Nitrogen stable isotopes composition in soil, plant leaves and response to seasonal changes along an urban-rural environmental gradient

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Thành phần đồng vị ổn định nitơ trong đất, lá cây và phản ứng với sự thay đổi theo mùa dọc theo khuynh độ môi trường thành thị - nông thôn

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ABSTRACT

Nitrogen stable isotopes ($\delta^{15}N$) abundance provides useful information on nitrogen input, transformation, and output, which indirectly reflect the characteristics of nitrogen cycling in terrestrial ecosystems. Here, our primary objectives were to: (i) examine how seasonal changes affect the $\delta^{15}N$ values in various soil depths, as well as in tree leaves and (ii) compare differences among three forest sites (Shushan National Forest Park_DSS), Zipengshan National Forest Park_ZPS, and Wanfoshan National Forest Park_WFS) representing urban, suburban, and rural environmental conditions in Anhui Province, China. Soil and plant foliar samples were collected seasonally from Quercus acutissima Carruth. forests among the three sites from July 2017 to June 2018. The study results revealed that: (1) There was a significant difference in the $\delta^{15}N$ values between the 0-10 cm and 10-20 cm soil depths in all three experimental sites. (2) The seasonal change trends of $\delta^{15}N$ in DSS at 0–10 cm soil depth demonstrated that values were highest in fall 2017 and lowest in summer 2017, whereas values at ZPS and WFS sites were highest in summer 2017 and lowest in spring 2018. At 10-20 cm soil depth, DSS and ZPS had maximum $\delta^{15}N$ values in Fall 2017 and lowest in summer 2018. (3) The $\delta^{15}N$ values in both young and mature leaves were notably greater at the urban sites than those at the suburban and rural sites. The findings from this study revealed that seasonal and site variation significantly increased the Nitrogen stable isotope composition.

Keywords:
$\delta^{15}N$, site variation, seasonal variation, urban to rural gradients.

TÓM TẮT

Sự phong phú của các đồng vị ổn định Nitơ ($\delta^{15}N$) cung cấp thông tin hữu ích về đầu vào, sự biến đổi và đầu ra của nitơ, phản ánh gián tiếp các đặc điểm của chu trình N trong hệ sinh thái trên cạn. Ở đây, mục tiêu chính của chúng tôi là: (i) kiểm tra xem những thay đổi theo mùa ảnh hưởng như thế nào đến giá trị $\delta^{15}N$ ở các độ sâu tầng đất khác nhau (0-10 và 10-20 cm), cũng như trong lá cây và (ii) so sánh sự khác biệt giữa ba khu rừng (Công viên rừng Quốc gia Dashushan_DSS), Công viên rừng Quốc gia Zipengshan_ZPS và Công viên

Từ khóa:
$\delta^{15}N$, biến đổi theo mùa, biến đổi theo khu vực, khuynh độ từ thành thị đến nông thôn.

rừng quốc gia Wanfoshan_WFS) đại diện cho các điều kiện môi trường thành thị, ngoại ô và nông thôn ở tỉnh An Huy, Trung Quốc. Các mẫu đất và lá thực vật được thu thập theo mùa từ rừng Sồi giữa ba địa điểm nghiên cứu từ tháng 7 năm 2017 đến tháng 6 năm 2018. Kết quả nghiên cứu cho thấy: (1) Có sự khác biệt đáng kể về giá trị $\delta^{15}N$ giữa các độ sâu tầng đất 0-10 cm và 10-20 cm ở ba địa điểm thí nghiệm của chúng tôi. (2) Xu hướng thay đổi theo mùa của $\delta^{15}N$ trong DSS ở độ sâu tầng đất 0–10 cm chứng minh rằng giá trị cao nhất vào mùa thu năm 2017 và thấp nhất vào mùa hè năm 2017, trong khi giá trị tại các địa điểm ZPS và WFS cao nhất vào mùa hè năm 2017 và thấp nhất vào mùa xuân năm 2018. Ở độ sâu tầng đất 10-20 cm, DSS và ZPS đều có giá trị $\delta^{15}N$ cao nhất vào mùa thu năm 2017 và giá trị thấp nhất vào mùa hè năm 2018. (3) Giá trị $\delta^{15}N$ ở cả lá non và lá thành thục đều lớn hơn đáng kể ở các khu vực thành thị so với các khu vực ngoại ô và nông thôn. Những phát hiện từ nghiên cứu này cho thấy sự thay đổi theo mùa và địa điểm khu vực đã làm tăng đáng kể thành phần đồng vị ổn định Nitơ.

