

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of moss *Haplocladium microphyllum* (Hedw.) Broth. for indicating growing environment variation and canopy retention on atmospheric nitrogen deposition

Xue-Yan Liu^{a,b}, Hua-Yun Xiao^{a,*}, Cong-Qiang Liu^a, You-Yi Li^{a,b}

^aState Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, CAS, Guiyang 550002, China

^bGraduate University of the Chinese Academy of Sciences, Beijing 100039, China

Received 10 October 2006; received in revised form 10 January 2007; accepted 5 February 2007

Abstract

Mosses have been recognized as a useful tool for biomonitoring atmospheric deposition and assessing regional environment. This study was carried on whether the same moss growing in areas with identical regional atmospheric deposition while under different growing environments would have the same indicating signals.

Similar variations in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were found between mosses collected from five habitats, with an increasing sequence from mosses under canopies to epilithic mosses, indicating that habitats were potentially regulating $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mosses. Drier habitats (lower water availability) and input of more aerosol N were the main reasons for higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mosses at open sites (especially for epilithic species), while more negative values of mosses under canopies were attributed to their wetter habitats and less uptake of aerosol N. Additionally, $\delta^{15}\text{N}$ values not $\delta^{13}\text{C}$ varied linearly with canopy thickness from -7.84‰ (1 m) to $-4.71 \pm 0.7\text{‰}$ (4 m), suggesting $\delta^{15}\text{N}$ was more sensitive for indicating canopy retention.

Consequently, isotopic data of mosses under different environments could not be compared for atmospheric deposition research with each other even collected at the same site. Moss $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were affected not only by regional atmospheric N sources but also by their growing environments. $\delta^{15}\text{N}$ of epilithic *Haplocladium microphyllum* at open sites can be taken as confident bio-indicator of atmospheric N deposition, which would deepen the application of stable nitrogen isotope of bryophytes in atmosphere–plant system study.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Atmospheric nitrogen deposition; Indicating; Canopy; Moss; Retention

1. Introduction

Isotopic data for atmospherically derived nitrogen are increasingly used in assessing the sources of inputs to a wide variety of soil and plant environ-

ments (Evans and Ehleringer, 1993; Durka et al., 1994), and much progress has been made in understanding the mechanisms that control terrestrial plant and foliar $\delta^{15}\text{N}$ when acquiring those atmospheric/allochthonous nitrogen sources (Stewart et al., 1995; Hietz et al., 1999; Evans, 2001), because some of them causing ^{15}N -depletion or ^{15}N -enrichment of nitrogen pools, and other leaving

*Corresponding author. Fax: +86 851 5891609.

E-mail address: xiaohuayun@vip.skleg.cn (H.-Y. Xiao).

no isotopic legacy (Handley et al., 1999). Therefore, $\delta^{15}\text{N}$ values of most natural samples (e.g. canopies, epiphytes, etc.) are not simply tracers to reflect utilization of isotopically different nitrogen sources, but the integrating result of many biogeochemical processes.

Canopies represent a distinct and natural barrier of light and atmospheric deposition to under-growing species. Earlier study has reported that the canopy retained 50–70% of the deposited free acidity and nitrogen, and dry deposition was believed as an important atmospheric source to canopies (Lindberg et al., 1986). Later more and more studies proved that canopy retention resulted in the chemical composition of atmospheric nitrogen deposition that are markedly different from those on the floor (Hanson and Garten, 1992; Lumme, 1994), and foliar absorption do influence the $\delta^{15}\text{N}$ of bulk precipitation (Stewart et al., 1995; Wilson and Tiley, 1998; Hietz et al., 2002). These studies mainly focused on the atmospheric-derived samples such as rain, throughfall or stemflow, but $\delta^{15}\text{N}$ of gaseous compounds and aerosol were rather limited because of sampling difficulties.

Without rooting system to acquire nutrients from substratum and lack of a cuticular barrier to atmospheric inputs, naturally growing mosses have entered the vision of bio-monitoring and have been extensively employed for surveying atmospheric deposition and assessing its environmental effects in the past few decades (Press et al., 1986; Gerdol et al., 2002). However, these related studies mainly focused on investigation of heavy metals deposition, e.g. Berg et al. (1995), Berg and Steinnes (1997) have brought moss biomonitoring from qualitative to quantitative tool for estimation of atmospheric deposition. In fact, mosses are also very sensitive to direct atmospheric nitrogen deposition, because most of the mosses (e.g. pinnate branched and weft-building species) rely on direct uptake of the atmospherically derived nitrogen for growing, and received nitrogen can be efficiently retained and assimilated over the whole shoot and leaf surface (Soares and Pearson, 1997). Some species have been estimated as excellent biomonitors of national and regional atmospheric nitrogen deposition, such as *Pleurozium schreberi* (Brid.) Mitt. and *Scleropodium purum* (Hedw.) Limpr. (Solga et al., 2005), *Sphagnum* from ombrotrophic mires (Press et al., 1986; Pitcairn et al., 1995) and also mixtures species (Pearson et al., 2000). The ^{15}N method has been

recently adopted in some of these studies (e.g. Pearson et al., 2000; Solga et al., 2005).

Although suitability of under-growing mosses for indicating canopy retention of heavy metals deposition was determined by Čeburnis and Steinnes (2000), yet our understanding of whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of underlying mosses can respond to different habitats and reflect the canopy retention remains limited. This lack of habitats understanding will trouble for our sampling selection and mislead our interpretation of atmospheric nitrogen deposition. In this study, we focused on biologically important stable isotope pairs ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of mosses (*Haplocladium microphyllum*) under five habitats (epilithic species on rocky surfaces, terricolous species under three canopies and in open fields). It was designed to investigate whether and how isotopic signatures of underlying mosses respond to their habitats and canopy retention, and secondly to evaluate $\delta^{15}\text{N}$ of *H. microphyllum* for further biomonitoring atmospheric nitrogen deposition.

