +2.07‰ to +7.30‰), which was supported by that local atmospheric N species was dominated by oxidized N (NO_x–N) from industrial and traffic emission, and Gerdol et al. (2002) also observed similar evidence at Ferrara, northern Italy. Thus, it was concluded that atmospheric N deposition in Guiyang city was dominated by NH_x–N, not NO_x–N.

However, at Mt. Gongga, the average $\delta^{15}N$ of mosses was −1.3‰ and −1.0‰ for new and old tissues, respectively (Fig. 4b), these less negative values (around 0‰) mainly indicated little influences from anthropogenic N pollutants at background sites. As there was much less data on N deposition at background sites in SW China, we could only find the atmospheric NH_3 concentration at Emei Mountain (Fig. 1) reported by Sun and Wang (1997), they found the atmospheric NH_3

Table 1
Average concentrations of NH_x–N and NO_x–N in atmospheric N deposition in the studying area of Guiyang

Deposition	Sampling time	NH_x–N	NO_x–N	References
Wet ($\mu eq\,L^{-1}$)	1984	60.56	10.00	Galloway et al. (1987)
Wet ($\mu eq\,L^{-1}$)	July 2001	69.44	13.87	Xiao and Liu (2002)
Wet ($\mu eq\,L^{-1}$)	October 2006	126.11	26.77	Liu Xueyan (unpublished data)
Aerosol ($\mu eq\,m^{-3}$)	2003 (annual)	0.21	0.05	Xiao and Liu (2004)
Gaseous ($\mu g\,m^{-3}$)	2005 (annual)	0.42 (NH_3)	0.32 (NO_2)	Xiao Huayun (unpublished data); Guiyang Environmental Protection Bureau (2006)

concentration varied from 2.3 to 3.8 $\mu g\,m^{-3}$ at sites <1300 m, but it was undetectable at the altitude of 3000 m (our sampling sites was at 3276 ± 16 m). Obviously, evidences from mosses N and $\delta^{15}N$ showed the atmospheric components were not disturbed by regional anthropogenic pollution. For these sites, annually continuous biomonitoring is necessary to detect the variation of atmospheric N sources and predict regional environment change.

5. Conclusions

The approach of mosses isotope was promising in terms of regional environment and atmosphere research, and open large perspectives in the field of biomonitoring. The present study mainly showed a simple way to indicate anthropogenic CO_2 and identify atmospheric N source. Some principal conclusions could be drawn as below:

(1) Nitrogen in new tissues of *H. microphyllum* could quantify the level of atmospheric N deposition more reliably, showing a value of about 30.18 $kg\,N\,ha^{-1}\,yr^{-1}$ at Guiyang and 8.46 $kg\,N\,ha^{-1}\,yr^{-1}$ at Mt. Gongga. More negative $\delta^{15}N$ (−9.4‰ to −5.0‰) of mosses at Guiyang mainly indicated that atmospheric NH_3 released from excretory wastes and sewage disposal should be stressed in modern cites with higher discharge but lower treatment. While mosses $\delta^{15}N$ at Mt. Gongga (mean = −1.3‰ to −1.0‰) mainly indicated little disturbance of anthropogenic N pollution in background area.

(2) Due to the physiological malfunction and nutrients translocation, old tissues presented lower C and N concentrations than new tissues, but there was no significant difference between N of new and old tissues at background area with lower N deposition. Besides, correlation between C and N was observed only for urban mosses, showing that higher atmospheric N supply would enhance moss C fixation, which might disappear under lower N deposition.

(3) More negative $\delta^{13}C$ of urban mosses (−30.2‰) indicated the influence of anthropogenic CO_2 sources in the city, which was isotopically more ^{13}C-depleted and caused relatively higher CO_2 partial pressure in city atmosphere. Besides, higher $\delta^{13}C$ for mosses at Mt. Gongga (−26.5‰) was related to higher altitude and lower annual temperature.

(4) There was no significant isotopic difference between new tissues and old tissues in both urban and background area, suggesting that no substantial isotopic fractionation occurred during the senescence of *H. microphyllum*. Compared with tracheophytes species, it most possibly was related to the simple and nonvascular structure of mosses physiologically.

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