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Monitoring atmospheric nitrogen pollution in Guiyang (SW China) by contrasting use of *Cinnamomum Camphora* leaves, branch bark and bark as biomonitors☆

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ABSTRACT

Moss (as a reference material) and camphor (*Cinnamomum Camphora*) leaf, branch bark and bark samples were systematically collected across an urban-rural gradient in Guiyang (SW China) to determine the efficacy of using these bio-indicators to evaluate nitrogen (N) pollution. The tissue N concentrations (0.13%–2.70%) and $\delta^{15}N$ values (−7.5‰ to +9.3‰) of all of these bio-indicators exhibited large spatial variations, as they recorded higher values in urban areas that quickly decreased with distance from the city center; moreover, both soil N concentrations and soil $\delta^{15}N$ values were found no significant differences within each 6 km from the urban to the rural area. This not only suggests that the different N uptake strategies and variety of N responses of these bio-indicators can be reflected by their different susceptibilities to variations in N deposition but also reveals that they are able to indicate that urban N deposition is mostly from traffic and industry (NO_x-N), whereas rural N deposition is mainly from agriculture (NH_x-N). Compared to previously collected urban moss and camphor leaf samples, the significantly increased $\delta^{15}N$ values in current urban moss and camphor leaf samples further indicate a greater contribution of NO_x-N than NH_x-N to urban N deposition. The feasibility of using the N concentrations and $\delta^{15}N$ values of branch bark and bark as biomarkers of N deposition thus was further confirmed through the comparative use of these bio-indicators. It can be concluded that vascular plant leaves, branch bark and bark can be used as useful biomonitoring tools for evaluating atmospheric N pollution. For further study, quantitative criteria for the practical use of these bio-indicators in response to N deposition should be developed and the differences in the $\delta^{15}N$ values of different plant parts should also be considered, particularly in urban environments that are severely disrupted by atmospheric pollution.

1. Introduction

The high density of traffic, population and industries have contributed massive inputs of reactive N to the atmosphere; therefore, many regions of China are experiencing intense air pollution, which has been further exacerbated by enhanced agricultural activities (Liu et al., 2011; Kan et al., 2012). N pollutants emitted into the atmosphere can be removed from the atmosphere by wet or dry deposition to lakes, soil and plants (Goulding et al., 1998; Zhang et al., 2010), which can lead to lacustrine and estuarine eutrophication, toxic metal activation, soil acidification, an imbalance in the availabilities of cations, changes in the biodiversity of terrestrial ecosystems, and the degradation of human health (Schulze, 1989; Galloway et al., 2004; Richter et al., 2005; Stevens et al., 2009). However, determining the origin of N pollutants and investigating the level of atmospheric N pollution is extremely difficult, as N emissions are usually derived from different anthropogenic sources and the deposition of N includes a variety of N compounds that exist in aerosols, gas phases, and precipitation. In contrast, bio-monitors represent an inexpensive, effective and reliable method of evaluating atmospheric N pollution.

Vegetation is one of the most important biological sinks for

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atmospheric N, which occurs through the processes of both dry and wet deposition. It is generally accepted that the highly consistent responses of plant tissues (e.g., mosses, lichens, vascular plant leaves) to increased atmospheric N inputs yield significantly higher tissue N concentrations (Hicks et al., 2000; Pitcairn et al., 2003; Liu et al., 2013). Mosses, which are mainly reliant on atmospheric N inputs as their source of N, have been more frequently used in studies of atmospheric N deposition than vascular plant leaves. The robust correlation between moss N concentrations and total atmospheric N deposition has been established in field studies (Xiao et al., 2010a). Additionally, the fact that the N concentrations of some vascular plant leaves (e.g., *Calluna vulgaris*, *Nardus stricta* and *Deschampsia flexuosa*) increase linearly with N deposition was also observed by Hicks et al. (2000) and Pitcairn et al. (2001). These bio-indicators (especially mosses) can therefore be suitable for determining the levels of regional and even national N deposition. Differently, the outermost tree bark of a tree trunk is primarily used to investigate local or regional atmospheric heavy metal pollution because of its constant exposure to the atmosphere (Bellis et al., 2001; Saarela et al., 2005; Suzuki, 2006). Schulz et al. (1997) determined that a relationship exists between the NO_3^- and NH_4^+ concentrations in pine tree barks and throughfall concentrations, such that the concentrations of oxidized and reduced N species in pine tree barks can be used to quantify throughfall rates. This indicates that bark and branch bark N concentrations may be closely linked to atmospheric N deposition. In addition, the findings obtained by Mitchell et al. (2005) and Boltersdorf et al. (2014) also suggested that tree bark can represent another resource with which to assess N deposition. However, studies of the use of bark and branch bark N concentrations as indicators of N deposition are still quite limited compared to those of mosses and tree leaves, which demonstrates the urgent and important need to study the susceptibility of bark and branch bark to increased N deposition.

It has been proposed that measuring N isotopic compositions in plants and soils via a single sampling (so that the system would not be disturbed by adding a ^{15}N tracer) might not only offer instantaneous information about N sources but may also yield the advantage of providing insights into the N cycling history of a region (Robinson, 2001; Pardo et al., 2007). Additionally, the isotopic compositions of atmosphere-derived N have been increasingly used to identify the potential N sources of inputs into various plant and soil environments (Evans and Ehleringer, 1993; Durka et al., 1994; Pearson et al., 2000; Redling et al., 2013). As well known, NO_x-N and NH_x-N are the two main sources for N pollution, which can be distinguished isotopically because higher $\delta^{15}N$ values were generally reported for NO_x compared to NH_x (Table S1). Therefore, results from many literature have shown that the analysis of $\delta^{15}N$ values in moss tissues can be used to distinguish the contributions of various N emission sources to regional atmospheric N deposition, because the N isotopic effects during N uptake are very low or even absent, such that the effectiveness of N emission reductions can be assessed (Bragazza et al., 2005; Solga et al., 2005; Pitcairn et al., 2006; Xiao et al., 2010b). Our knowledge of the use of vascular plants to evaluate atmospheric N pollution, by contrast, remains relatively poor, because many complex factors that affect $\delta^{15}N$ values in vascular plant tissues should be taken into account, namely, differences in N sources (e.g., soil organic N, soil NH_4^+, soil NO_3^-, and atmospheric N), root depth, plant mycorrhizal status, the transpiration efficiency of net N uptake, the influence of canopies, and fractionation in soil-plant systems (Högberg, 1997; Michelsen et al., 1998; Evans, 2001; Cernusak et al., 2009). Although this complexity does exist, several previous studies have revealed that the $\delta^{15}N$ values of vascular plant leaves can be affected by N deposition (Jung et al., 1997; Köchy and Wilson, 2001; Stewart et al., 2002; Kuang et al., 2011). Previous studies that have used the N isotopic compositions of vascular plant leaves as indicators of atmospheric N pollution have mainly focused on coniferous trees in non-urban ecosystems (mainly forest ecosystems) (Gebauer et al., 1994; Ammann et al., 1999; Bukata and Kyser, 2007; Kuang et al., 2011); however, thus far, relatively little attention has been paid to the response of vascular plants on atmospheric N pollution in urban environments. Bark, as a passive bio-indicator can directly adsorb N compounds or other pollutants from the atmosphere or from throughfall or stemflow. This characteristic of bark would cause a change in the chemical composition of its surface layer. Such changes can thus be used to evaluate the extent to which a given region has been subjected to atmospheric pollution (Poikolainen, 1997; Schulz et al., 2001; Suzuki, 2006). Although the determination of pine bark $\delta^{15}N$ values has been successfully applied in indicating NH_3 sources from agricultural activities (Schulz et al., 2001; Boltersdorf et al., 2014), a better understanding of the impacts of atmospheric N deposition on bark and branch bark $\delta^{15}N$ is still required. In particular, different bio-indicators have different N acquisition pathways and mechanisms, it implies the existence of their different N responses following the influence of N pollution. But until now, no study has systematically investigated the differences of $\delta^{15}N$ value variation in different plant parts (e.g., leaves, branch bark and bark) or different bio-indicators (e.g., leaves, branch bark, bark and mosses) in response to atmospheric N pollution.