2. Materials and methods

2.1. Field sites

In August 2005, the study was carried out in a small woodland with an area of about 0.25 km² in the southeast of Guiyang downtown (26°34'N, 106°43'E), Southwestern China (Fig. 1). Elevation within sampling area is 990 ± 3 m and there is no high building or covering around. The dominant higher plants were *Cryptomeria japonica*, *Osmanthus Fragrans*, and *Platanus acerifolia* within the woodland.

Guiyang has a subtropical monsoon climate with an annual average temperature of 15.3 °C. Annual rainfall averages 1197 mm (900–1500 mm yr⁻¹) and concentrated in summer (Xiao and Liu, 2004). The major anthropogenic nitrogen sources of atmospheric deposition are coal combustion and vehicle exhausts, which accounts for over 90% of energy consumption of the whole city. Average NH_4^+ and NO_3^- in precipitation were 1.25 and 0.86 mg l⁻¹ in 2001 (Xiao and Liu, 2002), and water-soluble inorganic N in TSP was high as 3.81 ± 1.64 μg m⁻³ for NH_4^+ and 3.03 ± 2.48 μg m⁻³ for NO_3^- (Xiao and Liu, 2004). Besides, gaseous NH_3 concentration was 7–8 μg m⁻³, averaging 7.6 μg m⁻³ in 2003 (Xiao and Liu, unpublished data). All these samples were collected at this woodland and it was estimated that

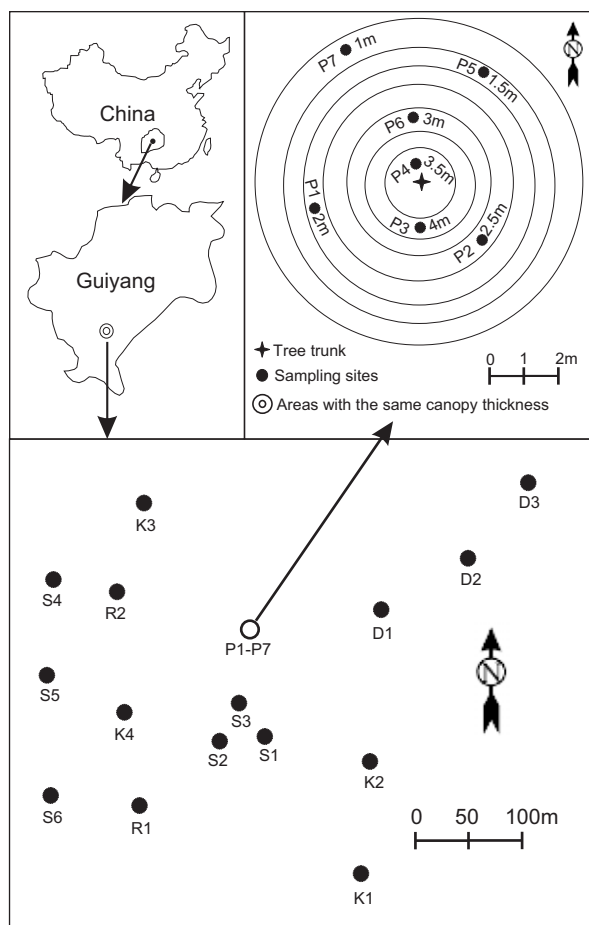


Fig. 1. Sampling sites within the study area at Guiyang. S represents mosses under *C. japonica*, P under *Osmanthus fragrans* Lour, D under *P. acerifolia*, K and R for terricolous and epilithic mosses in open fields, respectively (P1–P7 was under an *O. fragrans* Lour tree).

the critical loads for N deposition are still high in this city (Xiao and Liu, 2004).

2.2. Species collection and tissue treatment

H. microphyllum was chosen based on its larger presence under different growing conditions in the studying area, which has made our study possible. Besides, characterized by pinnate branches, quick growth rates, high pollution endurance and wide distribution, *H. microphyllum* has been considered as an efficient accumulator of air pollutants (An et al., 2006).

The amount of required mosses (*H. microphyllum*) was limited within such a small area, so the number of samples widely varied especially under

some smaller canopies. Firstly, mosses of the same species growing at different conditions was needed, and mosses in open fields (R1–R2; K1–K4) must not be under any canopy or overhanging vegetation. Secondly, the collection of mosses was restricted at a slightly higher place (10 cm) above around ground level to avoid the influence of surface water splashing, and samples near obvious bird roosts and possibly influenced by human disturbance were also excluded. Canopy thickness was directly measured by pole or stick, the thickest canopy of *C. japonica*, *O. fragrans* and *P. acerifolia* were 10, 4 and >10 m, respectively. Other description of sampled species and canopies are listed in Table 1.

Fresh mosses were stored in cleaned plastic bags before it was transported to laboratory. As we focused on the tissue N in mosses, all samples for total N and isotopic determination were gently rinsed with 1.5 mol l⁻¹ HCl solution, then sonicated and washed with deionized water (Milli-Q) for several times until no N was detected in the washed water, the main purpose of this washing procedure was to remove adsorbed pollutants on leaves thoroughly. Samples were dried in a vacuum oven at 70 °C and re-dried after ground.

2.3. Element analysis and isotopic determination

Total N and C/N ratio were determined by elemental analyzer (PE2400II, USA) with an analytical precision of 0.1%. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured on a Finnigan MAT 252 gas isotope ratio mass spectrometer after purification with liquid nitrogen. Nitrogen isotope measurement of mosses was conducted following the method of Kendall and Grim (1990). From three to five replicated measurements per sample (about 20–30 mg, DW) were carried out, and values are presented as the average of these measurements. IAEA-C₃ ($\delta^{13}\text{C} = 24.91\text{‰}$, cellulose) was used as a standard for $\delta^{13}\text{C}$ and the analytical precision ($n = 5$) was $\pm 0.1\text{‰}$. Analysis of potassium nitrate standard (MOR2386-01, 1.92‰) provided by Shoko Co. Ltd. (Tokyo, Japan), gave a mean (\pm SD) $\delta^{15}\text{N}_{\text{air}}$ value of $1.9 \pm 0.2\text{‰}$ ($n = 5$).

