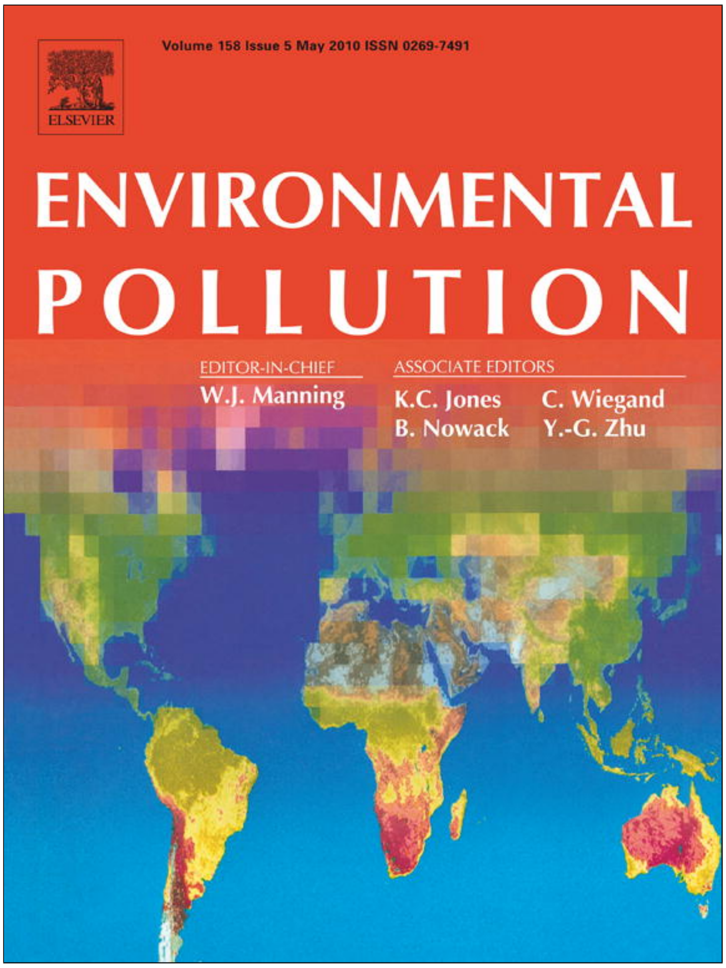
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Homogeneity of $\delta^{15}N$ in needles of Masson pine (*Pinus massoniana* L.) was altered by air pollution

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Values of $\delta^{15}N$ in needles of Masson pine (Pinus massoniana L.) were uneven and affected by air pollution.

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ABSTRACT

The present study investigated the changes of $\delta^{15}N$ values in the tip, middle and base section (divided by the proportion to needle length) of current- and previous-year needles of Masson pine (*Pinus massoniana* L.) from two declining forest stands suffering from air pollution, in comparison with one healthy stand. At the healthy stand, $\delta^{15}N$ in the three sections of both current- and previous-year needles were found evenly distributed, while at the polluted stands, $\delta^{15}N$ values in the needles were revealed significantly different from the tip to the base sections. The results implied that the distribution of $\delta^{15}N$ among different parts or sections in foliages was not always homogeneous and could be affected by air pollution. We suggested that the difference of $\delta^{15}N$ values among pine needle sections should be reconsidered and should not be primarily ignored when the needle $\delta^{15}N$ values were used to assess plant responses to air pollution.

1. Introduction

Nitrogen (N) is an essential nutrient for plant growth and there have been numerous studies investigating the behavior of N in forest ecosystems. Anthropogenic N emission into the atmosphere has increased N deposition dramatically over the last century, resulting from increased fossil fuel combustion and fertilizer use (Erisman et al., 2003; Galloway et al., 2003). Elevated levels of N deposition have inevitably become prominent sources of regional N pollution, which have been attributed to numerous detrimental plant responses and have caused serious consequences for forest health (Jung et al., 1997; Schaberg et al., 2002; Krupa, 2003). Thus, it is crucial to understand processes to determine how N is transformed, used, and recycled in forest ecosystems at the background of globally elevated N deposition.