This study thus utilizes mosses, leaves, branch bark and tree bark as indicators of N deposition; furthermore, through the determination of N concentrations and $\delta^{15}N$ values in these four bio-indicators, which were collected across an N deposition gradient in the Guiyang area, their abilities to reflect atmospheric N pollution were compared. The questions that were further discussed included the following: (1) Whether the evaluation conclusion of spatial distribution of N deposition in the study area is consistent via N concentration data of the four bio-indicators? (2) Are there any differences in the $\delta^{15}N$ values between these bio-indicators; if so, what affects them? (3) Does N deposition leave a recognizable N isotopic signal in bark and branch bark? (4) What are the main sources of N deposition in the current Guiyang area, and has the importance of different N pollution sources changed over time, according to the $\delta^{15}N$ values in these bio-indicators?

2. Materials and methods

2.1. Study area

Guiyang city of southwest China is situated in a wide karst valley basin with an average altitude of 1250 m. The city is characterized by a subtropical monsoon climate with an annual mean temperature of 15.3 °C and an annual mean rainfall of 1174.7 mm; the prevailing wind direction is southeast in the summer (Guiyang Environmental Protection Bureau, 2006). The main lithological feature in this study area was classified as carbonate; the soil is dominated by strongly weathered, acidic yellow soil, which records high aluminium concentrations and low base saturation (Larssen et al., 1998; Han et al., 2011).

The city center, which is located in the southern region of Yunyan and the northern region of Nanming, contains more than 100,000 vehicles and has a population density of 30,000/km^2 (Li et al., 2012). The total motor vehicle population in the city increased by 344%, from 225,400 in 2005 to 1,000,000 in 2015 (He, 2013; Traffic Management Bureau of Guiyang, 2016). Vehicles are regarded to represent the main source of NO_x-N pollution. A typical example is that the NO_x from vehicle emissions in Guiyang in 2010 accounted for 56.2% of total NO_x emissions (36.6 kt yr^{-1}) (He, 2013; Tian et al., 2013). In the past, low rates of wastewater treatment

(17.2% in 2004 and 20% in 2005) led to substantial NH_3 emissions (Guiyang Environmental Protection Bureau, 2006), which decreased the NO_3^--N/NH_4^+-N ratios (molar ratio) recorded in the N deposited in Guiyang city (Liu et al., 2008b). However, in recent years, the rate of wastewater treatment has increased to over 90% due to the implementation of centralized wastewater treatment (Guiyang Environmental Protection Bureau, 2015). The decrease in NH_3 emissions caused by centralized wastewater treatment and the rapidly increasing NO_x emissions produced by vehicles have therefore caused the N deposited in the Guiyang area to record higher NO_3^--N/NH_4^+-N ratio (molar ratio). For example, the NO_3^--N/NH_4^+-N ratios produced by wet N deposition in 1984 and 2001 were 0.17 and 0.20, respectively (Galloway et al., 1987; Xiao and Liu, 2002). However, this ratio has increased to 0.51 in 2007 (Han et al., 2011). In general, Guiyang city is experiencing rapid industrial expansion and extensive urbanization, which have caused NO_x-N emissions to increase more rapidly than NH_x-N emissions. In 2010, emissions of NH_3 were already 12 times lower than those of NO_x throughout Guizhou Province (Ministry of Environmental Protection, 2011). In addition, the NH_3-N and NO_x-N emission fluxes reported by Qu et al. (2016) were 37.07 kg N ha^{-1} yr^{-1} and 70.56 kg N ha^{-1} yr^{-1} in Guiyang in 2014, respectively.

2.2. Sample collection and treatment

Camphor trees (*Cinnamomum Camphora*) were chosen because they are widely distributed in the southern cities and represent a superior species for urban greening. Leaf, branch bark and bark samples from camphor trees that were approximately 15 years old and approximately 8 m in height were collected in June 2016. The geographical distribution of sampling sites is shown in Fig. 1. Previous moss and camphor leaf samples were collected in April 2006 and June 2009, respectively (Liu et al., 2008b; Wang, 2012). Sampling was conducted in both sunny and cloudy weather. Approximately 5–6 g of mature current-year leaves (with surface areas ranging from 10 to 30 cm^2) were collected from outer branches in the east, south, north, and west directions (from approximately 7 m above the ground). The external surfaces of branch barks (approximately 2 mm thick) were taken from the area beneath each sampled leaf sample (the main branch) using a drawknife. Similarly, the external surface of each bark (approximately 2 mm thick) was collected from a non-weathered area of the trunk at a height of approximately 1.5 m above the ground level. We collected respectively 1–3 representative for each type of sample from each selected tree. During the collection of the above mentioned samples at each site, three replications of each moss sample (mainly *Haplocladium* (*H. microphyllum*); only including a small amount of *Eurohypnum* (*E. leptothallum*) and *Erythrodontium* (*H. plumaeforme* and *E. julaceum*)) were simultaneously also collected on-site. At most of the sampling sites, moss samples were taken from bare rocks without canopy coverage within 1.5 km from trees chosen for sampling. In the city center, each sampling site was situated at least 60 m from main roads and pollutant sources. Plant samples in rural areas were collected at least 200 m from main roads and at least 500 m from fertilized cornfield or rice field. At each site, approximately 100 g of soil samples were taken from the rooting zone at a depth of 0–10 cm. The plant litters and roots were immediately removed. All sampling was performed using plastic gloves, and all samples were placed into labelled plastic bags and stored in a chilled box.

Back in the laboratory, bark and branch bark samples were trimmed to a uniform thickness (approximately 2 mm thick for most of samples). After that, all plant samples were immediately washed and subsequently freeze-dried. Soil samples were dried at 80 °C to a constant weight. Finally, all samples were homogenized using a mortar. All samples were divided into two halves; half of each sample was preserved in liquid nitrogen, and the other half used in this study was stored in a desiccator.

2.3. Chemical analyses

N concentrations (%; dry weight) in samples were determined using a vario MACRO cube elemental analyzer (Elementar, Frankfurt, Germany) with an analytical precision of 0.02% (n = 3). The ^{15}N natural abundance of the samples was analysed using an EA/IRMS system (Flash EA 2000 (Thermo Scientific, Bremen, Germany) connected to a Thermo MAT253 (Thermo Scientific, Bremen, Germany)). The $\delta^{15}N$ values (with an analytical precision of 0.1‰; n = 3) were reported in per mil (‰) relative to air (atmospheric N_2). L-glutamic acid (USGS 40), ammonium sulfate (IAEA-N-1) and potassium nitrate (IAEA-NO3) were used to correct the measured $\delta^{15}N$ values. At least three measurements of each sample were carried out, and each reported data represents the average of these replicated measurements.

2.4. Total atmospheric N deposition estimate

Due to the sampling sites are situated in different geographical locations (including urban, suburban, semi-rural and rural locations), the study area is subdivided into six regions (each 6 km away from the urban center as a region). The total atmospheric N deposition (N_{dep}) within each 6 km from central Guiyang to the rural area was estimated using moss (*H. microphyllum*) N concentrations (N_m), based on the following significant and positive linear relationship between these two parameters (Xiao et al., 2010a): $N_{dep} = -14.03 + 19.23N_m$ ($R^2 = 0.70$, $P < 0.001$).

2.5. Statistical analyses

In order to establish relationships between response and explanatory variables, regression analysis was performed using Origin 9.0 (OriginLab Corporation, Massachusetts, USA). Differences in N chemistry between bio-indicators were examined using two-way ANOVA with Scheffé's post hoc test, and one-way ANOVA followed by a Tukey-HSD test was also used to evaluate differences between groups of the same samples. All statistical analyses were conducted using SPSS 19.0 (SPSS Science, Chicago, USA), and all graphs were plotted using Origin 9.0 (OriginLab Corporation, Massachusetts, USA).

