



Response of stable carbon isotope in epilithic mosses to atmospheric nitrogen deposition

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Photosynthetic ¹³C discrimination of bryophytes might increase with elevated N deposition.

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ABSTRACT

Epilithic mosses are characterized by insulation from substratum N and hence meet their N demand only by deposited N. This study investigated tissue C, total Chl and $\delta^{13}\text{C}$ of epilithic mosses along 2 transects across Guiyang urban (SW China), aiming at testing their responses to N deposition. Tissue C and total Chl decreased from the urban to rural, but $\delta^{13}\text{C}_{\text{moss}}$ became less negative. With measurements of atmospheric CO_2 and $\delta^{13}\text{CO}_2$, elevated N deposition was inferred as a primary factor for changes in moss C and isotopic signatures. Correlations between total Chl, tissue C and N signals indicated a nutritional effect on C fixation of epilithic mosses, but the response of $\delta^{13}\text{C}_{\text{moss}}$ to N deposition could not be clearly differentiated from effects of other factors. Collective evidences suggest that C signals of epilithic mosses are useful proxies for N deposition but further works on physiological mechanisms are still needed.

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1. Introduction

Over the 20th century, anthropogenic reactive N emissions have led to a drastic increase in atmospheric N deposition, which has potentially altered natural C and N cycles ranging from regional to global scales (Gruber and Galloway, 2008). Worldwide N deposition is of great concern as it has been estimated to increase still further during the 21st century, e.g. the level of N deposition by 2030 may increase by 50–100% relative to 2000, with the largest absolute increases occurring over East and South Asia (Reay et al., 2008; Galloway et al., 2008). It is valuable to strengthen researches on the sources and effects of N deposition in East Asia.

The terrestrial biosphere, closely interacting with the atmosphere, plays a critical role in natural C and N biogeochemical cycles. There is huge interest to intensify the search for C and N responses in terrestrial plants to N deposition (Wania et al., 2002; Reay et al., 2008). Related studies, largely undertaken in Europe and North America, have shown that N deposition has been an important factor influencing plant's C fixation (Högberg, 2007; Reay et al., 2008). Nevertheless, the positive and negative effects of N deposition on different plant communities are far from being understood in the field. There remain considerable uncertainties

and insight into the responses of plant C and N signals to N deposition is still limited due to the complexities of ecosystem and vegetation property.

Typically, urbanization is an important driver of increasing N deposition. Large amounts of urban-derived reactive N are thought to be responsible for regional elevated N deposition and related influences on both biotic and abiotic ecosystems within, surrounding, and even at greater distances from the urban environment (Grimm et al., 2008). Therefore, it is of both environmental and ecological importance to investigate the cause and effect relationship between urban-derived N and those atmospheric-sensitive recipient plants in urban ecosystems, which allow us to further understand the scale of urban-derived N deposition, and whether urban-derived N deposition is beneficial or detrimental to plant resources at risk from urban pollution.

Compared with vascular plants, mosses are characterized by higher sensitivities to atmospheric N supply due to the lack of a true root system to acquire N from substratum. Unlike some lichens and algae, mosses can almost not utilize atmospheric N_2 due to the lack of azotobacteria, but the deposited N. Among them, epilithic mosses represent a special community because of uniform insulation from substratum N, especially species inhabiting naked rocks.

Methodologically, $\delta^{13}\text{C}$ has been used extensively to examine physiological, ecological, and biogeochemical processes related to C cycles, which provides insights into the interactions between plants

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and environmental factors at a variety of temporal and spatial scales (Farquhar et al., 1989; Israeli et al., 1996). $\delta^{15}\text{N}$, on the other hand, has been recognized as an effective tool holding source-specific information for tracing the deposition of N pollutants and plants N availability (Robinson, 2001). Because of the close correlation between C and N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are recognized as a biologically important stable isotope pair and are frequently used in combination to investigate N supply and C fixation occurring from individual organism to ecosystem level (e.g. Hietz et al., 1999; Robinson et al., 2000).

Recent interest in moss %N and $\delta^{15}\text{N}$ enhanced our knowledge of the spatial variation, main sources and N species of N deposition (e.g. Bragazza et al., 2005; Zechmeister et al., 2008). However, after being uptaken by mosses, how and to what extent N deposition will influence C signals is only beginning to be realized (Koranda et al., 2007; Liu et al., 2008c). Some pioneer studies have explored the photo-synthetic pathway of mosses and showed the potential of $\delta^{13}\text{C}_{\text{moss}}$ to indicate environmental constraints (Rundel et al., 1979; Williams and Flanagan, 1996), but we could only find a few studies on the $\delta^{13}\text{C}_{\text{moss}}$ responses to water limitation and microhabitats (Rice, 2000), altitude (Ménot and Burns, 2001), temperature and atmospheric CO_2 (Fletcher et al., 2006; Skrzypek et al., 2007). How $\delta^{13}\text{C}_{\text{moss}}$ can respond to atmospheric N supply needs to be explored not only for the application of $\delta^{13}\text{C}_{\text{moss}}$ as indicator of anthropogenic N deposition but also to enrich N deposition effect on photosynthetic C fixation.

In the light of the above context, we undertook a survey on the responses of C signals in epilithic mosses along a gradient of N deposition from the urban to rural area at Guiyang (SW China), where N deposition levels, main sources and main N species have been discussed (Liu et al., 2008a,b, 2009). In combination with atmospheric CO_2 concentration and $\delta^{13}\text{C}_{\text{CO}_2}$ measurements, the objectives of the present paper are specifically to: (1) analyze the spatial variations tissue C, total Chl and $\delta^{13}\text{C}$ in epilithic mosses and discuss their implications for human-induced environmental changes (pollution, anthropogenic CO_2 emissions and/or urban-derived N deposition); (2) discuss the effect of long-term elevated N deposition on C fixation of epilithic mosses and associated C isotopic mechanisms.

2. Materials and methods

2.1. Study area

The city of Guiyang, the capital of Guizhou Province, is located in the Karst region of SW China. The landform of Guiyang area belongs to the hill-plateau basin, with mountainous areas (4218 km², 53%) and hilly areas (2842 km², 36%) as the main landforms, and smaller flatland areas of only 912 km² (11%). The average altitude of Guiyang area is 1250 m (506–1762 m) above sea level, the relative difference in elevation is about 100–200 m and the urban district lies in a Karst basin at 1071 m surrounded by low/medium hills and mountains.

Guiyang falls within the monsoon climate in the subtropical zone that is temperate and humid without pronounced winters or hot summers. Annually, the temperature averages 15.3 °C, rainfall is 1170 mm, and relative humidity (RH) is 86% in the studying area. With the mild climate, higher humidity and widespread naked carbonate rocks, there are abundant epilithic moss communities in Guiyang, thus it is an ideal place to investigate the responses of epilithic mosses to anthropogenic environmental change and atmospheric deposition.