1. INTRODUCTION

Today, around 50% of the global's population resides in urban areas, and that percentage is expected to reach 60% by 2025 [1]. This indicates that urbanization is on the rise worldwide. Biodiversity, ecological productivity, and biogeochemical cycles are all impacted by urbanization [2]. It also indirectly influences ecosystems across a variety of scales by altering abiotic environmental conditions, including atmospheric chemistry [3], climate, and soil properties [4]. However, urban regions are among the least known of all ecosystems and have received little attention from general ecological studies [5]. Urban areas are known to be the source of heat, air pollution, and greenhouse gases that are causing regional and global environmental change [6]. The air temperature, atmospheric CO_2 concentration, and nitrogen deposition are all higher in urban areas than in rural environments [7]. These factors are well acknowledged to be significant forces for global climate change. Remaining ecosystem patches in an urban-rural environmental gradient may be used as a natural laboratory to study how plants and ecosystems react to environmental changes [8]. Many ecosystem functions, such as primary productivity, have been observed to alter systematically along an urban to the wildland gradient [9]. Compared with the developed countries in Europe and America, China's urbanization process started relatively late. However, the development is fast, as of 2014, the permanent urban population in China has increased from 170 million – at the beginning of reform and opening up – to 730 million [10, 11].

Urbanization is the predictable trend of human social as well as economic development, but the development of urbanization brings great pressure and challenges to the construction of the urban ecological environment [12]. Urban forests are frequently disturbed and destroyed, causing the decline of forest ecological function, due to the intensification of human activities. Urbanization changes the distribution of water and heat in cities, so that temperature, humidity, and other ecological factors in the urban, suburban, and rural ecological interface result in the formation of a corresponding increasing or decreasing gradient [13]. At these interfaces, the most prominent gradients include increases in air temperature, precipitation, atmospheric CO_2, and nitrogen (N) deposition [14]. It changes the carbon and nitrogen cycle and nutrient balance through the process of nitrogen mineralization in the urban forest soils [15]. The development of synthetic nitrogen fertilizer, burning of fossil fuels, and expansion of animal husbandry have led to an increase in atmospheric nitrogen which ultimately saturates the water bodies and terrestrial ecosystems [16, 17]. Concerns associated with nitrogen saturation have attracted extensive attention from scientists.

Although increased N deposits have the potential to boost overall agricultural productivity, excess N may harm terrestrial ecosystems by eutrophication of water sources, causing soil to become acidic, and reducing biodiversity [18]. Typically, nitrogen deposition components come from sources of ammonia or nitrogen oxide that are transported through air and the atmosphere. As a result of controls being implemented in numerous locations, NO_X emissions have been reduced including North America, Europe, and China. However, NH_3 emissions have not been highly regulated globally, although ammonium is the main source of inorganic N deposition [19, 20]. NH_3 emissions that are linked to operations involving fossil fuels and agriculture have distinct isotopic fingerprints of nitrogen. The natural abundance of ^{15}N (expressed by ^{15}N) of NH_4^+ in precipitation can help identify and quantify atmospheric NH_3 sources [21, 22].