3. Results

3.1. N concentrations of different bio-indicators and soils

The patterns in the N concentrations of different bio-indicators observed along the distance gradient from the city center to the rural area are presented in Fig. 2. The N concentrations in leaves ranged from 1.02% to 2.70%, with an average value of 1.71 ± 0.31% (n = 127). A wide range in N concentrations was detected in branch bark, ranging from 0.13% to 1.99%, with an average value of 0.99 ± 0.31% (n = 125). The N concentrations in bark ranged from 0.32% to 1.56%, and yielded the lowest observed average N concentration value (0.72 ± 0.23%; n = 90) of all of the bio-indicators. Comparing the N concentrations of leaves, bark and branch bark from all the sites reveals that they differ markedly from each other ($P < 0.001$) (Fig. 3). The highest average N concentration value (1.87 ± 0.37%; n = 109) among these bio-indicators was found in moss, with the concentrations of N in tissues ranging between 1.00% and 2.69%. The N concentrations in soils varied widely from 0.09% to 0.58%; however, no significant difference in the average

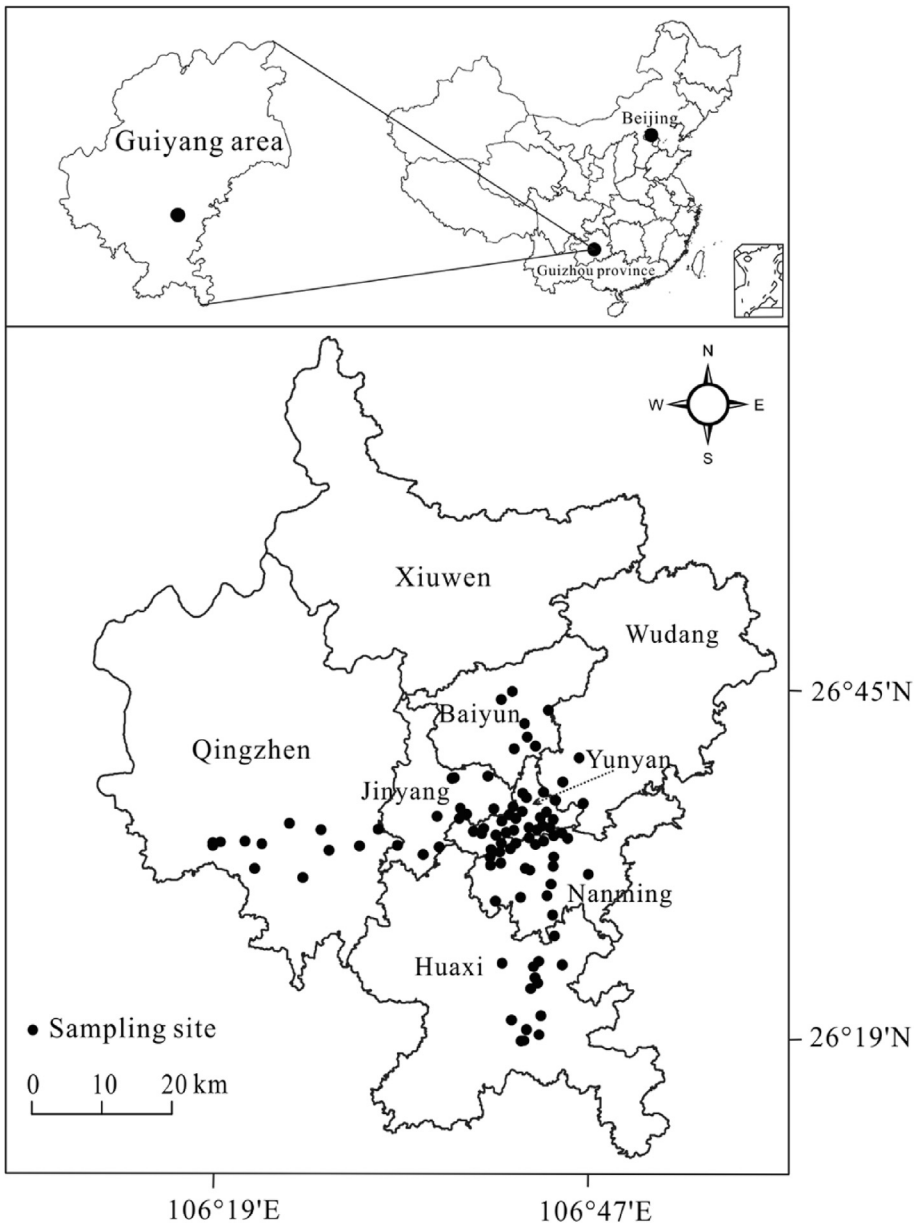

Fig. 1. Map showing the location of the Guiyang area and leaf sampling sites.

values of soil N concentrations was observed within each 6 km from the urban to the rural area ($P > 0.05$) (Table 1).

The general relationship between the N concentrations in all bio-indicators and distance from the city center exhibited a logarithmic decay within 25 km from the city center (Fig. 2). It is noteworthy that slightly higher N concentrations in these bio-indicators were found in sites at distances of greater than 25 km, although this increase is not significant (Fig. 2). The average leaf and bark N concentrations within each 6 km from the urban to the rural area showed a significant linear correlation with the estimated total N deposition ($P < 0.05$), while branch bark samples recorded only a positive trend between N concentrations and the estimated total N deposition ($P > 0.05$) (Fig. 4).

3.2. The $\delta^{15}N$ values of different bio-indicators and soils

The data presented in Fig. 5 show a similar trend, in which the $\delta^{15}N$ values of these bio-indicators decrease with increasing distance from the city center. Leaf, branch bark and bark samples recorded $\delta^{15}N$ values ranging between −4.5‰ and +8.0‰ (n = 127), −1.0‰ and +9.2‰ (n = 125) and −1.5‰ and +9.3‰ (n = 90), respectively. Although large ranges in $\delta^{15}N$ values (−7.5‰ to +5.3‰; n = 109) were also observed within moss samples, the average $\delta^{15}N$ values of leaf, branch bark and bark samples from all sampling sites were significantly higher than those of mosses ($P < 0.001$) (Fig. 3). Branch bark and bark samples were more enriched in ^{15}N than leaves, moreover, we found a lack of significant differences between the $\delta^{15}N$ values of branch bark and bark across all sites (Fig. 3). When analysing the $\delta^{15}N$ values of leaves and branch bark, a significant correlation was found between both their $\delta^{15}N$ values and the moss $\delta^{15}N$ values. Nevertheless, bark samples only exhibited a positive but non-significant trend between their $\delta^{15}N$ values and the moss $\delta^{15}N$ values (Fig. 6).

The measured $\delta^{15}N$ values of all soil samples varied from +1.1‰ to +8.1‰. Although we observed a slight decreasing trend in the average soil $\delta^{15}N$ value from the urban area to the rural area, the