Nitrogen deposition in Guiyang is characterized by a high proportion of NH_x and a significant decrease from the urban (mean = 31 kg N ha⁻¹ yr⁻¹) to rural area (mean = 14.3 kg N ha⁻¹ yr⁻¹) (Liu et al., 2009). Besides, it was once one of the cities with the highest acid deposition in SW China. However, in 1997 most of small-sized mills and coal mines as well as workshops with heavy pollution were forced to shut down and some heavy polluting industries were relocated. Currently over 49% of the city residents have replaced coal by natural gas (Xiao and Liu, 2004). A bio-monitoring study with epilithic mosses might be a valuable supplement for assessing current status of pollution.

2.2. Sampling of epilithic mosses

In April 2006, epilithic mosses were collected at 175 sites along four directions (NE, SW, NW and SE) from Guiyang urban to rural areas, ranging between 26°18' N–26°54' N latitude and 106°21' E–107°01' E longitude (Fig. 1). Sampling along the

SE direction was restricted by high mountains with thick vegetative cover. All samplings were performed under stable weather condition characterized by sunny or cloudy days.

Mosses eligible for this study were growing on natural naked rocks without soil, in open field without canopies or overhanging vegetation. Sampling was performed only at sites above ground level to avoid surface water splashes, and sites possibly disturbed by animals or pets were also avoided. At each site, 5–10 subsamples were collected and combined into one representative sample, and a subjective assessment of sample health and age (the green leafy shoot) was made to keep them as uniform as possible. In the urban area, epilithic mosses were mainly collected from sites on hills and mountains with higher elevations (1144.19 m, Table 2) than the urban central area where eligible samples were generally absent. Sampling sites away from the urban area were located at least 500 m from main roads and at least 100 m from other roads or houses.

Fresh mosses were stored in cleaned plastic bags en route to the laboratory. After identification, species that could not be found at every site were discarded, each sample was finally composed of four species: *Haplocladium microphyllum* (Hedw.) Broth, *Haplocladium angustifolium* (Hampe et C. Muell.) Broth, *Brachythecium salebrosum* (Web. et Mohr.) B. S. G and *Eurohypnum leptothallum* (C. Muell.) Ando.

2.3. Element analysis and isotopic determination

Epilithic mosses were gently rinsed with 1.5 mol L⁻¹ HCl solution, and then washed thoroughly with deionized water to remove adsorbed pollutants. Cleaned moss materials were dried in a vacuum oven at 70 °C and re-dried after being ground separately in liquid N into fine powders using a mortar and pestle.

Tissue C and N concentrations of epilithic mosses (% DW) were determined by elemental analyzer (Model PE2400, USA) with an analytical precision of 0.1%. The carbon isotope analysis of moss materials prepared with a sealed-tube combustion (at 850 °C) method requiring cryogenic purification of CO_2 with liquid N, then moss $\delta^{13}\text{C}$ was measured on a gas isotope ratio mass spectrometer (Model Finnigan MAT 252). Between three and five replicated measurements per sample were carried out, and values are presented as the average of these measurements. IAEA-C₃ ($\delta^{13}\text{C} = -24.97\text{‰}$, cellulose) was used as a standard for $\delta^{13}\text{C}$, with an analytical precision ($n = 5$) of $\pm 0.1\text{‰}$. The natural abundance of ^{13}C was calculated as $\delta^{13}\text{C}$ value in per mil (‰): $\delta^{13}\text{C} [\text{‰}, \text{V-PDB}] = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R denotes the ratio of mass 45/mass 44.

2.4. Analysis of moss chlorophyll

57 moss samples along the NE–SW transect were selected for total Chl (Chl *a* and Chl *b*) analysis because the amount of these samples could satisfy the need of all experiments. The Chl extractions of fresh mosses were quickly conducted under low light condition and low temperature (<30 h after sampling) to avoid the decomposition and loss of pigments.

After cleaning from impurities, mosses (≈ 0.2 g) were cut into pieces (1–2 mm) and mixed evenly with quartz sands plus a few mg of CaCO_3 powder. Then samples were ground with 2–3 mL 95% ethanol in a glass mortar and further ground with 10 mL ethanol until all tissues became white. After extracting for 3–5 min, the liquid was filtered into a 25 mL brown bottle and rinsed with deionized water until there was no green liquid on the filter paper and residue. Finally 95% ethanol was added to a constant volume of 25 mL for chlorophyll determination.

The contents of Chl were measured with a UV spectrophotometer (Unico2000) by using 95% ethanol as blanks, and quantifying the absorbance at 665 nm and 649 nm for Chl *a* and Chl *b* respectively. The equations used to calculate the concentrations of Chl (mg g⁻¹) were:

$$\rho_{\text{Chla}} = 13.95A_{665\text{nm}} - 6.88A_{649\text{nm}}$$

$$\rho_{\text{Chlb}} = 24.96A_{649\text{nm}} - 7.32A_{665\text{nm}}$$

$$\text{Pigment content (mg g}^{-1}\text{)} = \rho \times V \times N / m \times 1000$$

In which ' ρ ' represents Chl content (mg L⁻¹), ' V ' is the volume of extracting solvent (mL), ' N ' stands for the dilution factor and ' m ' is the weight of moss sample (g).

2.5. Sampling and analysis of atmospheric CO_2

In Dec. 2008, sampling of atmospheric CO_2 was conducted at 42 sites along the NE–SW transect through Guiyang urban (Fig. 1), trying to revisit locations where epilithic mosses were sampled. For examining the influence of CO_2 from urban basin on C signals of epilithic mosses, CO_2 samples were collected at both low-elevation sites and high-elevation sites on hills or mountains around the urban basin (Table 1). Sampling was conducted during 11:00am–14:30pm in order to reduce the daily variation. At each site, two CO_2 samples were collected with 1 L glass flasks for concentration and $\delta^{13}\text{C}$ analysis. Flasks were evacuated and fully flushed with air samples at each sampling site.