In addition to internal N cycling via mineralization, nitrification, leaching, gaseous losses [23], natural ^{15}N abundance is also influenced by extrinsic factors such as fertilization [24], seasonal changes [21], climate [25], soil depth, and urbanization [26]. To analyze the effects on forest soil N nitrogen isotopes, it is required to examine the impact of urbanization and seasonal variation in various regions.

The following were the specific objectives of this study: (i) to examine how seasonal variations influence the $\delta^{15}N$ values in various soil depths, as well as in tree leaves and (ii) to compare differences among three forest sites (i.e., Dashushan National Forest Park_DSS), Zipengshan National Forest Park_ZPS, and Wanfoshan National Forest Park_WFS) representing urban, suburban, and rural environmental conditions in Hefei city, Anhui province, China.

2. RESEARCH METHODS

2.1. Study area

This experiment was conducted in the natural secondary forests of Hefei City, Anhui Province. Hefei is the capital of Anhui Province, China. The population increased rapidly from 5.0 million in 2009 to 8.0 million in 2018. Hefei city had 2.34 million motor vehicles and the number of motor vehicles increased almost 9-fold between 2006 and 2018. The research region has a subtropical monsoon climate with a mild winter. The mean monthly winter temperature is 8°C in January, followed by a warm spring with a mean temperature of 23°C in April. Summer is hot with a mean temperature of 34°C in July, which in autumn drops to 23°C in October. The nearest meteorological station, which is in Hefei city, Anhui province, recorded annual average air temperatures of 15–16°C and annual average precipitation of 1100 mm .

Three typical sample plots of *Quercus acutissima* forests were selected in the urban area of Hefei (Dashushan National Forest Park), suburban area (Zipengshan National Forest Park), and rural area (Wanfoshan National Forest Park) as the research sites. The sampling area extends from Hefei central to Wanfoshan of Southwest Anhui province and it is separated into three parts from Hefei central (0-10 km, 10-40 km, > 40 km). The basic information of the sample plots is shown in Table 1.

Table 1. Fundamental characteristics of sampling plots in Dashushan National Forest Park, Zipengshan National Forest Park, and Wanfoshan National Forest Park

Characteristics	Study sites		
	DSS	ZPS	WFS
Mean altitude (m)	120	155	169
Mean slope (º)	12.5	17.5	20.3
Oak tree density (stem hm^{-1})	700±38	615±30	675±32
Oak tree height (m)	14.42±1.14	19.7±0.41	15.35±1.56
Oak diameter at breast height (cm)	24.63±2.27	33.86±2.29	21.01±2.50
Age of Oak forests (year)	120	175	150
Oak canopy density (%)	62±5.5	69±6.8	67±6.1

Notes: DSS, Dashushan National Forest Park; ZPS, Zipengshan National Forest Park; WFS, Wanfoshan National Forest Park. Values are means ± standard deviation (SD)

2.2. Field sampling and measurements

This study was carried out from July 2017 to June 2018. Representative natural secondary forests of *Quercus acutissima* with similar tree age, diameter at breast height, tree height, and canopy density (soil type is yellow-brown soil) were selected in the three study areas as research objects. Three plots (20m×20m) were initially established in each study site, and then four subplots (2m×2m) were set up randomly in an "S" shape in each plot. All sampling plots were at least 100 m from the roads. The soil samples were collected at two different depths (0-10 cm and 10-20cm) using a soil drilling sampler (5 cm diameter). The sampling time was July (summer) and October (autumn) in 2017 and March (spring) and June (summer) in 2018. No samples were taken in January 2018 because of continuous low temperatures and snowfall. In total, 288 soil samples were obtained throughout the study. Soil samples were air-dried naturally, and stored at room temperature. Roots and gravels were removed and sieved with a 2-mm mesh screen before being used for the determination of nitrogen stable isotopes (δ^{15} N).

In June 2018 (summer) fresh Oak leaves were collected and processed. At each location, four mature Oak trees without any symptoms of injuries and pathogen and insect infestation were cut down at the base. These trees were about one hundred years old. In total, 72 leaf samples were obtained throughout the research.