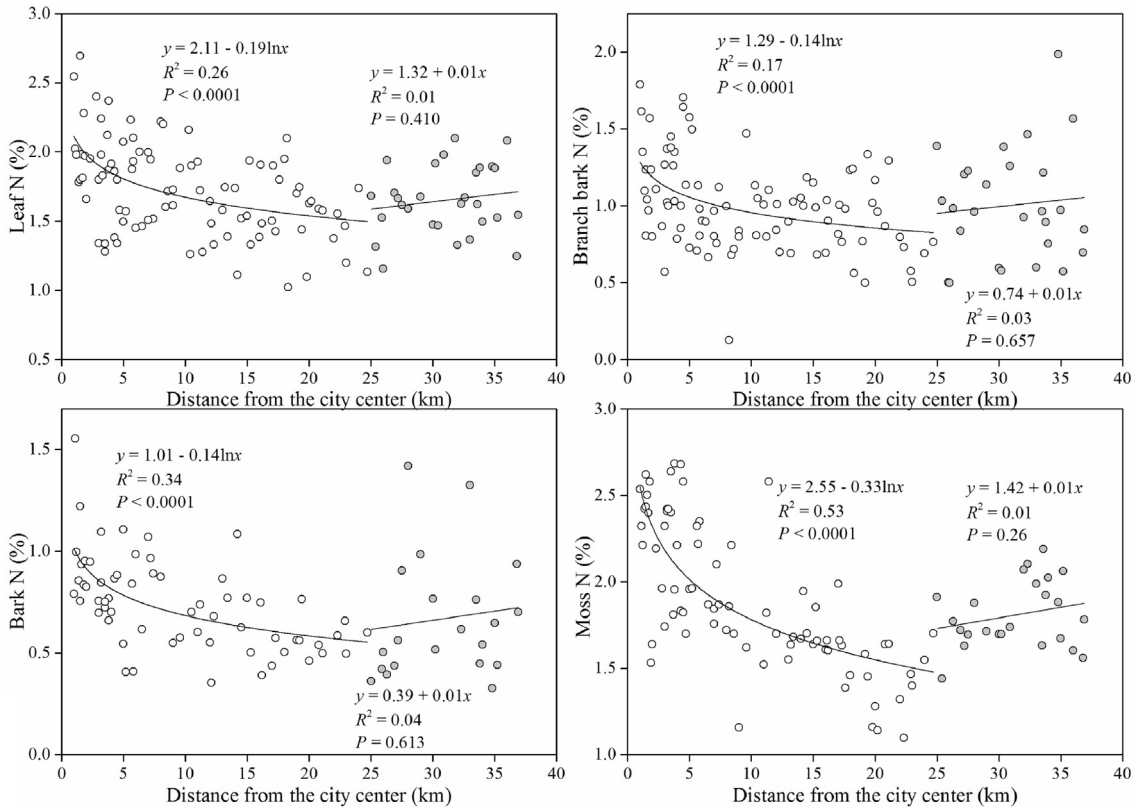

Fig. 2. Variations in N concentrations of leaves, branch bark and bark of camphor trees and mosses with distance from the city center. Unfilled circles represent the data from the area of 0–25 km; gray filled circles represent the data observed in sites over 25 km.

difference between these values is not significant ($P > 0.05$) (Table 1). Therefore, increasing differences between the $\delta^{15}N$ values of soil samples and the $\delta^{15}N$ values of leaf, branch bark and bark samples with increasing distance from the city center can be inferred.

4. Discussion

4.1. N concentrations of different bio-indicators and their relationship to N pollution

Atmospheric N deposition is regarded to be a decisive factor regulating the N concentrations of moss tissues, as areas that receive higher atmospheric N inputs record increasing moss N concentrations (Pitcairn et al., 2006; Pesch et al., 2008; Schröder et al., 2010; Harmens et al., 2011). In this study, moss is therefore considered as an excellent reference material to evaluate the ability of using vascular plant leaves, branch bark and bark to indicate N deposition. In comparison to mosses, which depend on atmospheric N as their main N source, vascular plants can obtain their nutrients from both atmospheric deposition and soils. Since the fractional contribution of atmospheric N to the N concentrations of vascular plant leaves is highly variable compared to that of soils, the variations in N deposition may be recorded by leaf N concentrations. For instance, many other studies reported robust correlations between vascular plant leaf N concentrations and N deposition (Hicks et al., 2000; Pitcairn et al., 2001; Power and Collins, 2010). In this study, no statistical differences in soil N concentrations are observed between sampling areas; therefore, leaf N concentrations significantly decreased with increasing distance from the city center, as did moss N concentrations (Fig. 2), which clearly confirms the feasibility of using vascular plant leaf N concentrations as a bio-indicator of N deposition. Large amounts of vascular plant leaf N concentration data from across all of China were compiled from the published literature by Liu et al. (2013); they found a 32.8% increase in N concentration in vascular plant leaves following an increase of approximately 60% in N deposition from the 1980s (averaged 13.2 kg N ha^{-1} yr^{-1}) to the 2000s (averaged 21.1 kg N ha^{-1} yr^{-1}). In addition, data from a total of 2094 leaf N concentration observations across China were compiled by Han et al. (2005) from previous studies; these data showed that the geometric mean leaf N concentration values were 1.57% for all trees (excluding herbs and shrubs), 1.41% for evergreen trees and 1.79% for broadleaves. In terms of the camphor (broadleaf) leaf N concentrations measured in this study, a geometric mean of 1.68% was computed, which is slightly less than the mean of 1.79% but higher than that of all trees and evergreen trees (Fig. S1). This means that the average level of N deposition in the Guiyang area may be close to the national average level (Fig. S1). According to the analysis of moss N concentrations, the estimated average level of atmospheric N deposition in the study area is approximately 20.6 kg N ha^{-1} yr^{-1}, which is similar to the reported average level of 22.2 kg N ha^{-1} yr^{-1} (derived from deposition monitoring network) in southwest China in the 2000s (Liu et al., 2013). However, it remains concerning that this average N deposition level is well above the critical load of N for some terrestrial ecosystems (5–10 kg N ha^{-1} yr^{-1} for heaths, cryptogams and bogs; 10–12 kg N ha^{-1} yr^{-1} or 3–14 kg N ha^{-1} yr^{-1} for coniferous forests; and 15 kg N ha^{-1} yr^{-1} for many habitats in Europe) (Schulze et al., 1989; Krupa, 2003; Pitcairn et al., 2003; Bobbink and Hettelingh, 2011). Therefore, we can speculate that most of the Guiyang area has been affected by anthropogenic N input to some extent.

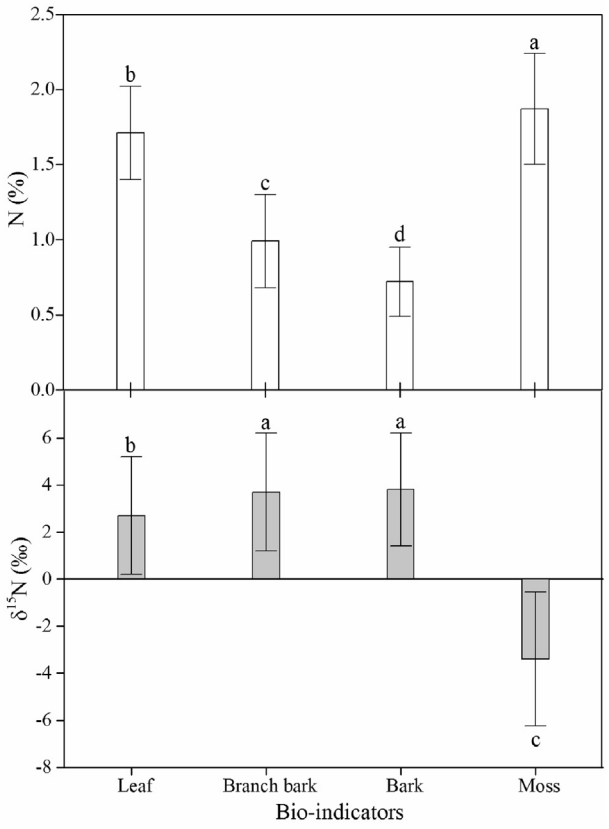

Fig. 3. Average N concentrations and $\delta^{15}N$ values of leaves, branch bark and bark of camphor trees and mosses at all sampling sites. The vertical lines represent standard deviations. Different letters above the bars indicate a significant statistical difference between means of the sample groups at $P < 0.05$.

The average N concentration of the bark samples (0.72 ± 0.23%) was relatively low compared with those measured in 16 areas in Germany (0.91 ± 0.25% for mixed tree species) (Fig. S1); however, these values were almost twice as large as those of Scots pine collected in areas affected by anthropogenic N in South and East Germany from 1988 to 1997 (Fig. S1). The measured N concentrations of oak bark across a N deposition gradient in Scotland were also relatively low (Fig. S1). Several studies conducted in Germany showed that the regional variations in bark N concentrations were positively correlated with elevated atmospheric N deposition, such that areas receiving less agriculture-derived NH_x-N influence can be effectively distinguished from areas receiving higher agricultural NH_3 input using bark N concentrations (Schulz et al., 2001; Boltersdorf and Werner, 2013; Boltersdorf et al., 2014). In fact, available comparisons of tree bark N concentration data are still rare, let alone collect information about branch bark N concentrations in response to N deposition. However, the above discussion clearly indicates that the general high bark N concentrations observed in certain areas may be associated with increasing atmospheric N deposition. Using estimated N deposition data based on moss N concentrations, we found that the tissue N concentrations in leaves and bark were remarkably correlated with N deposition and that branch bark also exhibited a positive correlation between increased tissue N concentrations and N deposition, although this correlation was lack of significance (Fig. 4). This result further strengthens the evidence linking vascular plant tissue N concentrations with N deposition.