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

$$\delta^{13}\text{C} [\text{‰ versus } V - \text{PDB}] = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

Table 1
Nitrogen contents, carbon and nitrogen isotopic composition of mosses under different growing conditions

| Site, sample (n) | Growing condition | Canopy species | Mean $\delta^{13}\text{C}$ and range (% vs PDB) | Mean $\delta^{15}\text{N}$ and range (% vs at-air) | Mean N content and range (% DW) |
|------------------|-------------------|--|---|--|---------------------------------|
| S1–S6 (n = 6) | Terricolous | <i>Cryptomeria japonica</i> (Linn. f.) D. Don. (evergreen) | -30.82 ± 1.36 (–32.92–29.74) | -8.15 ± 0.83 (–9.12–7.21) | 2.49 ± 0.20 (2.20–2.74) |
| P1–P7 (n = 16) | Terricolous | <i>Osmanthus fragrans</i> Lour. (evergreen) | -30.13 ± 0.72 (–31.73–28.97) | -6.25 ± 1.20 (–7.95–3.78) | 2.40 ± 0.24 (1.72–2.67) |
| D1–D3 (n = 3) | Terricolous | <i>Platanus acerifolia</i> (Ait.) Willd. (deciduous) | -30.26 ± 1.06 (–31.43–29.36) | -5.39 ± 2.20 (–7.85–3.62) | 2.58 ± 0.19 (2.44–2.80) |
| K1–K4 (n = 4) | Terricolous | No canopy (open field) | -29.94 ± 0.88 (–30.94–29.17) | -4.53 ± 0.58 (–5.40–4.20) | 1.78 ± 0.44 (1.24–2.30) |
| R1–R2 (n = 2) | Epilithic | No canopy (open field) | -28.19 ± 0.36 (–28.45–27.94) | -2.79 ± 0.91 (–3.43–2.15) | 2.60 ± 0.22 (2.45–2.76) |

Values represent means \pm SD of n samples range.

$\delta^{15}\text{N}$ [‰ versus at – air]

$$= (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

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 $\delta^{13}\text{C}/\delta^{15}\text{N}$ values under different growing conditions. N content, $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ difference under different canopy thickness was analyzed by one-way analysis of variance (ANOVA).

3. Results

3.1. Nitrogen content

Tissue N of mosses measured in this study ranged between 1.24% (K1) and 2.80% (D3) (Table 1). Epilithic mosses in open fields had the highest mean value ($2.60 \pm 0.22\%$), then terricolous mosses under deciduous *P. acerifolia* ($2.58 \pm 0.19\%$), under evergreen *C. japonica* ($2.49 \pm 0.20\%$) and under *O. fragrans* (evergreen) ($2.40 \pm 0.24\%$). Terricolous mosses at open sites had the lowest mean tissue N ($1.78 \pm 0.44\%$).

For mosses under one *O. fragrans* canopy, nitrogen content varied significantly along the canopy thickness transect, but there was no linear relationship between moss N and canopy thickness (Fig. 2). The maximum value of 2.65% occurred at the edge of canopy (canopy thickness = 1 m) (Table 2).

3.2. Carbon and nitrogen isotopic natural abundance

The average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of mosses showed similar variations (Fig. 3). Generally, mosses at open sites had higher mean values than those under canopies, and the highest and lowest values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were observed for epilithic mosses and those under *C. japonica* respectively. Based on multiple comparison, mean $\delta^{13}\text{C}$ between S, P, D and K were not significantly different (Fig. 3a). For

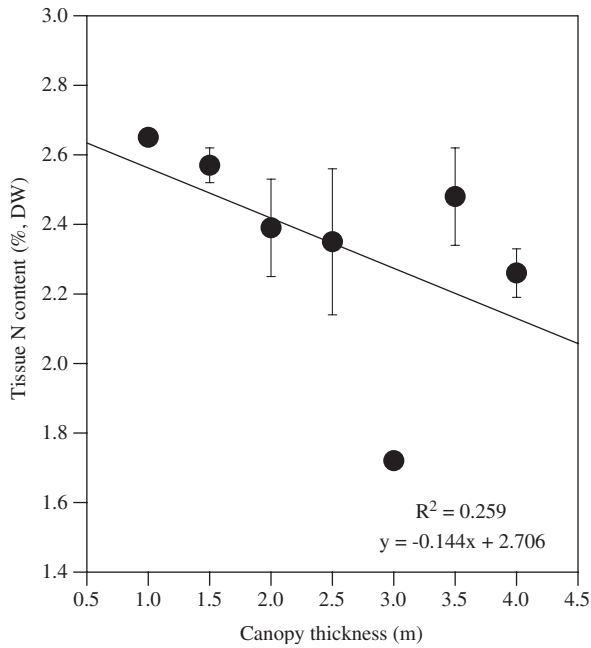


Fig. 2. Nitrogen contents plotted against canopy thickness. Data are expressed means \pm SD of n samples range ($P > 0.05$).

$\delta^{15}\text{N}$, except for those growing under *P. acerifolia* canopies (D), differences between mosses at open sites and under canopies were significant from each other (Fig. 3b). In addition, there was no linear relationship between N contents and N isotopic composition, but a significantly linear correlation was found between mosses mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Fig. 4).

There was no linear relationship between moss $\delta^{13}\text{C}$ and canopy thickness (Fig. 5a). But $\delta^{15}\text{N}$ signatures of mosses varied linearly with canopy thickness, with a magnitude from -7.84‰ at P7 (canopy thickness = 1 m) to $-4.71 \pm 0.7\text{‰}$ at P3 (canopy thickness = 4 m) (Fig. 5b).