2.3. Measurements of δ^{15}N

Soil and leaf samples for δ^{15}N determination were analyzed at the laboratory of soil analysis, Anhui Agriculture University using a DELTA V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Each analysis used a high-purity N_2 reference gas, and each analysis was repeated three times. Analytical precision (the standard deviation) for δ^{15}N was less than ±0.2‰. The stable isotopic composition's ^{15}N values were expressed in δ notation as the deviation from standards in parts per thousand (‰).

$$\delta^{15}N‰ = \left[\frac{R_{sample}}{R_{standard}} - 1\right] x\ 10^3$$

where,

R is the ratio $^{15}N/^{14}N$;

and the $R_{standard}$ value is based on atmospheric air nitrogen.

2.4. Statistical analysis

One-way ANOVA was performed to compare soil and leaf δ^{15}N in different urban-rural environmental gradients. Tukey's post hoc test was applied to compare significant differences between sampling sites and among four seasons at each soil depth. Statistically significant differences were defined as $p \leq 0.05$.

Prior to ANOVA, data normality and homogeneity of variance were tested. All the statistical analyses were carried out using the statistical software R for Windows Version 4.2.0 (R Development Core Team, 2022).

3. RESULTS

3.1. Soil $\delta^{15}N$ values

There were statistically significant differences ($p \leq 0.05$) in the $\delta^{15}N$ values between the two different soil depths (0-10 cm and 10-20 cm) in the same site (i.e., DSS, ZPS, and WFS) (Fig. 1). At the 0-10 cm soil depth, values of $\delta^{15}N$ were significantly lower ($p \leq 0.05$) than those in the 10-20 cm soil depth, and this tendency was observed at the three sites.

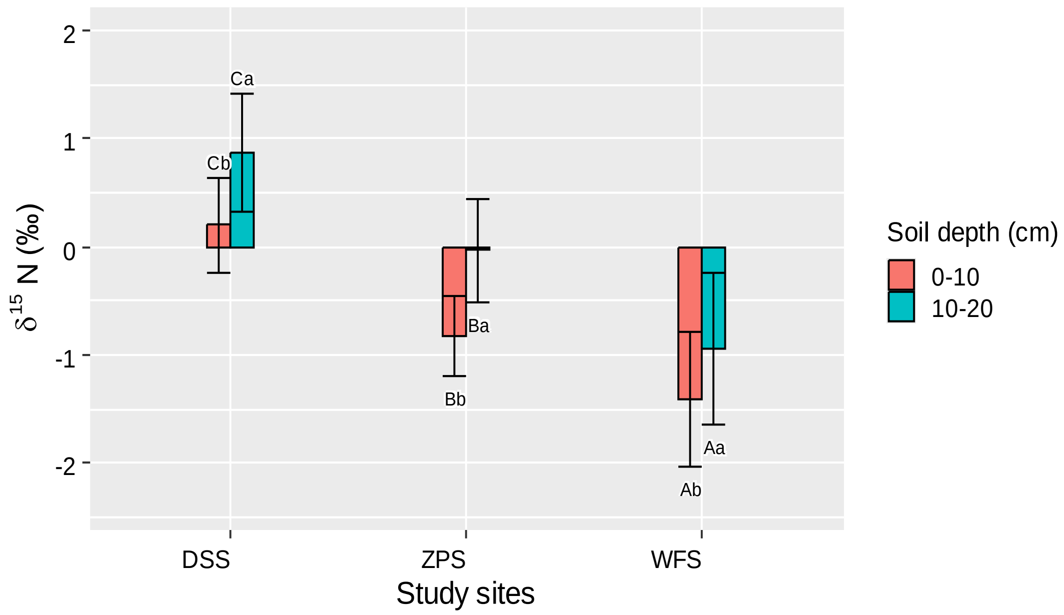

Figure 1. Soil $\delta^{15}N$ values at different soil depths (0-10 cm and 10-20 cm) in Dashushan Forest National Park (DSS), Zipengshan Forest National Park (ZPS), and Wanfoshan Forest National Park (WFS)

