Inter-Species and Inter-Organ Differences in Stable C and N Isotope Signatures of Macrophytes from the Largest Freshwater Lake in China

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Abstract: Macrophytes play important roles in shallow aquatic ecosystems. Stable isotope signatures of macrophytes indicate the environmental conditions and macrophyte contributions to food webs. However, macrophyte isotope signatures have been studied less than isotope signatures of other organisms. We determined the stable C and N isotope signatures of 10 aquatic plant species from Poyang Lake wetland (Wucheng, Yongxiu County) and Nanji wetland (Nanjishan, Xinjian County) in Jiangxi Province, which are Chinese national nature reserves, among different seasons. The isotope signatures for different species and seasons were significantly different. The dominant macrophyte species were *Potamogeton malaianus* and *Nymphoides peltatum.* The isotope signatures for different organs of these two species were determined. Both δ^{13}C and δ^{15}N values were higher for *P. malaianus* than for *N. peltatum* stems, roots and leaves, and δ^{13}C varied less for *N. peltatum* than for *P. malaianus* organs. The δ^{13}C and δ^{15}N values for *P. malaianus* organs increased in the order roots<stems<leaves. δ^{13}C values for *N. peltatum* organs decreased in the order roots>stems>leaves, and δ^{15}N values for *N. peltatum* organs increased in the order roots<stems<leaves. The stable C and N isotope signatures for *P. malaianus* and *N. peltatum* may be controlled by various factors including macrophyte life history, external sources of C/N, and the amount of water in the wetland. These results provided a theoretical reference and experimental data support for detecting the flow trend of C & N elements and environment changes in the lake by the δ^{13}C and δ^{15}N values of euhydrophytes .

Keywords: Macrophytes, δ^{13}C, δ^{15}N, *Potamogeton malaianus*, *Nymphoides peltatum*, Poyang Lake.

1. INTRODUCTION

Macrophytes are key components of shallow aquatic ecosystems that have many functions and provide many services. Aquatic macrophytes can remove N and P from water, absorb heavy metals from water, inhibit algal blooms, improve transparency of the water, and increase the dissolved oxygen concentration in the water. Macrophytes are very important for purifying and restoring aquatic environments (Jeppesen *et al*. 1998, Phillips *et al*. 2016). Aquatic macrophyte growth and metabolism can be affected by various environmental factors (Chappuis *et al*. 2017). The stable C and N isotope patterns in the tissues of macrophytes from different lakes can be markedly different. The characteristics of plant tissues (particularly leaves, which are the most active tissues) can be used to effectively monitor environmental changes in lakes.

Environmental water quality in many areas is becoming poorer, and this is increasingly being investigated by analyzing macrophytes because of the close relationships between macrophyte characteristics and environmental water quality. The roots, stems, and leaves of aquatic macrophytes are in intimate contact with the water the macrophytes inhabit. Degradation of aquatic macrophytes, particularly of submerged macrophytes, is an important sign of ecosystem degradation in shallow lakes (Zhou & Zeng, 2008; Hilt *et al*. 2017). The important relationships between aquatic macrophyte characteristics and environmental water quality are increasingly leading researchers to study aquatic macrophytes at the macro and micro scales, for example by analyzing stable C and N isotopes in aquatic macrophytes.

Aston made the first mass spectrometer and successfully detected isotopes in 1919. Mass spectrometry is now an effective method for analyzing isotopes, and stable C and N isotopes can be simply and effectively determined. Stable C and N isotopes in an aquatic macrophyte can indicate the way the plant photosynthesizes (C_3, C_4, or Crassulacean acid

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metabolism), the way the plant uses water, sources of water pollution, the degree of pollution and eutrophication of a lake, and other characteristics. This has led to stable C and N isotopes in macrophytes being analyzed in many studies. Liang (2014) found that the widespread aquatic plant species *Nymphoides peltatum* in Banghu Lake had a similar N isotope composition to the N isotope composition of sediment and that the N isotope composition of sediment in Banghu Lake was mainly affected by aquatic plants in the lake. LaZerte and Szalados (1982) found higher C isotope values for submerged plants than for floating-leaved plants and that the C isotope compositions of aquatic plants were affected by C in the water. Wu (2012) studied C isotopes of submerged plants in Erhai Lake, lakes in the Eastern Plain lake zones in China, and lakes on the Yunnan–Kweichow Plateau and found that the C isotope compositions of submerged plants were directly related to the C isotope compositions of organic matter in sediment and methane emission fluxes for the lakes and could be used to indicate C circulation in the lakes.