Different isotopic composition of naturally occurring and man-made N compounds has a distinct $^{15}N/^{14}N$ ratio and could potentially be distinguished by using variations in ^{15}N natural abundance (expressed as $\delta^{15}N$) (Heaton, 1986). Thus, stable isotope of ^{15}N is a useful indicator for investigating biogeochemical mechanisms that govern ecosystem N processes and it has been widely applied to elucidate research questions in environmental and ecological fields (Robinson, 2001). For example, $\delta^{15}N$ in plant tissue has been widely used to reveal long-term responses of forest species to atmospheric deposition (Gebauer and Schulze, 1991; Högberg, 1997; Näsholm et al., 1997; Emmett et al., 1998; Pardo et al., 2001, 2007). So far, most of the studies using foliage N isotope ratio have assumed that the values of $\delta^{15}N$ in different parts (or sections) of a foliage did not differ significantly and considered the whole foliage as a homogeneous sample when being used in isotopic studies or surveys (Nômmki et al., 1994; Buchmann et al., 1995; Högberg, 1997; Jung et al., 1997; Ammann et al., 1999; Pardo et al., 2001, 2007; Choi et al., 2005; Querejeta et al., 2008; Guerrieri et al., 2009). Actually, our previous research confirmed that elements in needles of *Pinus massoniana* were unevenly distributed (Kuang et al., 2007). As a result, the $\delta^{15}N$ values in different parts (sections) of foliage might not be evenly distributed when the plants were affected by air pollution. So far, this hypothesis has not been addressed in studies concerning N-isotope composition in vegetation.

This study was designed to investigate the difference of $\delta^{15}N$ value variation in different sections of needles of Masson pine

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(*P. massoniana* L.) sampled from 2 severely polluted and 1 healthy forest stands, in the Pearl River Delta of south China. The choice of Masson pine in this study was based on three factors: 1) it is one of the most extensively distributed conifer species throughout south China, where it dominates forest species in 18 provinces (Qin, 2002); 2) the high sensitivity of the pine family to acid deposition (Liu, 1996; Huang et al., 1998); and 3) The presently-occurring large scale decline in pine forest in the Pearl River Delta caused by severe acid deposition (Kuang et al., 2008). Over the past few decades, this pine species has been the most widely used for afforestation and reforestation schemes in large areas of south China. Our objectives were to determine:

1) If there are significant differences of $\delta^{15}N$ among different sections of different-aged pine needles; 2) the pattern of $\delta^{15}N$ variation along the pine needles from base to tip; and 3) the relevance of changed patterns of $\delta^{15}N$ distribution along the needle to the N deposition in the forest stands sampled?

2. Materials and methods

2.1. Sites selection and description

Three mixed Masson pine forest stands in the Pearl River Delta of south China, which consist of more than 60 percent broadleaved trees were selected for sampling pine needles, based on our recent research results (Kuang et al., 2008). The stands were growing in laterite soils developed from granite and sand shale under subtropical monsoon climates. At two of the forest stands, Xiqiaoshan (112°58′E, 22°55′N, XQS) and Dinghushan (112°10′E, 23°10′N, DHS), there were large number of pine trees with a substantial proportion of yellow needle and heavy needle loss (in particular at XQS) due to atmospheric pollution, which were categorized as polluted sites. It was reported that there were more than 90 ceramic factories and 300 burning kilns around XQS (Ma, 2002). Emissions from the ceramic industries caused severe damages on the vegetation (Wen et al., 2006). Atmospheric pollutants at DHS transported mainly by the southwest prevailing wind in the summer from the industrial areas in the Delta (Qi et al., 2001) decreased photosynthetic pigments and photochemical efficiency of pine needles there (Yu et al., 2005). The third stand, Nankunshan (113°51′E, 23°38′N, NKS), was sampled as a healthy stand, where pine trees generally had full crown and green needles. Details on the atmospheric pollution at the three stands were described previously and pine forests decline at XQS and DHS were confirmed recently by tree-ring analysis (Kuang et al., 2008). The co-dominant broadleaf species were *Schefflera octophylla*, *Schima superba*, *Machilus chinensi*, *Cinnamomum camphora*, *Aporosa dioica* at XQS, *S. octophylla*, *S. superba*, *Castanopsis chinensis*, *Randia canthioides* at DHS, and *S. superba*, *C. chinensis*, *Aporosa yunnanensis*, *Broussonetia papyrifela* at NKS (Fig. 1).