Interestingly, in the present study, we also found that the N concentrations in these four bio-indicators differ markedly from each other ($P < 0.001$) (Fig. 3). The differences in the tissue N concentrations of these bio-indicators may be more related to the different N acquisition mechanisms caused by species- and organ-specific. Substantial gaseous and aerosological atmospheric N species (e.g., NO_2, HNO_3, NH_4^+, NH_3 and RO_2NO_2) can generally be absorbed into vascular plant leaves through passive stomatal uptake and cuticular diffusion (Gebauer and Schulze, 1991; Calanni et al., 1999). Atmospheric N compounds absorbed by leaves can then be rapidly transformed into other N forms in order to maintain their metabolism and growth (Nussbaum et al., 1993; Ballmoos et al., 1993; Yoneyama et al., 2003). The lower N concentrations in bark and branch bark samples could be explained as follows. The bark is considered to be almost biologically or chemically inert in the presence of organic and inorganic substances due to the absence of metabolic processes; moreover, the bark surface is very porous, thus making it an excellent passive adsorbent of pollutants which are derived from the atmosphere or from throughfall or stemflow (Schulz et al., 1999; Odukoya et al., 2000). A similar characteristic may also exist in branch bark. However, canopies may be a key controlling factor in regulating the N concentrations of branch bark and bark, because the canopy interception of deposited N may reduce the nutrient supply to the underlying tissues to some extent (Liu et al., 2007). In contrast, mosses, which lack a cuticular barrier and do not have a root system, enable the free exchange of gases and solutions across their cell surfaces, such that they can actively absorb deposited N from the atmosphere, while the absorption rate of nutrients from the substrate is quite low (Turetsky, 2003; Bragazza et al., 2005; Wilson et al., 2009; Gerdol et al., 2014). Mosses as poikilohydric plant thus have a stronger N uptake ability than the other three bio-indicators.

While the N concentrations measured in leaves, branch bark and bark in this study differ markedly from each other, it can be concluded that the correlations between their N concentrations and N deposition (discussed above) further revealed their ability to indicate N pollution. Thus, the consistent patterns of rapid decreases in their N concentrations with increasing distance from the urban center can clearly be explained by the spatial variation in N deposition. In areas located less than 25 km from the urban center, atmospheric N deposition should present a significant declining

Table 1
The average N concentrations and $\delta^{15}N$ values of soils from different sampling areas (minimum and maximum values are given in parentheses).

Areas (km)	Number of samples	Soil N concentrations (%)	Soil $\delta^{15}N$ (‰)
0–6	17	0.21 ± 0.07 (0.11; 0.38) (a)	5.1 ± 1.8 (2.2; 7.0) (A)
6–12	18	0.27 ± 0.15 (0.13; 0.58) (a)	5.0 ± 1.5 (2.9; 8.1) (A)
12–18	16	0.21 ± 0.08 (0.13; 0.40) (a)	3.9 ± 1.6 (1.2; 7.2) (A)
18–24	14	0.21 ± 0.09 (0.10; 0.39) (a)	4.5 ± 1.6 (1.5; 7.3) (A)
24–30	12	0.25 ± 0.10 (0.09; 0.42) (a)	3.8 ± 1.5 (1.2; 6.5) (A)
30–36	10	0.23 ± 0.09 (0.12; 0.42) (a)	3.4 ± 1.5 (1.1; 6.2) (A)

Same letters indicate no significant difference between groups of samples (marked with lowercase letters for soil N concentrations and uppercase letters for soil $\delta^{15}N$ values) ($P < 0.05$).

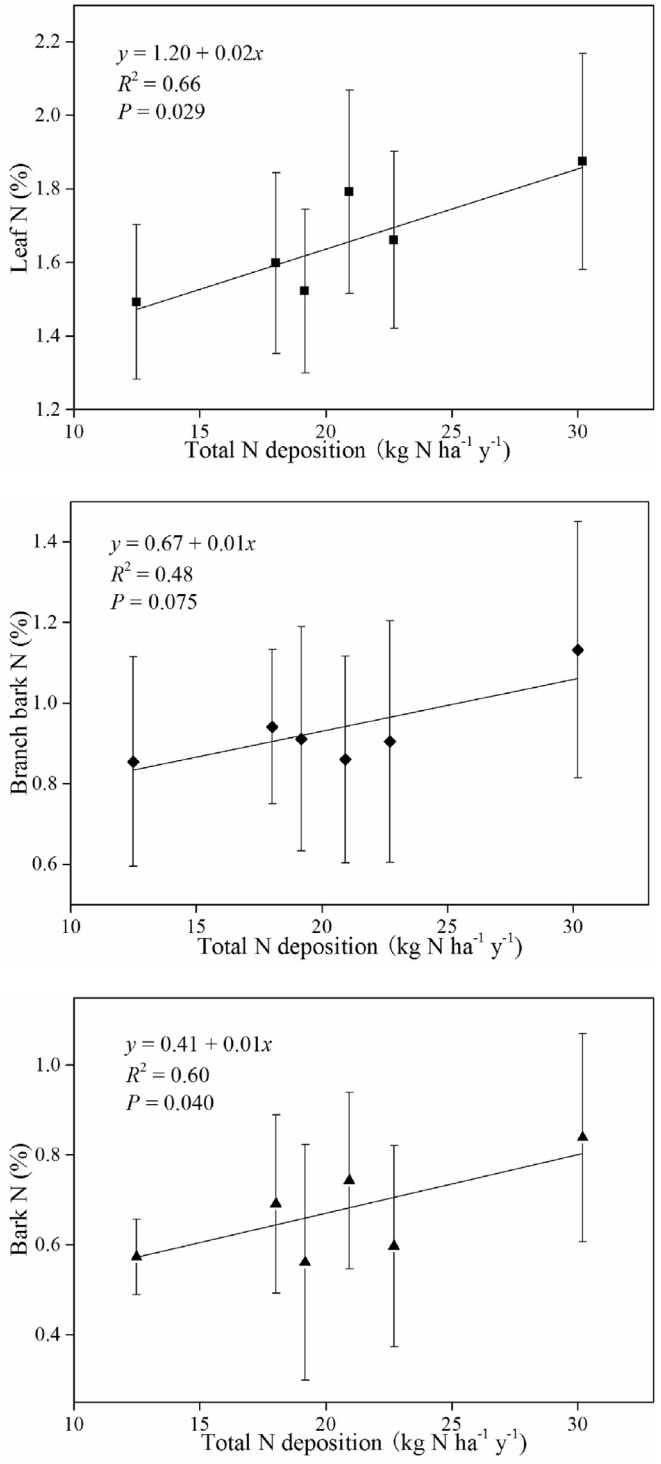

Fig. 4. The relationship between N concentrations of leaves, branch bark and bark of camphor trees and total N deposition derived from moss monitoring. The vertical lines represent standard deviations.

trend with the distance gradient, based on their N concentration patterns, which can also be confirmed by the analysis of moss N concentrations (Fig. 2) and a rapid decrease in atmospheric NO_2 concentration from central Guiyang to the rural area (Table S2). However, the slightly increased N concentrations observed in leaf, branch bark and bark samples in sites located more than 25 km from the city center may be attributed to the elevated N deposition caused by high NH_3 emissions from enhanced agricultural activities in rural areas. Liu et al. (2017) also reported that NH_3 emissions from agricultural activities (e.g., animal wastes, fertilizers and biomass burning) accounted for 61% of the total NH_3 emission, which is similar to the statistical result (more than 57% from livestock, poultry and fertilizer use) of Xiao et al. (2010). Additionally, atmospheric NO_2 concentrations are very low and somewhat similar in sites over 25 km from the city center (Table S2), indicating that the pollution signal (NO_x) may be only evident in urban areas (receiving substantial NO_x emissions from traffic and industry) but has almost disappeared in 25 km distance. Through the above discussion, we can conclude that the different abilities of these bio-indicators to accumulate atmospheric N compounds do not affect the overall trend of changes in tissue N concentrations in response to variations in N deposition. This reflects that leaf, branch bark and bark N concentrations are able to distinguish areas subjected to the influence of excess anthropogenic reactive N input.