4. Discussion

4.1. Moss N contents for indicating atmospheric N input

The mean N concentration of terricolous species was about $2.31 \pm 0.36\%$ in this study, similar to values (2.31%) found for *Pleurozium schreberi* (Brid.) Mitt. in Lower Saxony area characterized by intensive livestock farming (Mohr, 1999) and at Velmerstot in the western Germany with high bulk N deposition ($18.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$) (Solga et al., 2005). Additionally, Solga et al. (2005) estimated that the

Table 2

Nitrogen contents, carbon and nitrogen isotopic composition of mosses under an *Osmanthus fragrans* Lour tree

| Sample | P1 (n = 3) | P2 (n = 1) | P3 (n = 3) | P4 (n = 3) | P5 (n = 2) | P6 (n = 3) | P7 (n = 1) |
|--|--------------------------------------|------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|------------|
| Canopy thickness (m) | 2 | 3 | 4 | 3.5 | 1.5 | 2.5 | 1 |
| Mean N content and range (% DW) | 2.39 ± 0.14 (2.24–2.52) | 1.72 | 2.26 ± 0.07 (2.19–2.33) | 2.48 ± 0.14 (2.32–2.58) | 2.57 ± 0.05 (2.52–2.62) | 2.36 ± 0.21 (2.12–2.52) | 2.65 |
| Mean $\delta^{13}\text{C}$ and range (‰ vs PDB) | -29.81 ± 0.14 (–29.98––29.72) | –30.39 | -29.54 ± 0.51 (–29.93––28.97) | -29.70 ± 0.14 (–29.86––29.59) | -30.06 ± 0.22 (–30.21––29.90) | -31.40 ± 0.37 (–31.73––31.00) | –30.27 |
| Mean $\delta^{15}\text{N}$ and range (‰ vs at–air) | -7.29 ± 0.54 (–7.63––6.66) | –6.31 | -4.71 ± 0.82 (–5.32––3.78) | -6.00 ± 0.87 (–6.99––5.40) | -7.26 ± 0.97 (–7.95––6.57) | -5.91 ± 0.73 (–6.74––5.39) | –7.84 |

Values represent means \pm SD of n samples range.

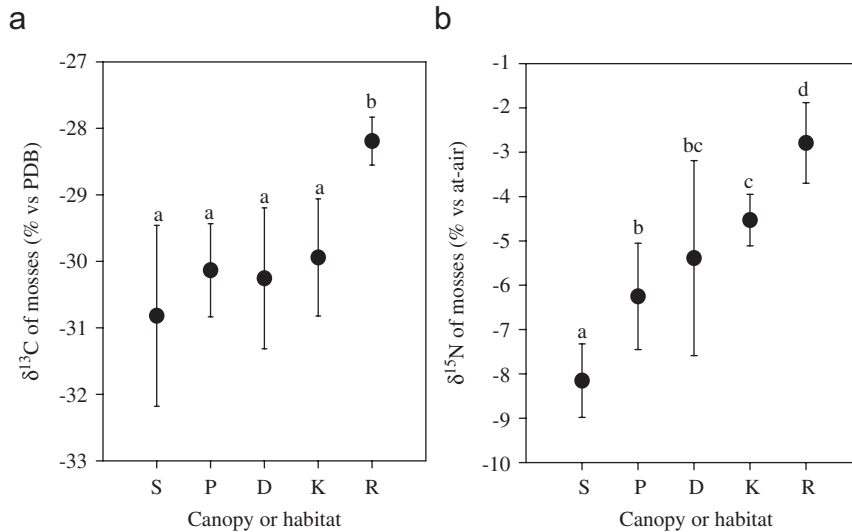


Fig. 3. Carbon (a) and nitrogen (b) isotopic signatures of mosses plotted against growing conditions. S represents mosses under *C. japonica* ($n = 6$), P under *O. fragrans* Lour ($n = 16$) and D under *P. acerifolia* ($n = 3$). K and R represent terricolous ($n = 4$) and epilithic species ($n = 2$) in open fields, respectively. Data are expressed means \pm SD of n samples range. Different letters above the bars indicate a significant statistical difference at level of $P < 0.05$.

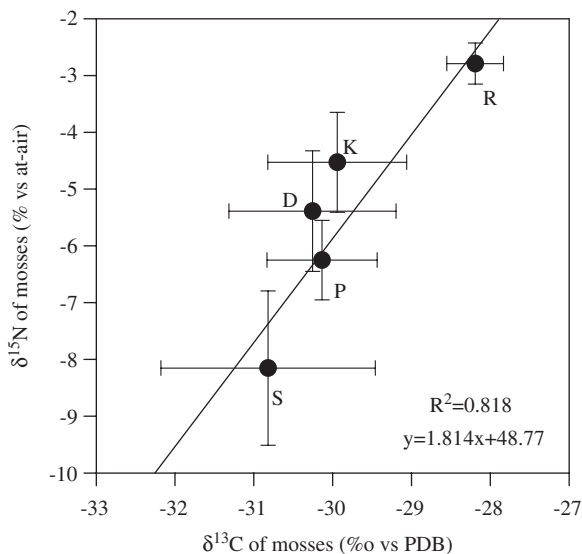


Fig. 4. Correlation between carbon and nitrogen isotopic signatures of mosses under different growing environments. Error bars are standard deviations ($P < 0.05$).

percent tissue nitrogen increased 0.066 for *P. schreberi* and 0.061 for *Scleropodium purum* (Hedw.) Limpr. for each $1 \text{ kg ha}^{-1} \text{ yr}^{-1}$ increase in bulk N deposition. Pitcairn et al. (2003) also reported that an elevated moss tissue N even reached about 3.5% in a woodland plot bordering on a poultry farm.

As a species of pinnate branched mosses, *H. microphyllum* is very sensitive to atmospheric N deposition (precipitation, dust, etc.) due to its larger surface area and higher growth rates (Wu et al., 2005). In this study, bulk N deposition was believed to be the controlling factor regulating moss N contents, with high moss N contents occurring in areas with high N deposition. General high N contents should be attributed to the high N deposition over Guiyang city (data shown in Section 2.1).