Atmospheric CO_2 concentration was measured on a gas chromatography (Model HP6890) and calculated based on external standards (gas standard came from National Center for the Certified Reference Materials, China). For $\delta^{13}\text{C}$ analysis, the

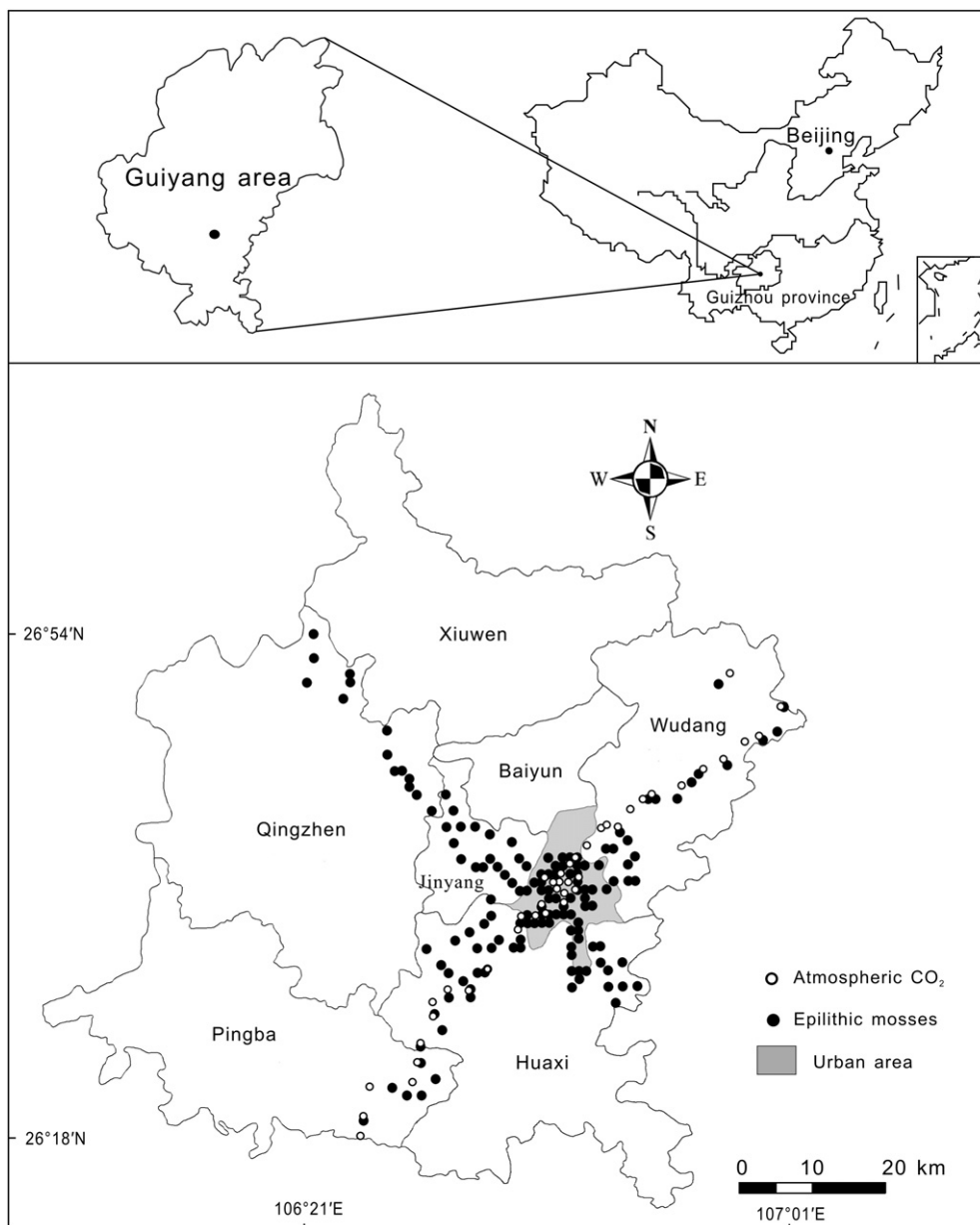


Fig. 1. Map showing the location of Guiyang area and sampling sites of epilithic mosses and atmospheric CO₂.

CO₂ was cryogenically extracted by liquid N and purified by circulation over an alcohol liquid N cold trap. Then $\delta^{13}\text{C}_{\text{CO}_2}$ values were measured on a gas isotope ratio mass spectrometer (Model Finnigan MAT 252) with reproducibility better than 0.15‰.

Table 1

Tissue C and $\delta^{13}\text{C}$ of epilithic mosses within every 5 km from the urban center at Guiyang area. Different letters (a, b) behind the values (means \pm SD) indicate a significant statistical difference at level of $P < 0.05$.

Distance (km)	Mean altitude (m)	Sample (n)	Tissue C (%)	$\delta^{13}\text{C}_{\text{moss}}$ (‰)
0–5	1144.19	48	45.60 \pm 3.27 ^a	-29.27 \pm 0.82 ^a
5–10	1163.73	43	45.66 \pm 3.78 ^a	-28.65 \pm 0.79 ^{ab}
10–15	1155.88	32	43.61 \pm 3.47 ^{ab}	-28.33 \pm 0.64 ^{ab}
15–20	1211.61	17	42.55 \pm 3.30 ^{ab}	-28.06 \pm 0.77 ^{ab}
20–25	1161.78	9	39.50 \pm 1.97 ^b	-28.07 \pm 0.55 ^b
25–30	1171.33	9	39.52 \pm 2.66 ^b	-27.93 \pm 0.44 ^b
30–35	1290.00	6	40.61 \pm 3.16 ^b	-27.52 \pm 0.36 ^b
35–40	1156.50	4	40.66 \pm 2.89 ^b	-27.27 \pm 0.68 ^b
40–45	1274.00	4	41.34 \pm 1.25 ^b	-27.52 \pm 0.43 ^b
45–50	1222.67	3	40.95 \pm 0.29 ^b	-27.61 \pm 0.39 ^b

2.6. Statistical analysis

Statistical analysis was conducted by using SPSS 12.0 software (SPSS Science, Chicago, USA). Differences between two groups were tested by a *t*-test, significant differences between mean values of three or more groups were determined by one-way ANOVA followed by a multiple comparison test (Tukey HSD, LSD). Regression analysis was performed to evaluate changes of tissue C, $\delta^{13}\text{C}_{\text{moss}}$, CO₂ concentration and $\delta^{13}\text{C}_{\text{CO}_2}$ with distance from the urban center. Correlations were analyzed by one-way analysis of variance (ANOVA), relationships were tested by regular Pearson product–moment correlation coefficients.