4.2. $\delta^{15}N$ patterns of different bio-indicators and source attribution

In many of the forest and agricultural ecosystems that have been studied, plant tissues have been found to record $\delta^{15}N$ values that are lower than those of soil N (Shearer and Kohl, 1986; Nadelhoffer and Fry, 1994; Van Kessel et al., 1994; Kuang et al., 2011). This difference is likely caused by the low N_2-fixing ($\delta^{15}N_{Air}$ values close to zero) capacity and incomplete mineralization of organic soil N in forest soils and the high N loss rates observed in agricultural soils (Handley and Raven, 1992; Gebauer et al., 1994), because major N loss pathways (e.g., denitrification, nitrate leaching and ammonia volatilization) and mineralization can drive long-term ^{15}N enrichment in the remaining soil N (Högberg, 1990). In this study, when comparing the $\delta^{15}N$ values in soil and the three bio-indicators (leaves, branch bark and bark), the highest $\delta^{15}N$ values were also observed in soils, with the exception of branch bark and bark samples that were collected from the city center. This might be induced by the causes below.

On the one hand, as mentioned above, the remaining soil N records strong ^{15}N enrichment because of soil N loss and mineralization. Nitrification can also cause a long-term ^{15}N enrichment of residual N (Högberg, 1990). This means that the products (e.g., ammonium and nitrate) of mineralization and nitrification have lower $\delta^{15}N$ values compared to original soil N. Previous studies have shown that there was little isotope effect during N uptake and assimilation by roots in most natural ecosystems and that mycorrhizal fungi can assimilate isotopically light N to plants (Shearer and Kohl, 1986; Michelsen et al., 1996, 1998; Evans, 2001), so lower plant tissue $\delta^{15}N$ values were found with the absorption of those N products (lower $\delta^{15}N$ values compared to original soil N) by plants. It is important to note that discrimination during N uptake by roots will occur when external N supply is relatively high (e.g., in urban environments) (Evans, 2001). However, under conditions when external N availability is high, processes involved in the assimilation and turnover of N in the soil-plant system were still found to tend to decrease the ^{15}N abundance of the original plant N sources (Ammann et al., 1999; Xiao et al., 2011). Additionally, canopies can largely decrease the input of atmospheric N into the understory environment, and throughfall at the bottom of the canopy is expected to be more ^{15}N-enriched than rainfall at the top of the canopy due to the occurrence of N isotope fractionation during the interception of N deposition by the canopies. A study by Heaton et al. (1997) revealed that NO_3^- washed from the canopy has more positive $\delta^{15}N$ values than that of rainfall and that the $\delta^{15}N$ values of NH_4^+ washed from the canopy are also higher in depositional environments recording high NH_3 concentrations. Similar issues of the discrimination against ^{15}N occurring during the foliar

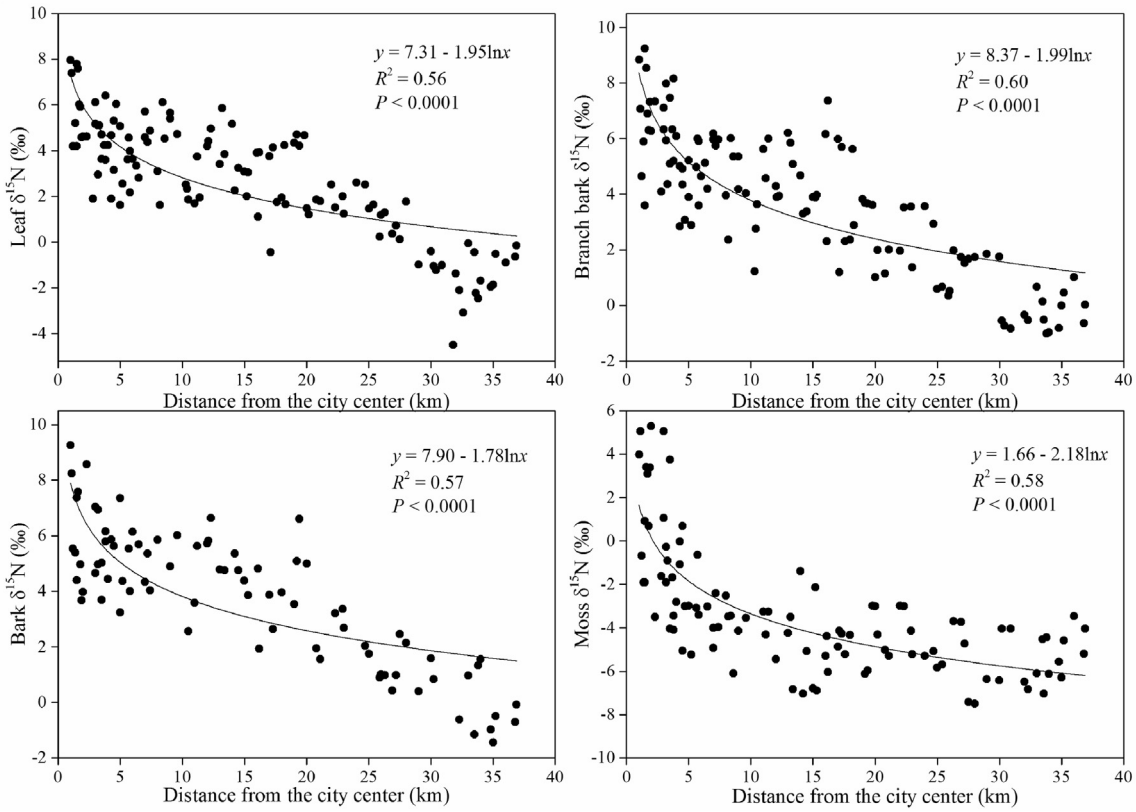

Fig. 5. Variations in the $\delta^{15}N$ values of leaves, branch bark and bark of camphor trees and mosses with distance from the city center.

retention of N deposition were also reported by Stewart et al. (1995) and Handley et al. (1999); thus, higher $\delta^{15}N$ values are found in throughfall, which implies that the N sources of understory environments should be isotopically heavier. Atmospheric N deposition (e.g., NO_x, HNO_3, NH_3, NO_3^- and NH_4^+) can contribute considerable amounts of available plant N sources to canopies. For example, Ammann et al. (1999) reported that only atmospheric NO_2 contributes approximately 25% needle N nutrition at the polluted sites. This also supports the theory that the net canopy exchange of N was largely controlled by the foliar uptake of dry deposition N although N provided by both dry and wet deposition can be incorporated into leaves (Lindberg et al., 1986; Garten et al., 1998). Thus, the deposition of N with differing $\delta^{15}N$ values can create differences in the $\delta^{15}N$ values of different plant parts (Gebauer et al., 1994; Kuang et al., 2011). The slight increase in the $\delta^{15}N$ values from leaves to bark in all sites (Fig. 7) may also be related to the $\delta^{15}N$ values of deposited N that is available for assimilation at the bottom of the canopy. This likely explains the presence of slightly higher branch bark and bark $\delta^{15}N$ values than soil $\delta^{15}N$ values in the city center (receiving a high ^{15}N-enriched NO_x input). It is thus clear that canopies are an important factor in changing the N concentrations and $\delta^{15}N$ values of deposited N (especially for throughfall), which fully agrees with the theory proposed by Handley and Raven (1992) and Wania et al. (2002). These authors found that changes in the $\delta^{15}N$ values of plant tissues were affected not only by N sources with different isotopic compositions but also by the presence of different N acquisition pathways and mechanisms. Therefore, isotopic fractionation occurred in soil-plant systems, and the influence of atmospheric N inputs may be responsible for the relatively low $\delta^{15}N$ values of plant tissues compared with those of soils.