2.2. Sampling and processing

Five pine trees (>40-year-old) per stand were selected by a distance of at least 100 m away from each other, which were visually assessed as the representative across the stand. Needles were collected from the sample trees at the end of the growing season by shooting down two well-exposed branches from the upper sun-crown. At DHS and NKS, current year (C) and previous-year (C + 1) needles were sampled, but at XQS, only were C needles obtained because of the severe defoliation of older needles induced by air pollution from industrial resources in the nearby. All needles were rinsed in deionized water and dried at 75 °C. After drying, each fascicle of the same-aged needle was divided into three sections (tip, middle, and base section) proportional to the needle length. In order to exclude the impact of sheath on the needle $\delta^{15}N$, the sheaths on the needles were carefully removed prior to the division. The same sections from the same-aged needles (per tree) were pooled as one sample. The dried samples were ground to pass 2 mm sieve and stored in a desiccator before analysis.

2.3. Analytical procedures

Bulk N isotope ratios were measured on a Finnigan isotope mass spectrometer (Delta Plus XL, ThermoFinigan, USA) coupled to a CE flash[1112] EA via a ConfloIII interface. High purity N_2 reference gas was run with each analysis. Three to five replicate measurements per sample were carried out, and values were averages of the replicated measurements. The analytical precision (SD, $n = 3$) for $\delta^{15}N$ was ±0.2‰. Isotope data were reported as $\delta^{15}N$ values, which calculated according to the following equation:

$$\delta^{15}N = \left[\frac{R_{sample}}{R_{standard}} - 1\right]1000$$

Where R_{sample} represents the isotope ratio ($^{15}N/^{14}N$), and $R_{standard}$ is $^{15}N/^{14}N$ for atmospheric N_2.

All experimental analyses were performed at the State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences.

2.4. Statistical methods

Effects of forest stand, needle section and needle age and their interactions on N isotope ratios were evaluated with Univariate ANOVA using the SPSS statistical program. One-way ANOVA was performed to compare the difference of isotope

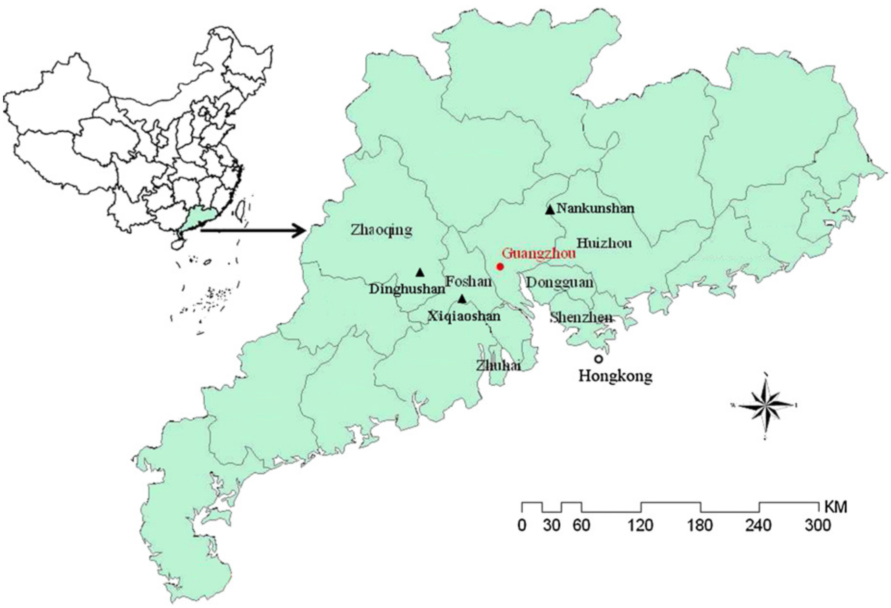

Fig. 1. Locations of study sites in the Pearl River Delta, south China.

Table 1
Results of analysis of variance for effects of stand, needle age, needle section and the interactions on δ^{15}N. df = degrees of freedom, F = F values based on Univariate ANOVA, p = probabilities. $p < 0.10$ was assumed to be significant.