3. Results

3.1. Spatial variations of tissue C, total Chl and $\delta^{13}\text{C}$ in epilithic mosses

Tissue C of epilithic mosses at Guiyang area (34.47–52.76%) showed an exponential decrease with distance from the urban center ($y = 9.206e^{-0.042x} + 38.024$) (Fig. 2). Urban mosses (0–5 km)

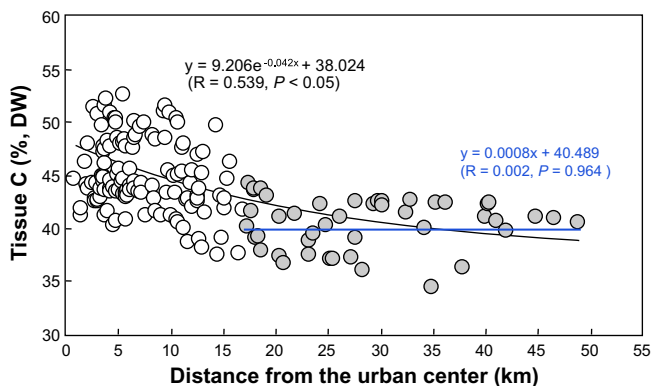


Fig. 2. The spatial variation of tissue C in epilithic mosses with distance from the urban center at Guiyang area (The linear regression was calculated taking into account samples over 17.18 km, $n = 44$).

exhibited significantly higher tissue C (mean = $45.60 \pm 3.27\%$) than those in suburban and rural area, where the lowest mean content was $39.50 \pm 1.97\%$ (Table 1). However, there was no substantial variation of tissue C over 10 (20) km from the urban center ($y = 0.0008x + 40.489$, Fig. 2), and no significant difference ($P < 0.05$) was found between mean C contents beyond 20 km (Table 1).

Similar to tissue C, higher total Chl concentrations were also observed in urban mosses ($2.96 \pm 0.68 \text{ mg g}^{-1}$, 0–5 km), and there was no significant difference between mean values out of the urban area, ranging between $2.01 \pm 0.49 \text{ mg g}^{-1}$ and $2.42 \pm 0.96 \text{ mg g}^{-1}$ (Fig. 3).

$\delta^{13}\text{C}_{\text{moss}}$ ranged between -30.69‰ and -26.42‰ and became less negative with distance from the urban center to the rural area [$y = 0.674 \ln(x) - 30.03$, Fig. 4]. Urban mosses (0–5 km) exhibited significantly more negative $\delta^{13}\text{C}_{\text{moss}}$ ($-29.27 \pm 0.82\text{‰}$) than those in suburban and rural area, and the highest $\delta^{13}\text{C}_{\text{moss}}$ ($-27.27 \pm 0.68\text{‰}$) occurred at 35–40 km. There was also no significant difference ($P < 0.05$) between average $\delta^{13}\text{C}_{\text{moss}}$ values in the non-urban area (Table 1).

3.2. Spatial variations of atmospheric CO_2 and $\delta^{13}\text{CO}_2$

Atmospheric CO_2 concentration at Guiyang area ranged between 379 and $554 \mu\text{mol mol}^{-1}$, and $\delta^{13}\text{CO}_2$ varied from -9.65‰

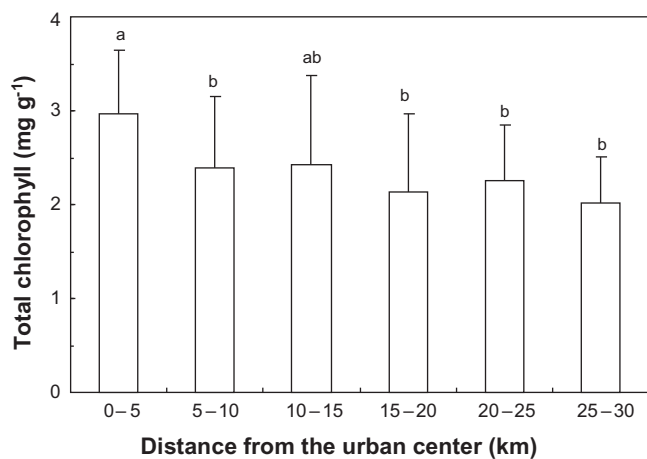


Fig. 3. Comparison of total Chl in epilithic mosses within every 5 km from Guiyang urban. Different letters above the bars indicate a significant statistical difference between values (means \pm SD) at the level of $P < 0.05$ (0–5 km, $n = 7$; 5–10 km, $n = 19$; 10–15 km, $n = 14$; 15–20 km, $n = 9$; 20–25 km, $n = 5$; 25–30 km, $n = 3$).

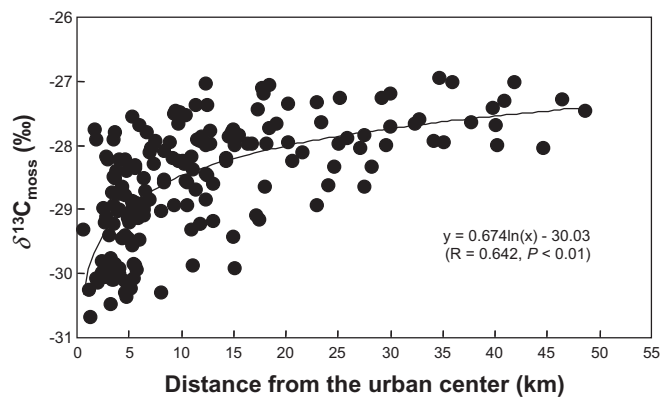


Fig. 4. The spatial variation of $\delta^{13}\text{C}_{\text{moss}}$ with distance from the urban center at Guiyang area.

to -14.41‰ . As listed in Table 2, low-elevation sites in the urban basin showed significantly higher atmospheric CO_2 level ($484.36 \pm 39.52 \mu\text{mol mol}^{-1}$) and more negative $\delta^{13}\text{CO}_2$ ratio ($-13.54 \pm 0.47\text{‰}$) than sites with higher elevations on hills or mountains around the urban area ($409.62 \pm 9.62 \mu\text{mol mol}^{-1}$, $-10.98 \pm 0.71\text{‰}$). However, for zones where epilithic mosses were largely distributed and sampled, there was no substantial variation of atmospheric CO_2 and $\delta^{13}\text{CO}_2$ with distance from the urban to rural area (Fig. 5), and both atmospheric CO_2 and $\delta^{13}\text{CO}_2$ did not show significant differences between the urban and rural areas (Table 2).

3.3. Correlations between C and N parameters in epilithic mosses

Tissue C of epilithic mosses correlated positively with tissue N (0.85–2.97%) (Fig. 6). $\delta^{13}\text{C}_{\text{moss}}$ correlated negatively with both tissue C and N (Fig. 7). Total Chl of epilithic mosses correlated positively with both tissue C and N, but negatively with C/N ratio and $\delta^{13}\text{C}_{\text{moss}}$ (Fig. 8).