Differences in moss tissue N between sites should be more related to their growing environments (substratum and canopy influence) since atmospheric N deposition level within such a small area could be considered uniform. In this study, canopy thickness was found an important factor in regulating moss N contents. Although no linear relationship was seen in Fig. 2, it is still suggested that canopy uptake of deposited N influences the nutrient supply to underlying mosses. The higher N contents in mosses at more exposed sites should be due to the higher dry deposition N, which was lower for mosses under canopies (Table 1).

4.2. Responses of moss $\delta^{13}\text{C}$ signatures to habitats

Foliar $\delta^{13}\text{C}$ was believed to mainly reflect the extent to which primary CO_2 assimilation was limited by carboxylation and/or CO_2 diffusion in plant leaves (Robinson et al., 2000). ^{13}C abundance

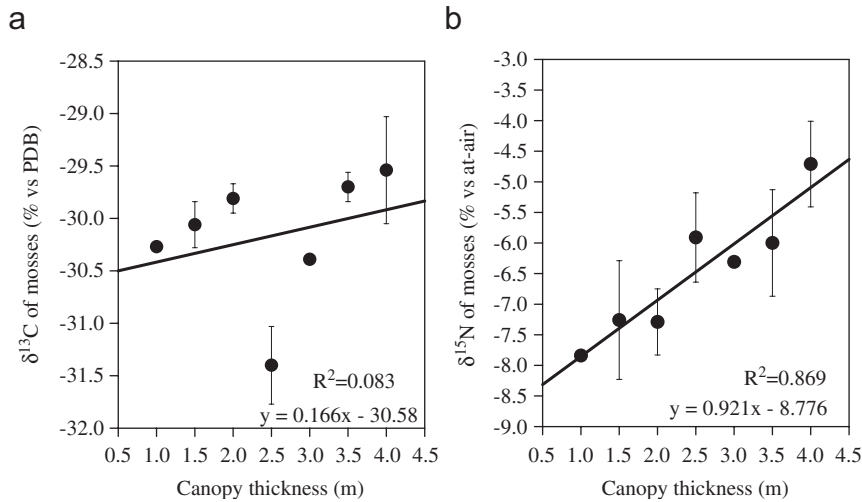


Fig. 5. Carbon (a) ($P > 0.05$) and nitrogen (b) ($P < 0.05$) isotopic signatures plotted against canopy thickness. Error bars are standard deviations.

of mosses measured in this study varied from -32.9‰ to -27.9‰ (Table 1), within the $\delta^{13}\text{C}$ range of C_3 plant (i.e. -20‰ to -35‰). C_3 photosynthetic pathways would be modified by many environmental factors, such as water (rain) availability, light intensity, etc. which through some dominant physiological processes results in explicable ^{13}C variation ultimately via effects on the balance between stomatal conductance and carboxylation (Ehleringer et al., 1993). Because only one species of mosses was investigated in this study, genetic difference and photosynthetic pathways effect could be eliminated, moss $\delta^{13}\text{C}$ could be considered mainly as an integrator of the variations of environmental factors.

From Fig. 3a, ^{13}C abundance of mosses varied significantly between growing environments. We focused on the water availability (due to canopy effect and substratum) of different growing conditions as the decisive factor. Dryer condition with lower RH for open sites and wetter habitats under canopies are considered as main reasons for mosses $\delta^{13}\text{C}$ difference. The highest $\delta^{13}\text{C}$ of epilithic mosses mainly indicated the poor water availability on rock surface where mosses inhabit. Moreover, the dry and harsh growing condition would weakened the capacity of moss photosynthesis, which further lead to higher $\delta^{13}\text{C}$ of epilithic mosses. This is in agreement with the fact that plant (*Hordeum spontaneum* C. Koch.) will express less negative $\delta^{13}\text{C}$ values under low water availability resulting from drought stress (Robinson et al., 2000). Oppositely, stomatal conductance of leaves will

largely increase in environments amenable to high RH, which will increase the CO_2 concentration of leaves and make $\delta^{13}\text{C}$ of photosynthetic products more negative (Tieszen and Boutton, 1988). Therefore, wetter substratum and abundant ground water under canopies for terricolous mosses should be responsible for their more negative $\delta^{13}\text{C}$ values.

The above discussion is actually consistent with the general theory that C_3 plants need moderate light and abundant water habitat (Handley et al., 1994), which in the case of moss was preserved substratum.

However, Israeli et al. (1996) had concluded that higher light levels would increase $^{12}\text{CO}_2$ uptake rates and decrease ^{13}C discrimination. According to this, $\delta^{13}\text{C}$ of mosses sheltered by canopies should be higher because of poor light intensity. Therefore, we must point out that substrate water under canopies played a more important role for moss photosynthetic activity than light did in this study, because only very thick and enveloping canopies could cause $\delta^{13}\text{C}$ of the same magnitude. So water availability is the potential reason for moss $\delta^{13}\text{C}$ differences in this study, and hence, moss $\delta^{13}\text{C}$ could be a sensitive tool for further indicating mosses habitats in various ecological scales.

4.3. Moss $\delta^{15}\text{N}$ for indicating canopy retention on atmospheric N deposition

Atmospheric N deposition retention on canopy broadly refers to N uptake via adsorption, absorption, assimilation, or other processes. Both simulated experiments (e.g. Lumme, 1994) and field

studies (e.g. Lovett and Lindberg, 1993; Garten et al., 1998) on N cycling indicated that almost half of the total atmospheric nitrogen deposition to some forests was 'consumed' by the forest canopies. Moreover, ^{15}N tracer studies (e.g. Garten et al., 1998) and earlier studies (e.g. Lindberg et al., 1986; Hanson and Garten, 1992) on different chemical forms of N deposition indicated that dry N deposition uptake by canopy was a major contributor to the net canopy exchange of N in both deciduous and coniferous forests.

All sampling sites in our study were concentrated within an area of about 0.25 km^2 to ensure there was no spatial variation in nitrogen deposition level. So ^{15}N enrichment extent of underlying mosses relied on the input of dry deposition N and ^{14}N incorporation capacity of upper canopies. Although there was no significant $\delta^{15}\text{N}$ difference between P and D, D and K, generally higher values at open sites than mosses under canopies were still seen, and epilithic mosses expressed the highest $\delta^{15}\text{N}$ value of $-2.79 \pm 0.91\text{‰}$ (Fig. 3b).