Source	df	F	p
Stand	2	327.34	0.000
Needle age	1	1.24	0.271
Needle section	2	9.90	0.000
Stand × age	1	0.20	0.657
Stand × section	4	1.56	0.20
Age × section	2	0.25	0.783
Stand × age × section	2	0.28	0.758

values in different needle sections from the same age class at each stand. Effects with probabilities of $p < 0.10$ and $p < 0.05$ were considered to be significant and extremely significant, respectively.

3. Results

The main effects of stand, needle age, needle section and their interactions on isotope ratios were summarized in Table 1. Forest stand and needle section had significant effects on the dependent variable analyzed. Effects of needle age and interactions between stand and age, stand and section, age and section, however, were not found significantly for the δ^{15}N values. Needle δ^{15}N were mainly typical of individual forest stands and needle sections. Thus, further determination of the effects of the needle section and stand on the isotope ratios was very necessary.

Values of section δ^{15}N along the needles at the three forest stands were presented in Table 2. Needle section δ^{15}N values at DHS and XQS were completely different from those at NKS. At the two polluted stands DHS and XQS, needle δ^{15}N values were negative, while at the relatively healthy stand NKS, the values were positive. Patterns of the section δ^{15}N values along the needle did not differ between the polluted stands and the healthy stand, with the same trend decreasing from tip to base section of the needles. At DHS, δ^{15}N values were −2.22 to −1.40‰ for tip, −2.53 to −1.70‰ for middle and −2.66 to −1.99‰ for base section in C needles, and −2.03 to −0.90‰ for tip, −2.20 to −1.20‰ for middle, −2.60 to −2.03‰ for base section in C + 1 needles. At XQS, section δ^{15}N values patterned similarly with the ones at DHS although only C needles were analyzed. However, the section δ^{15}N values at XQS were found more negatively than the corresponding sections at DHS, with the values ranging from −3.09 to −1.79‰ for tip section, −3.58 to −2.40‰ for middle section, and −3.78 to −3.39‰ for base section. Mean values of the δ^{15}N in tip section differed significantly from the ones in base section at the polluted stands DHS and XQS. In contrast, section differences in the mean δ^{15}N value at NKS were not revealed statistically different for both C and C + 1 needles, though the values also decreased from 1.41‰ in tip section to 0.20‰ in base section.

Table 2
Mean values and standard deviations (S.D.) of δ^{15}N in the needle sections of each age category at the stands. C + 1 needles from XQS were not available because only C needles were remaining on the branches at the time of sampling. Difference of isotope values among the needle sections in the same age at each stand was obtained by One-way ANOVA. Effects with probabilities of $p < 0.10$ and $p < 0.05$ were assumed to be significant and extremely significant, respectively.

Stand	Section	N	Mean value and S.D. (‰)	
			C needle	C + 1 needle
DHS	Tip	5	−1.82 ± 0.45a*	−1.51 ± 0.45A
	Middle	5	−2.13 ± 0.33ab	−1.75 ± 0.46A
	Base	5	−2.35 ± 0.32b	−2.41 ± 0.24B
	p value		0.09	0.01
XQS	Tip	5	−2.46 ± 0.55a	–
	Middle	5	−2.98 ± 0.62ab	–
	Base	5	−3.01 ± 0.65b	–
	p value		0.01	–
NKS	Tip	5	0.93 ± 0.40	1.01 ± 0.40
	Middle	5	0.87 ± 0.55	0.97 ± 0.58
	Base	5	0.73 ± 0.61	0.83 ± 0.57
	p value		0.89	0.92

*The significant differences among the sections along the same-aged needles were marked with different letters after the Mean ± S.D.