(Data are shown as means and standard deviation. Different capital letters (A, B, C) indicate significant differences between different sites in the same soil layer ($p \leq 0.05$), and different lowercase letters (a, b, c) indicate significant differences between different soil layers in the same site ($p \leq 0.05$)

Furthermore, there were significant seasonal differences in the soil $\delta^{15}N$ in urban, suburban, and rural forest soils (Fig. 2, $p \leq 0.05$). Specifically, the values of $\delta^{15}N$ in DSS at the 0-10 cm soil depth were highest in fall 2017 (0.83‰), but they were lowest in summer 2017 (-0.10‰) (Fig. 2a). The greatest values of $\delta^{15}N$ in ZPS and WFS were recorded in the summer of 2017 (-0.29‰ and -0.56‰, respectively). In spring 2018, the WFS had the lowest δ15N values (-2.00%), whereas the ZPS had the lowest $\delta^{15}N$ values (-1.11‰) in summer 2018. At the 10-20cm soil depth, the $\delta^{15}N$ values were highest in DSS and ZPS in fall 2017 (1.47‰ and 0.54‰), respectively, but they were lowest in summer 2018 (0.18‰ and -0.64‰), respectively (Fig. 2b). In WFS, there was no significant distinction in $\delta^{15}N$ in comparison with the 0-10 cm soil depth.

In both soil depths, values of $\delta^{15}N$ were always highest in DSS, but they were always lowest in WFS. There was a decreasing trend in $\delta^{15}N$ values at 0-10cm and 10-20 cm soil depth among three sites. The highest values were found in DSS, followed by ZPS, and the lowest in WFS (Fig. 2a-b).

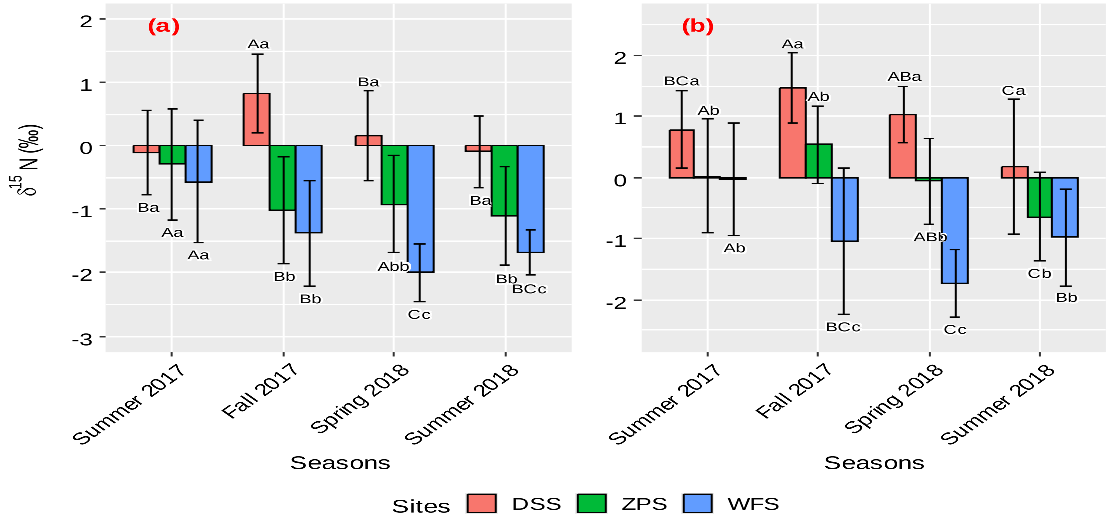

Figure 2. Season changes of δ15N values at the 0-10 cm (a) and 10-20 cm (b) soil depths in Dashushan National Forest Park (DSS), Zipengshan National Forest Park (ZPS), and Wanfoshan National Forest Park (WFS)