Dry deposition was usually the main form N deposition in many areas. Plant foliar can uptake large amount of deposited N (both dry and wet), and uptake of dry deposition N was relatively a major contributor to the net canopy exchange of N in both deciduous and coniferous forests (Lindberg et al., 1986; Garten et al., 1998). It was shown that average NH_4^+ and NO_3^- concentration in TSP of 2003 was 3.81 ± 1.64 and $3.03 \pm 2.48\text{ mg}^{-1}\text{ m}^{-3}$, respectively (Xiao and Liu, 2004), which is generally higher than the data of aerosols in England (Yeatman et al., 2001a, b), and gaseous NH_3 concentration sampled at this woodland in 2003 was high, averaging $7.6\text{ }\mu\text{g m}^{-3}$ ($7\text{--}8\text{ }\mu\text{g m}^{-3}$) (Xiao and Liu, unpublished data). More importantly, $\delta^{15}\text{N}$ in aerosols was believed to be higher than that in rainfalls. $\delta^{15}\text{N}$ signatures of rainfalls over Guiyang city were $+2.0 \pm 4.4\text{‰}$ for $\delta^{15}\text{NO}_3^-$ and $-12.2 \pm 6.7\text{‰}$ for $\delta^{15}\text{NH}_4^+$ (Xiao and Liu, 2002), less positive than $\delta^{15}\text{N}$ -aerosol in southern ($+10.0 \pm 3.0\text{‰}$) and northern ($+15.0 \pm 3.0\text{‰}$) UK (Yeatman et al., 2001a). Similar issues on higher $\delta^{15}\text{N}$ of dry N deposition were summarized in earlier researches (e.g. Heaton, 1986; Russell et al., 1998).

Thus the relatively high $\delta^{15}\text{N}$ values of mosses in open fields (R and K) could be explained by: canopies have consumed large amount of aerosols N with less negative $\delta^{15}\text{N}$ values (Yeatman et al., 2001a), mosses at open sites were exposed to

original wet and dry deposited N, whose $\delta^{15}\text{N}$ values were higher than those of throughfall or stemflow under canopies.

As the contribution of dry deposition N was decisive for mosses $\delta^{15}\text{N}$ difference in this study, the above discussion actually does not contradict the theory that foliar retention/uptake expresses different discrimination against ^{15}N (preference for ^{14}N incorporation) (Stewart et al., 1995; Handley et al., 1999; Evans, 2001), which results in more ^{15}N in throughfall and thus N sources for mosses under canopies should have become heavier.

Besides, foliar retention and assimilation are rather selectively associated with canopy types, leaf morphology and surface cutin or trichomes, which would affect foliar wettability and nitrogen residence time and further lead to different foliar absorption. In this study, significantly lower $\delta^{15}\text{N}$ for mosses under *C. japonica* (S) was found than other two canopies (Fig. 4b), suggesting different canopy would perform differently on changing $\delta^{15}\text{N}$ of atmospheric nitrogen deposited to under growing mosses. *P. acerifolia* (deciduous) had relatively shorter annual canopy time than other two evergreen species (*O. fragrans* and *C. japonica*), more exposure to dry deposition N (high $\delta^{15}\text{N}$ values) under the deciduous trees should be responsible for mosses higher $\delta^{15}\text{N}$ values. The lowest $\delta^{15}\text{N}$ ($-8.15 \pm 0.83\text{‰}$) of mosses might be attributed to the higher obstructing effect of *C. japonica* canopies, whose compact leaves would largely reduce the input of atmospheric N into the understory environment.

4.4. Correlation between moss $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures

Fig. 4 showed a good correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of mosses from the five different growing environments. As discussed above, $\delta^{13}\text{C}$ of mosses mainly reflected that different substratum, water availability, light intensity and drought or humidity, etc. Poor water availability was the driver to higher $\delta^{13}\text{C}$ of epilithic mosses, and more negative $\delta^{13}\text{C}$ values of mosses under canopies was a response to higher water availability correlated with canopy and substratum (soil). Whereas, $\delta^{15}\text{N}$ of mosses primarily indicated atmospheric deposition N input, especially for dry deposition. This was also suggested in Hietz et al. (1999) that $\delta^{15}\text{N}$ of epiphytes (including mosses) was a better indicator of atmospheric-derived N sources.

However, plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were also regulated by some concurrent factors. For example, photosynthesis was correlated with water availability, poor water availability would decrease the ability of photosynthesis (C assimilation), further result in reduction of productivity and N requirement or assimilation, in which different $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variations occurred (Yoneyama et al., 2001). Wong (1979) also concluded that nutrition supply strongly influenced foliar response to atmospheric CO_2 (photosynthesis). Therefore, the correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of mosses growing under different environments indicates that environmental factors interacted with each other, suggesting that the physiological responses of mosses to changing habitats and N inputs are correlated through C–N interactions. So $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, as biologically important stable isotope pairs, together is a useful tool to decode many biogeochemical mechanisms.

4.5. Moss δ values reflecting effect of canopy thickness

Canopy thickness was an important factor to influence underlying environments (Hietz et al., 2002). It also played a key role in changing N content (Filoso et al., 1999) and $\delta^{15}\text{N}$ of atmospheric N deposition (particularly throughfall) (Heaton et al., 1997).

Our investigation showed that $\delta^{15}\text{N}$, not $\delta^{13}\text{C}$ of mosses was linearly correlated with canopy thickness (Fig. 5), suggesting that $\delta^{15}\text{N}$ of mosses was more sensitive to the canopy thickness effect than $\delta^{13}\text{C}$ which was mainly controlled by other factors (discussed in Sections 4.2 and 4.4). For $\delta^{15}\text{N}$, thicker canopy meant larger N retention and more ^{14}N assimilation (Stewart et al., 1995; Handley et al., 1999; Evans, 2001), which would cause throughfall with less negative $\delta^{15}\text{N}$ ratio for underlying mosses. As a result, the thicker canopy was, the less negative $\delta^{15}\text{N}$ of beneath mosses became (Fig. 4b). This mechanism corresponded to the natural ^{15}N abundance variations of epiphytes within canopy and throughfall at different positions under canopy (Wania et al., 2002).