4. Discussion

4.1. Implications of moss C and $\delta^{13}\text{C}$ variations

Tissue C and $\delta^{13}\text{C}$ signals of plants are commonly influenced by multiple genetic, climatic, environmental and geographical factors (Farquhar et al., 1989). However, the urban area of the studying city is relatively small in size and the urban district lies in a Karst basin surrounded by low/medium hills and mountains (Introduced in 2.1). Moss samples were mainly collected at sites on hills or mountains with higher elevations than urban central area, the annually mean rainfall, relative humidity and temperature show no difference from the urban to rural areas. Besides, all samples showed uniform species composition, substratum (naked carbonate rocks in open fields), light intensity and water conditions. Accordingly, the genetic (photosynthetic pathway), climatic and meteorological parameters may not be the main factors influencing the spatial variations of tissue C (varied by 6%) and $\delta^{13}\text{C}_{\text{moss}}$ (by 2‰) from the urban to rural area (Table 1).

The average altitude of sampling sites was $1169.69 \pm 78.31 \text{ m}$, thus the altitude effect on $\delta^{13}\text{C}_{\text{moss}}$ can be negligible because $\delta^{13}\text{C}$ of plant leaves has been shown to be ^{13}C -enriched by about $+1.1$ – $+1.37\text{‰}$ with each 1 km increase of altitude (Körner et al., 1991). Accordingly, the spatial variations of tissue C and $\delta^{13}\text{C}_{\text{moss}}$ in this study were mainly induced by differences of anthropogenic factors between the urban and rural area, including atmospheric pollution, anthropogenic CO_2 emissions and atmospheric N deposition.

Table 2

Concentrations of atmospheric CO₂ and δ¹³CO₂ at different locations of Guiyang area. Different letters (a, b) behind the values (means ± SD) indicate a significant statistical difference at level of *P* < 0.05.

Location	Mean altitude (m)	Sample (<i>n</i>)	Atmospheric CO ₂ (μmol mol ⁻¹)	δ ¹³ CO ₂ (‰)
Urban area	1062.71	7	484.36 ± 39.52 ^a (445.84–553.62)	-13.54 ± 0.47 ^a (-14.41 to -12.91)
Hills or mountains around the urban	1137.12	8	409.62 ± 9.62 ^b (395.73–426.80)	-10.98 ± 0.71 ^b (-12.10 to -9.65)
Suburb and rural area	1146.56	27	400.85 ± 9.66 ^b (378.67–425.43)	-10.63 ± 0.38 ^b (-11.44 to -9.77)

4.2. Biological evidences for reflecting reduced SO₂ pollution

Atmospheric pollution will negatively affect leaf photosynthesis and commonly leads to a lower C concentration. Vingiani et al. (2004) observed that tissue C of *Sphagnum capillifolium* decreased by 2% with exposure to urban SO₂ pollution as photosynthesis was influenced notably. Likewise, Garty et al. (1993) reported that the decrease of total Chl in *Ramalina duriaei* was related to SO₂ pollution. Oppositely, higher moss C and Chl concentrations were observed in present study (Figs. 2 and 3), the photosynthesis and CO₂ fixation of urban mosses did not respond to supposed SO₂ pollution as expected.

During 1981–1990, the concentration of ground SO₂ at Guiyang reached 400–500 μg m⁻³, which was 3 times higher than the average of 10 major cities in northern America (Huang et al., 1995; Xiao and Liu, 2004), even the average level (300 μg m⁻³) in 1996 was about 6 times above the WHO annual guideline of 50 μg m⁻³. However, most of small-sized mills and mines as well as workshops with heavy pollution were forced to shut down since 1997. Moreover, some heavy polluting industries were relocated, and over 49% of the city residents replaced coal by natural gas. In 2003, only 87 μg m⁻³ of SO₂ was determined at Guiyang and a lower level of 70 μg m⁻³ was found in 2005 (Xiao and Liu, 2004). Therefore, tissue C and Chl measurements provide biological evidences for reduced SO₂ pollution in the urban area, and changes in moss C signals from urban to rural environment was not determined by air pollution.

4.3. Anthropogenic CO₂ was not the main factor regulating tissue C and δ¹³C_{moss}

Anthropogenic CO₂ emissions from various fossil-fuel combustions sources may cause higher ambient CO₂ concentrations in the urban atmosphere, thus would improve leaf-level photosynthesis and C assimilation of urban plants, by which discrimination against heavier isotope (¹³C) in leaf cells would increase (O'Leary, 1981). Such a pattern has also been observed in mosses (e.g. Ménot and Burns, 2001; Fletcher et al., 2006). On the other hand, anthropogenic CO₂ is isotopically far more ¹³C-depleted than normal atmosphere (Widory and Javoy, 2003); the average δ¹³C of mixed CO₂ in urban areas might be more ¹²C-enriched than non-urban areas; thus directly leading to more negative δ¹³C_{moss}. Based upon the above mechanism, we hypothesized that anthropogenic CO₂ emission in the urban area was a primary cause of observed variations.

Unexpectedly, although both CO₂ and δ¹³CO₂ in the study area, even in the supposedly pollution-free rural area, were generally higher and more negative than those of normal atmospheric CO₂, only a weak decline of CO₂ concentration and a negligible increase of δ¹³CO₂ were observed from the urban to rural sites (Fig. 5). Both atmospheric CO₂ and δ¹³CO₂ did not differ significantly between urban moss sampling sites (409.62 ± 9.62 μmol mol⁻¹, -10.98 ± 0.71‰) and rural sites (400.85 ± 9.66 μmol mol⁻¹, -10.63 ± 0.38‰) (Table 1). This pattern was opposite to that in the Paris metropolitan area where atmospheric CO₂ was found to decrease from 951 μmol mol⁻¹ at urban sites to 387 μmol mol⁻¹ at rural sites, and the mixing of fossil-fuel-derived CO₂ (δ¹³C = -40.5‰ to -24.6‰) resulted in the δ¹³CO₂ increasing from -19.8‰ in the urban to -8.6‰ in the rural areas (Widory and Javoy, 2003). In the Guiyang urban area, only low-elevation sites had significantly higher CO₂ concentration (484.36 ± 39.52 μmol mol⁻¹) and more negative δ¹³CO₂ (-13.54 ± 0.47‰) (Table 1). This is because complex terrains such as urban mountain basins strongly affect the height of atmospheric boundary layer and of urban CO₂ mixing ratios (Pataki et al., 2005). Thus anthropogenic CO₂ mixing may mainly occur at low-elevation layers (<1144.19 m, Table 1) in the urban basin of Guiyang. Our