4. Discussion

Atmospheric N deposition has affected forest ecosystems in the Pearl River Delta for a long term (Fang et al., 2006). The relative importance of different sources of N for the plant N budget can be derived at the leaf level by using the N isotope (Siegwolf et al., 2001). In this study, we first explored the potential of using needles δ^{15}N of Masson pine to indicate the difference of plant-available N among the stands. Significant differences of the needle δ^{15}N values were observed from the relatively healthy and the polluted stands (Table 1). We found that needle δ^{15}N at the NKS were much higher than those at XQS and DHS. This might be induced by two possible causes below. First, the differences of N inputs into the forest system. The polluted stands in this study (DHS and XQS) were severely affected by intensive industrial N emissions in the Pearl River Delta since the 1980s. The mean annual N wet deposition was reported to be 35.57 kg hm^{-2} a^{-1} in 1989–1990 (Huang et al., 1994) and 38.40 kg hm^{-2} a^{-1} in 1998–1999 (Zhou and Yan, 2001) in the geographic region. While NKS, far away from the industrial pollution source, was hardly affected by atmospheric pollution. In industrial areas, compounds of nitrogen oxides (NO_X) and ammonia gases (NH_X) contribute a significant N input via dry and wet deposition to plant and soils, and to the gaseous uptake by the plant stomata. Nitrogen oxides have been revealed as the dominant component in the wet N deposition at DHS for many years (Fang et al., 2008). As a result, the decrease δ^{15}N at the polluted stands sampled in the present study may have resulted from increased input of isotopically light N (NO_X) from the atmosphere, because increased atmospheric deposition of isotopically light N could decrease foliage δ^{15}N values (Gebauer et al., 1994; Poulson et al., 1995). The other possible cause of needle δ^{15}N decrease at the polluted stands is changes in plant-available N in the soil. Changes in the isotopic composition of available N sources and the relative proportion of N taken up from different sources lead to changes in needle δ^{15}N (Nadelhoffer and Fry, 1994). In this study, values of δ^{15}N in the topsoil (0–5 cm mineral layer) at NKS (+6.9 to +7.5%) were much higher than those found at DHS (+2.10 to +2.56‰) and XQS (−1.7 to −0.65‰) (*data unpublished*), implying that heavier ammonium (NH_X) were more incorporated into the ecosystems at NKS, which increased the δ^{15}N of the tree N pools either directly through root uptake or indirectly after microbial recycling. Variations in δ^{15}N in the soil–plant system have been related to fractionation during the transformation from soil N to plant-available N, or during N absorption, or N fractionation in the plant (Gebauer and Schulze, 1991).

The behavior of N in forest ecosystems has been of particular concern due to the increasingly large amounts of N delivered to the ecosystems by atmospheric deposition. Trees not only passively absorb considerable amounts of gaseous and aerosolic N deposition (such as NO_X) through the stomates, but also take up N such as ammonium and nitrate ions by the roots when those compounds were deposited to the soil. The isotopic ratios of various N sources varied depending on the isotopic fractionation processes that were associated with soil N transformations (Mariotti et al., 1982). Therefore, the δ^{15}N value of a plant sample was primarily determined by the isotope ratio of the N source in numerous studies. However, it is generally accepted that δ^{15}N values of plant or soil samples could not be used as sole indicators of specific processes because the values are affected not only by N-isotope composition of external sources but also by several N isotope fractionation processes associated with N dynamics in the soil–plant system (Nadelhoffer and Fry, 1994; Högberg, 1997; Chang and Handley, 2000; Choi et al., 2003).

Based on the available data suggesting that there was little fractionation during N uptake by roots (Shearer and Kohl, 1986) and during other translocation processes within plants, e.g. during reabsorption and retranslocation of N from senescing and older age-classed of foliage (Garten, 1993), many researchers used δ^{15}N as indicators of long-term plant responses to atmospheric deposition, with the assumption that δ^{15}N values in different parts (sections) of a foliage were homogeneous. In the present study, we revealed two important points: 1) at the polluted stands (DHS and XQS), values of δ^{15}N in the base section were significantly lower than those in the tip section, suggesting an non-uniform distribution of δ^{15}N across the foliage; and 2) at the relatively healthy stand (NKS), section values of δ^{15}N in both C and C + 1 needles were evenly distributed from tip to base of the needle, which is consistent with findings of some researchers (Shearer and Kohl, 1986; Garten, 1993). A possible explanation to these different patterns between the pine forest stands was not just N absorption (reabsorption) from soil to plant or from atmosphere to plant and the N translocation (retranslocation) from the base section to tip section, but also N fractionation was affected by air pollution. Our results confirmed that the distribution of δ^{15}N was uneven along the needle and was affected by pollution. Thus, the future use of foliage δ^{15}N as an indicator of plant responses to N deposition from industrial emission should be reconsidered and the δ^{15}N difference in different needle parts (sections) should not be primarily ignored, particularly in the case when the forests were adversely affected by air pollution. The reasons responsible for the variation of δ^{15}N in the needles may be related to many factors, which should be further investigated in future studies.

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