Canopy thickness was found to be an important factor regulating atmospheric N deposition in this study. In fact, this supported that the variations in plant N isotopic composition were not simply caused by utilization of isotopically different N sources, but also by different mechanisms during N

acquisition processes (Handley and Raven, 1992; Wania et al., 2002).

5. Conclusions

This study has uniquely investigated variations of isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of one species (*H. microphyllum*) under five growing environments within a niche. Some principal conclusions drawn from this study are presented as follows:

- (1) Drier habitats and direct exposure to dry deposition are mainly responsible for higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of mosses in open fields (especially for epilithic species). While for mosses under canopies, higher water availability correlated with canopy shading and wetter substratum could explain their more negative $\delta^{13}\text{C}$ values, and less aerosol N input could be the main reason for their lower $\delta^{15}\text{N}$ values.
- (2) Moss $\delta^{13}\text{C}$ mainly reflects their habitats and physiological response (e.g. water availability, photosynthesis and N demand), and $\delta^{15}\text{N}$ variations clearly indicate that canopy retention would change the input of atmospheric N sources into the under canopy environment. Their good correlation reflects that they are regulated by some correlating environmental factors.
- (3) Linear relationship between moss $\delta^{15}\text{N}$ and canopy thickness ($y = 0.921x - 8.776$) suggests that canopy thickness is an important factor regulating atmospheric N deposition and should be considered in sampling. From this study, we suggest that $\delta^{15}\text{N}$ of epilithic *H. microphyllum* could be further taken for biomonitoring atmospheric N deposition, which would enlarge our knowledge on atmospheric N input and its ecological effect.

Acknowledgements

The authors gratefully acknowledge Dr. Li lin (College of Life Sciences, Hebei Normal University) for her help with species identification. This study work was kindly financially supported by the Ministry of Science and Technology of China through Grants 2006CB403200 (C.Q. Liu) and by the National Natural Science Foundation of China through grants 40573006 and 40373039 (H.Y. Xiao).

References

- An, L., Cao, T., Yu, Y.H., 2006. Heavy metals contents in *Haplocladium* and their relationships with Shanghai city environment. Chinese Journal of Applied Ecology 17 (8), 1490–1494 (in Chinese with English abstract).
- Berg, T., Roysset, O., Steinnes, E., 1995. Moss (*Hylocomium splendens*) used as biomonitor of atmospheric trace element deposition: estimation of uptake efficiencies. Atmospheric Environment 29 (3), 353–360.
- Berg, T., Steinnes, E., 1997. Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute deposition values. Environmental pollution 98 (1), 61–71.
- Čeburnis, D., Steinnes, E., 2000. Conifer needles as biomonitors of atmospheric heavy metal deposition: comparison with mosses and precipitation, role of the canopy. Atmospheric Environment 34, 4265–4271.
- Durka, W., Schulze, E.D., Gebauer, G., Voerkelius, S., 1994. Effects of forest decline on uptake and leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements. Nature 372, 765–767.
- Ehleringer, J.R., Hall, A.E., Farquhar, G.D., 1993. Stable Isotopes and Plant Carbon–Water Relations. Academic Press, London.
- Evans, R.D., 2001. Physiological mechanisms influencing plant nitrogen isotope composition. Trends in Plant Science 6, 121–126.
- Evans, R.D., Ehleringer, J.R., 1993. A break in the nitrogen cycle in arid lands? Evidence from $\delta^{15}\text{N}$ of soils. Oecologia 94, 313–317.
- Filoso, S., Williams, M.R., Melack, J.M., 1999. Composition and deposition of throughfall in a flooded forest archipelago (Negro River, Brazil). Biogeochemistry 45, 169–195.
- Garten Jr., C.T., Schwab, A.B., Shirshac, T.L., 1998. Foliar retention of ^{15}N tracers: implications for net canopy exchange in low- and high-elevation forest ecosystems. Forest Ecology and Management 103, 211–216.
- Gerdol, R., Bragazza, L., Marchesini, R., Medici, A., Pedrini, P., Benedetti, S., Bovolenta, A., Coppi, S., 2002. Use of moss (*Tortula muralis* Hedw.) for monitoring organic and inorganic air pollution in urban and rural sites in Northern Italy. Atmospheric Environment 36, 4069–4075.
- Handley, L.L., Raven, J.A., 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant, Cell and Environment 15, 965–985.
- Handley, L.L., Odee, D., Scrimgeour, C.M., 1994. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ patterns in savanna vegetation: dependence on water availability and disturbance. Functional Ecology 8, 306–314.
- Handley, L.L., Austin, A.T., Robinson, D., Scrimgeour, C.M., Heaton, T.H.E., Raven, J.A., Schmidt, S., Stewart, G.R., 1999. The ^{15}N natural abundance ($\delta^{15}\text{N}$) of ecosystem samples reflects measures of water availability. Australian Journal of Plant Physiology 26, 185–199.
- Hanson, P.J., Garten, C.T., 1992. Deposition of H^{15}NO_3 vapour to white oak, red maple and loblolly pine foliage: experimental observations and a generalized model. The New Phytologist 122, 329–337.
- Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology 59, 87–102.
- Heaton, T.H.E., Spiro, B., Robertson, S.M.C., 1997. Potential canopy influences on the isotopic composition of nitrogen and sulphur in atmospheric deposition. Oecologia 109, 600–607.
- Hietz, P., Wanek, W., Popp, M., 1999. Stable isotopic composition of carbon and nitrogen and nitrogen content in vascular epiphytes along an altitudinal transect. Plant Cell and Environment 22, 1435–1443.
- Hietz, P., Wanek, W., Wania, R., Nadkarni, N.M., 2002. ^{15}N natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131, 350–355.
- Israeli, Y., Schwartz, A., Plaut, Z., Yakir, D., 1996. Effects of light regime a $\delta^{13}\text{C}$, photosynthesis and yield of field-grown banana (*Musa* sp., Musaceae). Plant, Cell and Environment 19, 225–230.
- Kendall, C., Grim, E., 1990. Combustion tube method for measurement of nitrogen isotope ratios using calcium oxide for total removal of carbon dioxide and water. Analytical Chemistry 62 (5), 526–529.
- Lindberg, S.E., Lovett, G.M., Richter, D.D., Johnson, D.W., 1986. Atmospheric deposition and canopy interactions of major ions in a forest. Science 231, 141–145.
- Lovett, G.M., Lindberg, S.E., 1993. Atmospheric deposition and canopy interactions of nitrogen in forests. Canadian Journal of Forest Research 23, 1603–1616.
- Lumme, I., 1994. Nitrogen uptake of Norway spruce (*Picea abies* Karst.) seedlings from simulated wet deposition. Forest Ecology and Management 63, 87–96.
- Mohr, K., 1999. Passives Monitoring von Stickstoffeinträgen in Kiefernforsten mit dem Rotstengelmoss (*Pleurozium schreberi* (Brid.) Mitt.). Zeitschrift für Umweltchemie und Ökotoxikologie 11, 267–274.
- Pearson, J., Wells, D., Seller, K.J., Bennett, A., Soares, A., Woodall, J., Ingrouille, J., 2000. Traffic exposure increases natural ^{15}N and heavy metal concentrations in mosses. The New Phytologist 147, 317–326.
- Pitcairn, C.E.R., Fowler, D., Grace, J., 1995. Deposition of fixed atmospheric nitrogen and foliar nitrogen content of bryophytes and *Calluna vulgaris* (L.) Hull. Environmental Pollution 88, 193–205.
- Pitcairn, C.E.R., Fowler, D., Leith, I.D., Sheppard, L.J., Sutton, M.A., Kennedy, V., Okello, E., 2003. Bioindicators of enhanced nitrogen deposition. Environmental Pollution 126, 353–361.
- Press, M.C., Woodin, S.J., Lee, J.A., 1986. The potential importance of an increased atmospheric nitrogen supply to the growth of ombrotrophic *Sphagnum* species. The New Phytologist 103, 45–55.
- Robinson, D., Handley, L.L., Scrimgeour, C.M., Gordon, D.C., Forster, B.P., Ellis, R.P., 2000. Using stable isotope natural abundances ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. Journal of Experimental Botany 51, 41–50.
- Russell, K.M., Galloway, J.N., Macko, S.A., Moody, J.L., Scudlark, J.R., 1998. Sources of nitrogen in wet deposition to the Chesapeake Bay region. Atmospheric Environment 32 (14–15), 2453–2465.
- Soares, A., Pearson, J., 1997. Short-term physiological responses of mosses to atmospheric ammonium and nitrate. Water, Air and Soil Pollution 93, 225–242.
- Solga, A., Burkhardt, J., Zechmeister, H.G., Frahm, J.P., 2005. Nitrogen content, ^{15}N natural abundance and biomass of the