Although the many steps involved in the assimilation and turnover of N mentioned above may finally cause the depletion or enrichment of ^{15}N in vascular plant tissues (Högberg, 1997; Robinson, 2001; Stewart et al., 2002), there is evidence that the $\delta^{15}N$ values of vascular plant tissues were directly related to the N isotopic compositions of atmospheric N pollutants when plants are exposed to heavy air pollution (Stewart et al., 2002; Saurer et al., 2004). The analysis of the N stable isotopic compositions of *calluna vulgaris* leaves across an urban-rural gradient in London also showed evidently greater $\delta^{15}N$ values in leaves in an urban area than those in a rural area, which was attributed to the greater uptake of atmospheric nitrogen oxides by leaves in urban areas (Power and Collins, 2010). Xu and Xiao (2017) also suggested that the more positive $\delta^{15}N$ values observed in needles (*Pinus Massoniana* L.) in urban areas can be linked to emissions of NO_x-N. Similarly, the $\delta^{15}N$ values of bark collected from areas with high inputs of agriculture-derived NH_x-N have been shown to significantly differ from those of bark sampled in non-polluted areas (Schulz et al., 2001; Boltersdorf and Werner, 2013). A recent study conducted by Boltersdorf et al. (2014) in Germany further corroborated that bark samples record more negative $\delta^{15}N$ values in areas receiving relatively high agricultural NH_3 inputs than those in areas that are less affected by agricultural N. In this study, we provide more evidence to demonstrate the value of vascular plant leaves, branch bark and bark as bio-indicators of atmospheric N pollution.

It is well known that mosses can effectively take up deposited N with small isotopic fractionation (Bragazza et al., 2005; Solga et al., 2005). In the present study, a positive correlation between the $\delta^{15}N$ values of leaves, branch bark and bark and those of mosses was observed (lack of a significant isotopic relationship between bark and moss samples) (Fig. 6), which revealed the visible contribution of atmospheric N with different N isotope compositions to the N concentrations of camphor leaves, branch bark and bark (such that their isotopic compositions were changed); moreover, we did not

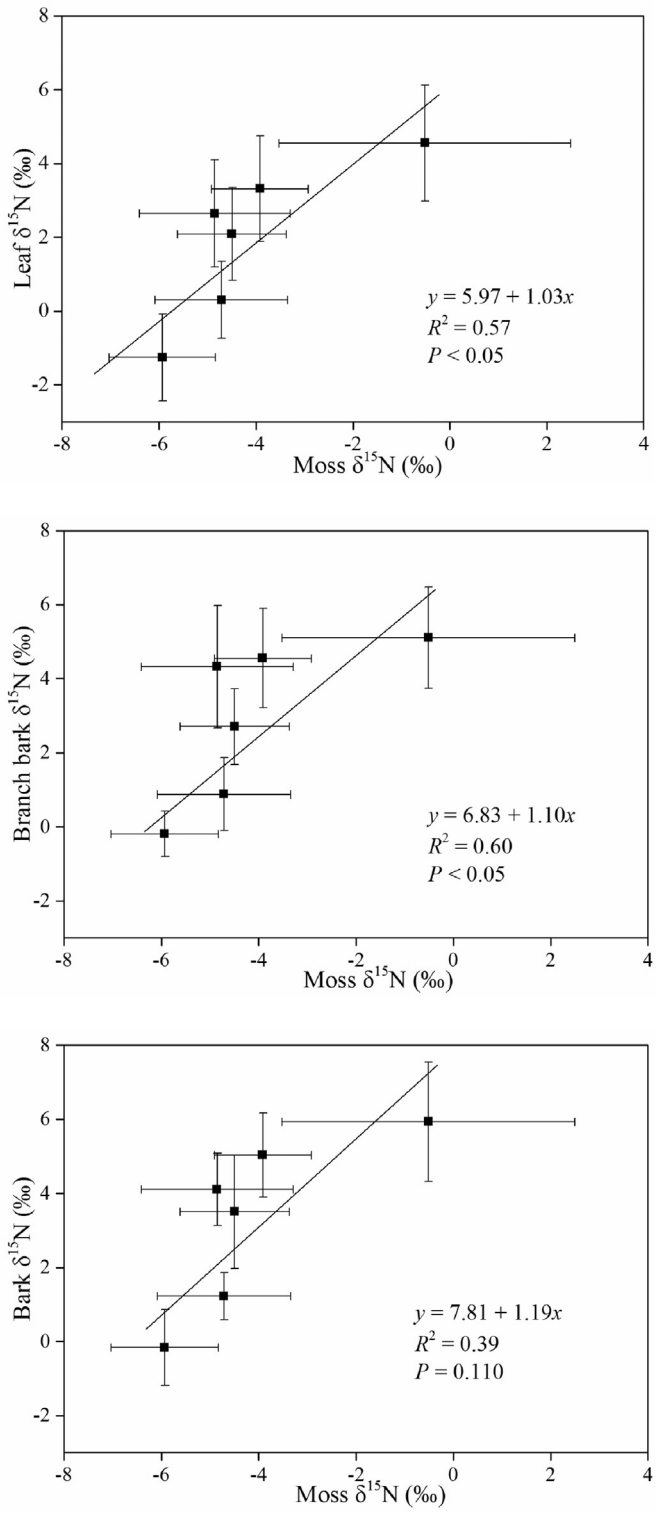

Fig. 6. The relationship between $\delta^{15}N$ values of leaves, branch bark and bark of camphor trees and $\delta^{15}N$ values of mosses. The vertical and horizontal lines represent standard deviations.

found significant differences in both the soil N concentrations and soil $\delta^{15}N$ values in the study areas (Table 1). These findings clearly indicate that the isotope effect occurred in the soil-plant system as discussed above do not cause the significant differences between plant tissue (e.g., leaves or bark) $\delta^{15}N$ values in different sites. Thus,

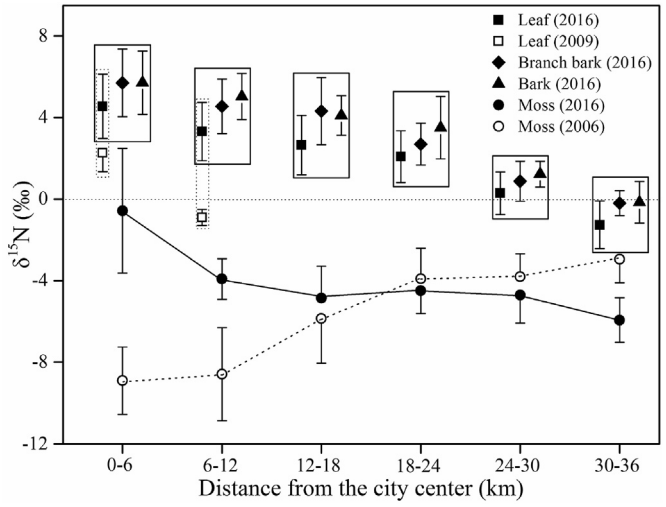

Fig. 7. The patterns of the average $\delta^{15}N$ values of mosses and camphor leaves, branch bark and bark within each 6 km from the urban to the rural area. The vertical lines represent standard deviations. The $\delta^{15}N$ data of moss (collected in April 2006) and camphor leaf (collected in June 2009) samples are cited from Liu et al. (2008b) and Wang (2012), respectively.

the wide ranges in $\delta^{15}N$ values measured within leaves (up to 12.5‰), branch bark (10.2‰) and bark (nearly 11‰) across an urban-rural gradient imply that these bio-indicators are very sensitive to the spatially variable N deposition. Despite canopies were considered to be a controlling factor which may have caused the $\delta^{15}N$ values of branch bark and bark to be relatively higher than those of leaves (because the canopy interception of deposited N can affect the isotopic compositions of N compounds supplied to the underlying environment) (Liu et al., 2007), the fact that these three bio-indicators and mosses (as a reference material) showed almost parallel decreases in $\delta^{15}N$ with increasing distance from the city center (Fig. 5) further suggests that vascular plant leaves, branch bark and bark can be used to map the dominant forms of atmospheric N deposition over much larger areas with the help of isotopic data.