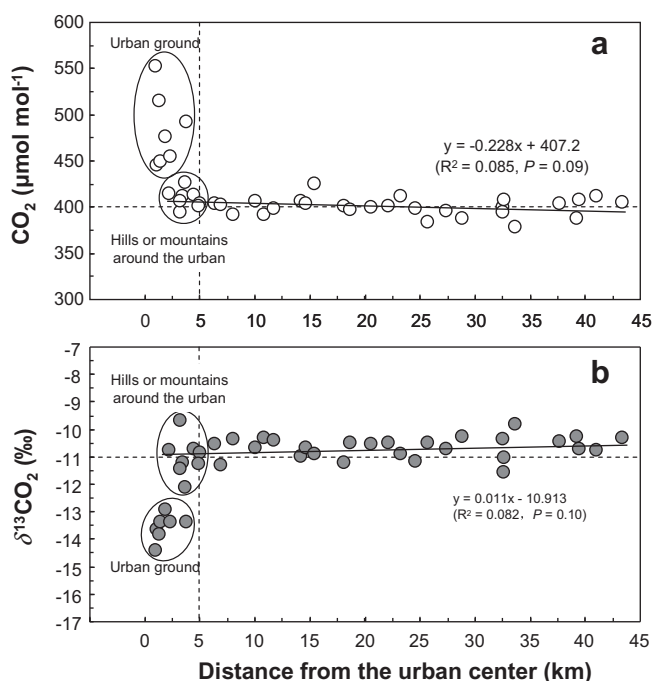


Fig. 5. Spatial patterns of atmospheric CO₂ (a) and δ¹³CO₂ (b) with distance from Guiyang urban.

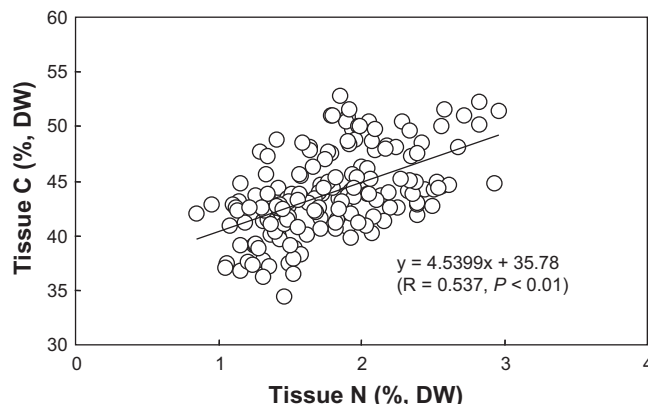


Fig. 6. Correlation between tissue N and tissue C of epilithic mosses.

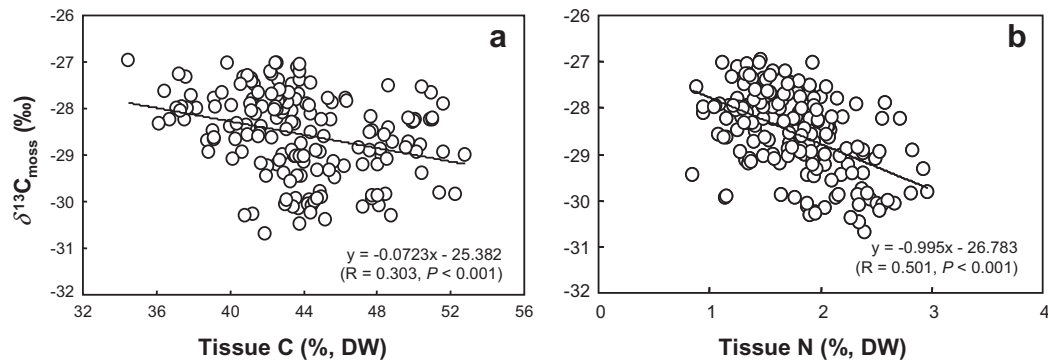


Fig. 7. $\delta^{13}\text{C}_{\text{moss}}$ of epilithic mosses plotted against tissue C (a) and tissue N (b).

previous study on mosses at lower sites of Guiyang urban (990 ± 3 m) showed significantly more negative $\delta^{13}\text{C}_{\text{moss}}$ ($-30.2 \pm 1.1\text{‰}$), most possibly influenced by anthropogenic CO_2 (Liu et al., 2008c). Accordingly, a relatively well mixed layer of daily CO_2 mixing ratio was estimated to be less than 100 m above ground level in Guiyang city, which was lower than that (>160 m) reported in the Salt Lake Valley, Utah (Pataki et al., 2005), most possibly due to the small urban size and low CO_2 emission intensity in Guiyang.

Therefore, a mountain urban basin effect greatly reduced the mixing of fossil-fuel-derived CO_2 , atmospheric CO_2 was not a major factor contributing to the observed variations of C signals in epilithic mosses that broadly reside on higher locations in the mountainous city area.

4.4. Fertilizing effect of N deposition on moss C fixation

As the only N source for moss photosynthesis, N deposition plays a very critical role in influencing C signals of epilithic mosses. Tissue N was found to be strongly correlated with tissue C (Fig. 6), elucidating that elevated N deposition and thereby higher N assimilation could enhance C fixation in epilithic mosses. Enhanced C fixation was suggested to be the resultant of improved synthesis of carboxylation enzyme (mainly Rubisco) (Guehl et al., 1995), expressing higher total Chl concentrations (Fig. 3). Decreasing tissue C of epilithic mosses was mainly attributed to the decrease of N deposition from Guiyang urban (mean = $31 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to rural ($14.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Based on the correlations between tissue C and tissue N ($y = 4.5399x + 35.78$, Fig. 6), tissue N and N deposition ($y = 0.052x + 0.7305$, Liu et al., 2008c), a relationship between N deposition and C concentration of epilithic mosses became apparent as ($y = 0.2361x + 39.10$), which indicated that each $1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ increase of N deposition could enhance tissue C by 0.2361% in epilithic mosses in the study area.

Higher N availability and N assimilation could facilitate the synthesis of carboxylation enzyme (mainly Rubisco), chlorophyll, proteins, carbohydrates and amino acids (Evans, 1989; Guehl et al., 1995). Moss C concentrations ($\geq 39.50 \pm 1.97\%$, Table 1) were generally higher than sites with relatively lower N deposition. Liu et al. (2008c) reported that moss C 38.2–38.9% was lower at Gongga Mountain (SW China) where N deposition was about $8.46 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Similarly, Koranda et al. (2007) indicated that elevated N supply caused increases of amino acid and carbohydrates in moss tissues. And a more recent experimental study by Liu et al. (2008) showed that enhanced N deposition significantly increased mosses photosynthetic rates and contents of starch, total N, soluble sugar and proteins.