(Data are shown as means and standard deviation. Different capital letters (A, B, C) indicate significant differences between different seasons in the same gradient ($p \leq 0.05$), and different lowercase letters (a, b, c) indicate significant differences between different gradients in the same season ($p \leq 0.05$)

3.2. Leaf δ15N values

δ15N values in the young leaves did not significantly differ ($p > 0.05$) from those in the mature leaves (Fig. 3). There was a statistical difference in the values δ15N in the two types of plant leaves among three sites ($p \leq 0.05$). The δ15N distribution trend in plant leaves under the urban-rural gradient was consistent with that found in soil. At three sites, the leaf δ15N was dropped from DSS to WFS due to dramatical reduction of nitrogen deposition (Fig. 3). In the two plant foliar types of three sites, the annual mean values of young leaves and mature leaves in WFS (-5.73 ‰, -6.12‰) were remarkedly lower ($p \leq 0.05$) than those in ZPS (-4.33‰, -4.58‰) and DSS (-3.57‰, -3.68‰) (Fig. 3).

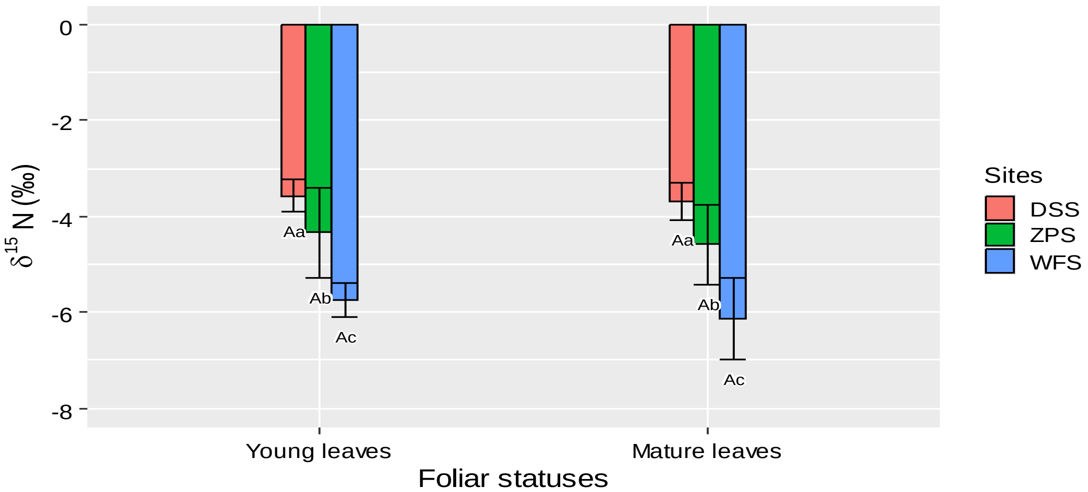

Figure 3. δ15N in plant leaves obtained from Dashushan National Forest Park (DSS), Zipengshan National Forest Park (ZPS), and Wanfoshan National Forest Park (WFS)

Notes: Data are shown as means and standard deviation. Different capital letters (A, B, C) indicate significant differences between different foliar statuses in the same site ($p \leq 0.05$), and different lowercase letters (a, b, c) indicate significant differences between different sites in the same foliar statuses ($p \leq 0.05$)