The high traffic densities in urban areas not only contributed substantially NO_x emissions, but also caused an increase in emissions of traffic-derived NH_3 (the increase in emissions is still larger for NO_x than for NH_3) (Liu et al., 2017). However, due to atmospheric NH_3 has a fast deposition velocity (with concentrations falling by 90% within the first 10 m from the roadside) (Cape et al., 2004), the contribution of traffic-derived NH_3 to the $\delta^{15}N$ values of plants which were mainly more than 20 m away from a major road (over 60 m in this study) is relatively small (Power and Collins, 2010). Furthermore, the traffic-derived NH_3 showed relatively negative $\delta^{15}N$ values (Table S1). In contrast, more positive $\delta^{15}N$ values were frequently determined in oxidized N species, which were mainly sourced from traffic and industrial emissions (Table S1). Thus, the higher $\delta^{15}N$ values observed in these bio-indicators in urban areas are the results of exposure to high NO_x-N environments, indicating that N pollution is dominated by ^{15}N-enriched atmospheric NO_x-N. This is supported by moss $\delta^{15}N$ analysis. The average moss $\delta^{15}N$ value of −0.6‰ measured in the city center was higher than that collected from Mt. Gongga (as a station of atmospheric background observation in Southwest China; with an average value of −1.3‰) (Liu et al., 2008a), which reflects that the higher contribution of NO_x-N to N deposition in urban areas. It is interesting to note that the other three bio-indicators had significantly more positive $\delta^{15}N$ values than mosses. Obviously, this difference can be well explained by the

different N acquisition pathways and mechanisms of these bio-indicators as discussed above.

In fact, the current rapid development of the regional economy in Guiyang city has caused NO_x emissions to sharply increase, and traffic NO_x emissions have accounted for more half of the total amount of NO_x emissions (He, 2013; Tian et al., 2013), driving high average atmospheric NO_2 concentrations in urban areas (with average atmospheric NO_2 concentrations of 19 μg m^{-3} in 2006, 34.9 μg m^{-3} in 2010 and 39.1 μg m^{-3} in 2016 (April 4th - July 4th)) (Tian et al., 2013; Guiyang Environmental Protection Bureau, 2007, 2016). The current level of NO_2 concentration was approximately three times higher than that measured in the northern coniferous forests (with a critical level of NO_2 of 10–15 μg m^{-3}) (Manninen and Huttunen, 2000) and was more than two times higher than the background NO_2 level (of 17.3 μg m^{-3}) measured in a low polluted area of London (Carslaw and Carslaw, 2007). Moreover, the implementation of centralized wastewater treatment substantially reduced the main urban NH_3 emission source (e.g., the rate of wastewater treatment was 17.2% in 2004, 20% in 2005 and over 90% in recent years) (Guiyang Environmental Protection Bureau, 2006, 2015). This change in the importance of different N pollution sources over time was also recorded by plant tissue $\delta^{15}N$ values. As shown in Fig. 7, the $\delta^{15}N$ pattern of moss (*H. microphyllum*) collected in 2006 (within 12 km from the urban center) contrasted with that of the current moss samples, thus revealing that the previous urban moss $\delta^{15}N$ values were primarily affected by the ^{15}N-depleted atmospheric NH_x-N derived from untreated wastes and sewage ($\delta^{15}NH_x$-N = −15‰ to −4‰) (Liu et al., 2008b), while the current urban moss $\delta^{15}N$ values were much more controlled by isotopically heavy NO_x-N from traffic emissions. Similarly, the current urban camphor leaves were enriched by 2–4 $\delta^{15}N$‰ units compared with the urban camphor leaves sampled in 2009 (Fig. 7). Therefore, the increasing leaf and moss $\delta^{15}N$ values and significantly positive $\delta^{15}N$ values observed in the branch bark and bark in urban areas not only reflect a proportionally greater influence of NO_x-N but also indicate the presence of temporally increasing sources of NO_x pollution in this area. The atmospheric diffusion of pollutants strongly reduced the concentrations of atmospheric N pollutants (e.g., NO_2 shown in Table S2); therefore, the sites near the urban area were less influenced by the ^{15}N-enriched NO_x-N, such that tissue $\delta^{15}N$ values at these sites may reflect the integrated influence of isotopically heavy and light N deposition. This can efficiently explain the variability of data observed in the combining areas between the urban and rural area (12–30 km).

Reduced N species associated with emissions produced by agricultural activity usually have negative $\delta^{15}N$ values (Table S1). The observed lower $\delta^{15}N$ values in plant tissues are thus typical of rural areas, where isotopically light NH_x-N are dominant (Solga et al., 2006; Boltersdorf et al., 2014). In this study, almost all plant samples collected from the more remote rural area were significantly depleted in ^{15}N compared with those collected from the urban area. Only a few leaf, branch bark and bark samples recorded the positive $\delta^{15}N$ values. A similar pattern in plant tissue $\delta^{15}N$ values was also reported by Pearson et al. (2000) and Power and Collins (2010), who observed the strong ^{15}N depletion of moss and *Calluna* leaves collected from rural areas and the relative ^{15}N enrichment of samples from urban areas. It has been documented that anthropogenic NH_3 emissions accounted for 99.85% of total NH_3 emissions (72.6 kt) in Guiyang city in 2006 and that livestock and fertilizer usage were regarded to be the most important source of anthropogenic NH_3 emissions (Xiao et al., 2010). Thus, it has been concluded that the greater uptake of NH_x-N from agricultural emissions may significantly contribute to the negative $\delta^{15}N$ values of these bio-indicators in rural environments. This biomonitoring result also matched the fact that NO_x emissions from traffic and industrial sources in urban areas contribute higher proportions (over a 2-fold difference) to total reactive N emissions than do NH_x emissions produced by agricultural activities in rural areas (Qu et al., 2016). Indeed, it was also observed that NO_2 levels decreased rapidly moving from the urban area to the rural area (Table S2). Therefore, the spatial patterns of prevailing N sources can be effectively reflected by the $\delta^{15}N$ values of these bio-indicators, showing the higher contributions of NO_x-N to the N deposition of urban areas and the greater importance of NH_x-N to the N deposition of rural areas.

5. Conclusion

This study represents the first attempt to systematically compare the differences in responses to atmospheric N deposition using leaves, branch bark, bark and mosses as bio-indicators. The fact that the N concentrations of these bio-indicators differ markedly from each other indicates that these bio-indicators have different N acquisition pathways and mechanisms. However, the consistent patterns of decreasing tissue N concentrations in these bio-indicators reported here clearly indicate that a decrease in atmospheric N deposition occurs from the city center to the rural area.

Changes in the $\delta^{15}N$ values of plant tissues represent a transformation, to some extent, in the relative contributions of NO_x-N and NH_x-N sources to plant N nutrition. The increasing trend of $\delta^{15}N$ values from leaves to bark suggests that canopy retention is an important factor affecting the input of deposited N into the under canopy environment. Additionally, the presence of significantly elevated $\delta^{15}N$ values in current urban mosses and camphor leaves compared with previously collected samples and the rapid decrease in the ^{15}N abundance observed with increasing distance from the urban center in all of these bio-indicators strongly indicates that proportionally higher tissue N uptake is derived from ^{15}N-enriched NO_x-N in the urban area, whereas agriculture-related NH_x-N deposition is dominant in the rural area. This can also lead to the general conclusion that the $\delta^{15}N$ analysis of branch bark and bark can be used to reflect different atmospheric N pollution sources. However, it is important to note that although the extant isotopic data for atmospherically derived N can be potentially used to distinguish the main N source on-site, some complementary data on reactive N emissions or their atmospheric concentrations still need to be referenced because there may be overlap between the isotopic ranges of NO_x-N and NH_x-N under some complex conditions.

Therefore, although it is complicated to apply different bio-indicators due to their different N uptake strategies, the use of plant species to monitor environmental N pollution is still an effective strategy, particularly mosses with the most favorable N acquisition pathways and mechanisms. Additionally, considering that the N deposition monitoring stations have tended to be scant in current China, the use of bio-monitors clearly offers an easy and low cost alternative to evaluate atmospheric N pollution. Further studies on this topic will likely enhance our knowledge of the impacts of anthropogenic N inputs on N cycles and plant physiology.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.10.005.

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