Therefore, higher tissue C in urban mosses actually showed a nutritional effect of elevated N supply on the productions of

organic C compounds during photosynthesis of epilithic mosses. Similarly, Von Arb et al. (1990) reported higher Chl ($>4 \text{ mg g}^{-1}$) of *Parmelia sulcata* in an urban area than those in suburban and rural areas, and pointed out a fertilizing effect of atmospheric N on lichen photosynthesis. Signal et al. (2008) found that total Chl of 6 moss species near a motorway (NO_2 , $25 \mu\text{g m}^{-3}$) was 11–75% higher than those at 150 m from motorway (NO_2 , $15 \mu\text{g m}^{-3}$), then concluded that higher N inputs served as a fertilizer causing 22% higher tissue N than those under lower N deposition.

In the Karst ecosystems with rocky desertification, epilithic mosses are perennial epiphytes with the potential to relegate atmospheric N to organic matter and soil production on rock surfaces. This study provided first insight into the relation between atmospheric N retention and moss C sequestration on naked rock surfaces, which is valuable for further studies on the habitats succession of epilithic mosses.

4.5. Increasing $\delta^{13}\text{C}_{\text{moss}}$ from the urban to rural environment

4.5.1. Moss photosynthesis and ^{13}C fractionation

Different from vascular plants, photosynthetic performances of mosses operate without stomata on gametophytes, they depend upon simple diffusion for CO_2 uptake and the internal CO_2 availability is often controlled by the thickness of water films on leaf surfaces (Proctor et al., 1992; Rice, 2000). However, photosynthetic C metabolism and ^{13}C isotopic discrimination for mosses are recognized being similar to mechanisms of C_3 plants (Williams and Flanagan, 1996). For vascular plants, ^{13}C isotopic discrimination varies negatively with the ratio of chloroplast CO_2 (C_c) and atmospheric CO_2 (C_a), changes in C_c/C_a and $\delta^{13}\text{C}$ are, therefore, a function of changes in conductance to CO_2 and/or photosynthetic capacity influenced by variable environmental and physiological factors (O'Leary, 1981; Farquhar et al., 1989). Similarly, the two primary processes that cause photosynthetic ^{13}C fractionation in mosses are diffusion and biochemical assimilation due to Rubisco, changes in $\delta^{13}\text{C}_{\text{moss}}$ occur in association with changes in the relative influence of diffusional and enzymatic fractionations (Rundel et al., 1979; Williams and Flanagan, 1996). Based on the two processes, the $\delta^{13}\text{C}_{\text{moss}}$ variation observed in the study area was interpreted as below.

4.5.2. Possible differences of diffusional fractionations caused by microhabitats or microclimates

Different from stomatal openness/closure, CO_2 conductance in mosses is mainly driven by a passively variable water layer causing limitation to CO_2 diffusion (Rice, 2000). Given the same morphological and anatomical traits, the resistance to inward CO_2 diffusion is dependent upon the retention and thickness of water layers. A

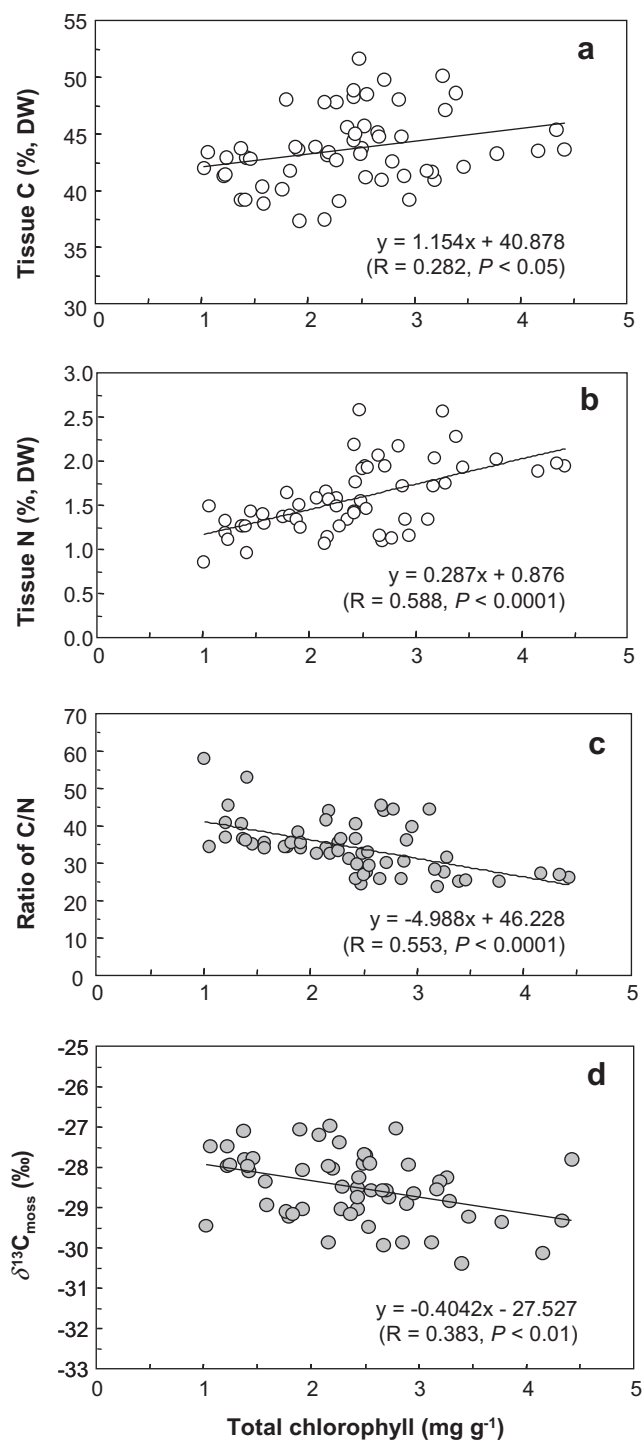


Fig. 8. Total Chl plotted against tissue C (a), tissue N (b), ratio of C/N (c) and $\delta^{13}\text{C}$ (d) of epilithic mosses.

mechanistic model has been used to examine the environmental regulation of photosynthetic CO_2 exchanges in mosses, showing the diffusional resistance by water films is a key factor controlling C_c and ^{13}C fractionation (Farquhar et al., 1982; Williams and Flanagan, 1998)

In this study, it is difficult to measure the true difference of water contents at so many sites in the field because mosses are poikilohydric with weather condition instantaneously. Although there is no significant difference of annual climatic parameters

within the studying city area, here we discuss the possibility that the changing $\delta^{13}\text{C}_{\text{moss}}$ was caused by the variability in diffusional resistance to CO_2 due to variable microhabitats or microclimates among the sampling sites.