- two pleurocarpous mosses *Pleurozium schreberi* (Brid.) Mitt and *Scleropodium purum* (Hedw.) Limpr. in relation to atmospheric nitrogen deposition. *Environmental Pollution* 134, 465–473.
- Stewart, G.R., Schmidt, S., Handley, L.L., Turnbull, M.H., Erskine, P.D., Joly, C.A., 1995. ^{15}N natural abundance of vascular rainforest epiphytes: implications for nitrogen source and acquisition. *Plant, Cell and Environment* 18, 85–90.
- Tieszen, L.L., Boutton, T.W., 1988. Stable carbon isotopes in terrestrial ecosystem research. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds.), *Stable Isotopes in Ecological Research*. Springer, New York, pp. 167–195.
- Wania, R., Hietz, P., Wanek, W., 2002. Natural ^{15}N abundance of epiphytes depends on the position within the forest canopy: source signals and isotope fractionation. *Plant Cell and Environment* 25, 581–589.
- Wilson, E.J., Tiley, C., 1998. Foliar uptake of wet-deposited nitrogen by Norway spruce an experiment using ^{15}N . *Atmospheric Environment* 32, 513–518.
- Wong, S.C., 1979. Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. *Oecologia* 44, 68–74.
- Wu, H.Y., Bao, W.K., Wang, A., 2005. Concentrations and characteristics of chemical elements in bryophytes. *Chinese Journal of Ecology* 24 (1), 58–64 (In Chinese with English abstract).
- Xiao, H.Y., Liu, C.Q., 2002. Sources of nitrogen and sulfur in wet deposition at Guiyang, Southwest China. *Atmospheric Environment* 36, 5121–5130.
- Xiao, H.Y., Liu, C.Q., 2004. Chemical characteristics of water-soluble components in TSP over Guiyang, SW China, 2003. *Atmospheric Environment* 38, 6297–6306.
- Yeatman, S.G., Spokes, L.J., Dennis, P.F., Jickells, T.D., 2001a. Comparisons of aerosol nitrogen isotopic composition at two polluted coastal sites. *Atmospheric Environment* 35, 1307–1320.
- Yeatman, S.G., Spokes, L.J., Dennis, P.F., Jickells, T.D., 2001b. Can the study of nitrogen isotopic composition in size-segregated aerosol nitrate and ammonium be used to investigate atmospheric processing mechanism? *Atmospheric Environment* 35, 1337–1345.
- Yoneyama, T., Matsumaru, T., Usui, K., Engelaar, W.M.H.G., 2001. Discrimination of nitrogen isotopes during absorption of ammonium and nitrate at different nitrogen concentrations by rice (*Oryza sativa* L.) plants. *Plant, Cell and Environment* 24, 133–139.