4. DISCUSSION

It is well known that temporal and spatial changes of the soil δ^{15}N depend on the uneven distribution of soil nitrogen, nitrification, denitrification, and mineralization in the soil. The organic layer of soils can add nitrogen to the soil minerals under normal conditions [27, 28]. Therefore, the surface soil layer (0 - 10 cm) can have an increased concentration of ^{15}N because of the exclusion of the heavier ^{15}N from the mineralization process [27]. Long-term enrichment of residual nitrogen in ^{15}N can be achieved by major soil nitrogen transformation processes such as ammonia volatilization, nitrification, denitrification, and microbial soil ammonium immobilization [29, 30]. In the present study, the values of δ^{15}N at the 0-10 cm soil depth in DSS were highest in the fall and lowest in the summer of 2017. Furthermore, the δ^{15}N values were highest in ZPS and WFS in the summer of 2017 and lowest in the Summer of 2018 and Spring of 2018, respectively. However, at 10-20 cm soil depth, δ^{15}N values were highest in fall 2017 but lowest in summer 2017, and summer 2018 in DSS and ZPS, respectively. Moreover, in WFZ, the change patterns of δ^{15}N in the 10-20 cm soil layer were consistent with that of the 0-10 cm soil layer. This seasonal pattern was similar to the findings of Ciezka et al. [31]. Due to the isotopic effects of equilibrium processes, greater values (δ^{15}N) - NH_4^+ were more frequently observed in summer than in winter. Higher concentrations of (^{15}N) aerosol NH_4^+ and precipitation NH_4^+ are mostly caused by the isotopic effects of equilibrium processes [32].

Numerous variables, including atmospheric nitrogen addition, microbial nitrification, root depth, and air nitrogen addicting can affect the ^{15}N values in the soil. It was challenging to demonstrate the direct relationship between atmospheric nitrogen deposition and soil ^{15}N in Hefei City due to a lack of extensive historical data, particularly in the isolated rural area. Rarely have reports on soil ^{15}N measurements been linked to atmospheric N deposition. Although Kuang et al. [33] claimed that amplified air nitrogen deposition from cars and other forms of industry in south China significantly influenced the changes in forest soil ^{15}N. The soil ^{15}N from the current study increased from WFS to DSS. We found a rising trend in soil ^{15}N from WFS to DSS area, which is probably due to N input sources with differing isotope compositions.

The soil δ^{15}N values were found to be higher in the subsoil than top soil depths, which aligns with most previous studies' results that δ^{15}N of soils increased with increasing soil depth [34, 35]. This process may be described by the ^{15}N-depletion that occurs during the microbial degradation of organic matter, which occurs concurrently with the leftover substrate moving lower over time [36].

To evaluate the ecological and environmental effects of anthropogenic N inputs and identify the sources of anthropogenic N inputs, it is necessary to first identify the sources of anthropogenic N inputs, the ^{15}N levels in plants and soil are frequently utilized [37]. The results of the study showed there was no significant difference between the ^{15}N values of mature and young leaves, which are in line with those of earlier reports [32, 33], but Li et al. [38] demonstrated that the age of the foliage had a substantial impact on ^{15}N values. Additionally, a major cause of acid rain in urban areas is the rapidly rising NO_x emissions brought on by an increase in coal use and motor vehicle use. Increased agricultural activity has significantly increased NH_3 emissions in rural regions. Several plant N sources may be modulated by increasing atmospheric N compound concentrations such as NO_x and NH_3 [39].

5. CONCLUSIONS

In all three experimental sites, there was a notable variation in the soil's ^{15}N values between the 0–10 cm and 10–20 cm soil depths. At all sites, the top soil layer's ^{15}N values in the forest soil were significantly lower than those in the deep soil layer. The values of δ^{15}N in DSS at 0–10 cm soil depth showed seasonal variable tendencies that were maximum in fall 2017 and maximum in summer 2017, while values at ZPS and WFS sites were highest in summer 2017 and lowest in spring 2018. At 10-20cm soil depth, DSS and ZPS had maximum δ^{15}N values in Fall 2017 and lowest in summer 2018. The urban sites had higher ^{15}N values in mature and young foliage than those at the suburban and rural sites. Season and site have a significant effect on nitrogen isotopes. The results obtained from the present study revealed that seasonal and site variation significantly increased the nitrogen isotopes.

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