Differences of temperature and moisture affecting evapotranspiration could cause differences in water films and therefore diffusional limitation in plants. Most possibly, higher temperature around the urban area caused less persistent water films and lower water contents therefore decreased diffusional limitation to CO_2 uptake in mosses, which was expressed as higher C_c and more negative $\delta^{13}\text{C}_{\text{moss}}$ (Rice and Giles, 1996; Rice, 2000). Besides, increased ^{13}C fractionation was expected during water loss until desiccation, when the diffusional limitation decreases due to diminishing water films and the first photosynthetic capacity remains at high levels until it declines below the optimum for assimilation (Williams and Flanagan, 1996)

Accordingly, more negative $\delta^{13}\text{C}_{\text{moss}}$ might be interpreted in terms of higher C_c caused by low diffusional resistance, which has partly offset the decreased ^{13}C discrimination effect (decreased C_c) triggered by enhanced photosynthetic rates

4.5.3. Increased ^{13}C discrimination induced by N deposition

Of course, it is more likely that water retention and contents for perennial epilithic mosses did not differ substantially from the urban to rural area because of the uniform genetic, substratum, annually climatic conditions and higher elevations than the urban central area (Table 2). This means that the variations of C_c/C_i ratio and the $\delta^{13}\text{C}_{\text{moss}}$ were caused by relatively more influences from enzymatic fractionation than the diffusional fractionation

Based on discussions in the section of 4.4, N deposition played an unequivocal fertilizing effect on the photosynthesis of epilithic mosses. Evidences from negative correlations between tissue C and $\delta^{13}\text{C}_{\text{moss}}$, tissue N and $\delta^{13}\text{C}_{\text{moss}}$ indicated that increased ^{13}C discrimination occurred under higher N deposition in the urban area (Figs. 7 and 8). The variation of total Chl sensed the change of photosynthesis, especially the negative correlation between total Chl and $\delta^{13}\text{C}_{\text{moss}}$ showed that there was an unevadable effect of N deposition on photosynthetic ^{13}C discrimination. These evidences elucidated that some other mechanisms during elevated N assimilation must more strongly counteract the expected decline in C_c/C_i and ^{13}C discrimination caused by enhanced photosynthesis. The major one is that the biochemical C assimilation processes discriminate against the heavier isotope ($^{13}\text{CO}_2$) more than the lighter isotope ($^{12}\text{CO}_2$) because $^{12}\text{CO}_2$ is more reactive and likely to be diffused and assimilated into the photosynthetic products more easily than $^{13}\text{CO}_2$ (reviewed in Dawson et al., 2002). Due to the increase of C fixation triggered by N deposition, more $^{12}\text{CO}_2$ during the metabolic and biosynthetic reactions was assimilated in urban mosses

This interpretation did not contradict with the mechanism that lower C_c and isotopic fractionation occurred under increased photosynthesis due to elevated N deposition because $\delta^{13}\text{C}_{\text{moss}}$ in some urban sites with higher N deposition indeed showed more positive or comparable signatures with those in rural area (Fig. 4). Therefore, the general pattern of $\delta^{13}\text{C}_{\text{moss}}$ (2‰ depletion from rural to urban area) actually showed a long-term spatial-integrated result of more $^{12}\text{CO}_2$ assimilation than C_c/C_i decline in moss tissues.

4.5.4. $\delta^{13}\text{C}_{\text{moss}}$ for indicating urban-derived N deposition

The N deposition gradient in the studying area is mainly caused by the decrease of urban-derived NH_x from the urban to rural areas, which is mainly released from excretory wastes and sewage (Liu et al., 2008a,b, 2009). Therefore, it is essential to discuss the relationship between the variation of $\text{NH}_x\text{-N}$ and changes in moss C signals for understanding the effect of N deposition.

Moss C did not change with distance over 10 (20) km from the urban center ($y = -0.0008x + 40.489$) (Fig. 2), and no statistical difference was observed for both tissue C and $\delta^{13}\text{C}_{\text{moss}}$ within each 5 km increment beyond 20 km (Table 1). These features illustrated that the influence of elevated N deposition on moss C signatures was mainly within 20 km, which was coincident with that urban-derived NH_x was mainly deposited within 20 km from the urban center (Liu et al., 2008c). The reason why C signals could respond sensitively to the distribution of urban-derived N deposition was also related to the fertilizing effect of $\text{NH}_x\text{-N}$ on plant C fixation. Van Hove et al. (1991) observed that *Populus euramericana* exhibited higher CO_2 assimilation rate under enhanced NH_x deposition ($64 \mu\text{g m}^{-3} \text{NH}_3$). Van der Eerden and Pérez-Soba (1992) found that increased NH_3 absorption in leaves improved photosynthetic enzyme and CO_2 uptake. These results highlighted that N deposition was the prominent candidate being responsible for observed changes in C signals of epilithic mosses.

5. Conclusions

Increasing N deposition has been involved as an important phenomenon in global changes. This study explored responses of C signals in epilithic mosses to the effect of elevated N deposition, and revealed the applicability of $\delta^{13}\text{C}_{\text{moss}}$ for indicating anthropogenic N deposition.

The decreasing concentrations of tissue C and total Chl from urban to rural environment suggested that SO_2 pollution in Guiyang was not harmful to moss photosynthesis. Physiological parameters of epilithic mosses were therefore useful for assessing human-induced environmental changes due to urbanization or administrative policies. The urban–rural patterns of CO_2 concentration and $\delta^{13}\text{CO}_2$ pointed out an urban mountain basin effect on the mixing of anthropogenic CO_2 emissions in Guiyang city. Nitrogen deposition was therefore inferred as the main factor regulating spatial variations of moss C signals from the urban to rural areas.

A nutritional effect of N deposition on photosynthesis and C fixation of epilithic mosses was observed, and a quantitative relationship between N deposition and tissue C content was estimated as $y = 0.2361x + 39.10$. As epilithic mosses play important roles in constantly changing their rocky habitats through biological activities and transferring atmospheric-derived substances to substratum after death, our results provided useful insights into the effect of N deposition in driving moss C and N sinks on rock surfaces, which is helpful for understanding the vegetation succession and restoration in the Karst ecosystems with rocky desertification, especially in Southwestern China.

The $\delta^{13}\text{C}_{\text{moss}}$ variation suggested that ^{13}C discrimination in epilithic mosses can respond to elevated N deposition, $\delta^{13}\text{C}_{\text{moss}}$ was therefore useful for reflecting urban-derived N at spatial scales and N deposition effect on plant C fixation. However, due to the complexity of influences from environmental or climatic factors on moss photosynthesis, further studies are strongly needed to clarify mechanisms of N deposition effect on C isotopic fractionation.

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