Few studies of the characteristics of stable C and N isotopes in macrophytes in Poyang Lake (the largest freshwater lake in China) and particularly stable C and N isotopes in macrophytes of different types and the distributions of stable C and N isotopes in different tissues (roots, stems, and leaves) of macrophytes have been performed. In this study, the stable C and N isotope distributions in macrophytes from Poyang Lake were investigated. The C and N isotope distributions in different macrophyte tissues and species and in macrophytes from different locations and collected in different seasons were determined.

2. MATERIALS AND METHODS

Macrophytes were collected from the main sub-lakes and wetland areas in the Poyang Lake wetland (Wucheng, Yongxiu County) and Nanji wetland (Nanjishan, Xinjian County) national nature reserves of China in 2014. Macrophytes of 10 species in nine genera were collected. The submerged macrophytes were of nine species in eight genera, and the only floating-leaved macrophyte was *N. peltatum*.

Lake Poyang is the largest freshwater and river-connected lake in China and a biodiversity hotspot that is rich in aquatic organisms. Lake Poyang supports many macrophytes (28 families, 56 genera, and 95 species; Jian *et al*., 2001). Macrophytes were collected from the main sub-lake of Poyang Lake and the area surrounding Poyang Lake wetland national nature reserve and from the Nanji wetland national nature reserve during the mid-water-level period in spring (April), wet season in summer (July), mid-water-level period in fall (October), and dry season in winter (December) in 2014. The sampling sites were in the main sub-lake and surrounding wetland area of Poyang Lake national nature reserve and Nanji wetland national nature reserve, including in Changhu Lake, Changhuchi Lake, Dachahu Lake, Dahu Lake, Donghu Lake, Meixihu Lake, Shahu Lake, Zhanbeihu Lake, and Zhonghu Lake in the Poyang Lake area. The sampling sites are shown as white dots in Figure **1**.

Macrophytes that grew well and had consistent morphologies (labeled N later) were selected. Fresh macrophytes were collected directly from shallow water. The macrophytes were washed and then stored

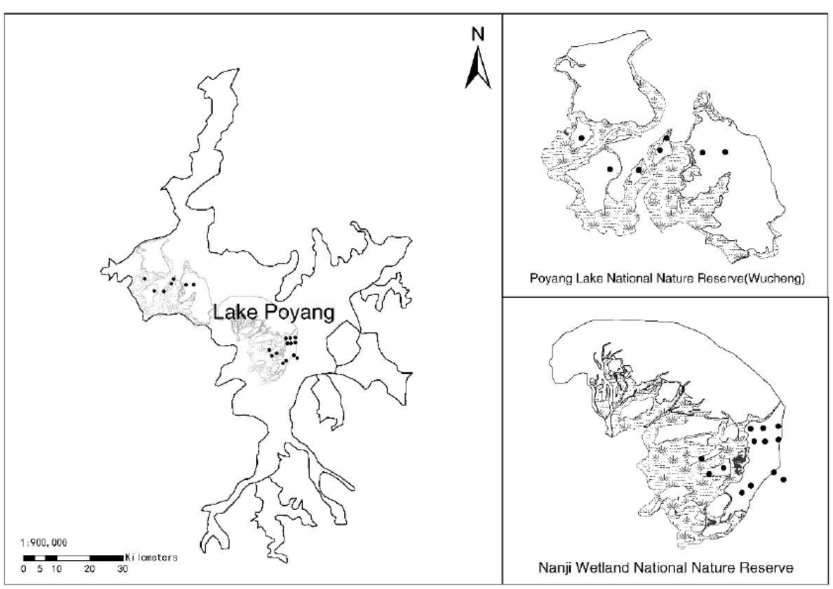

Figure 1: The sketch of sampling sites in Poyang Lake (Jiangxi, China).

at a low temperatures. The macrophytes were transported to the laboratory and the C and N isotope contents were determined.

2.1. Sample Pre-Treatment

The macrophytes were transported to the laboratory in refrigerated containers. Algae attached to the surfaces of the macrophytes were removed, then the macrophytes were washed three times with deionized water. The roots, stems, and leaves were then separated and dried at 80 °C for 48 h until each sample reached a constant weight. Each sample was then ground to a fine powder using a mortar and pestle and stored in a tube.

2.2. Analytical Methods and Instruments

The total carbon (TC) content, δ^{13}C, total nitrogen (TN) content, and δ^{15}N of each sample were determined using a Carlo Erba EA-1110 elemental analyzer (Carlo Erba, Italy) and a Finnigan Delta Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Chinese Academy of Science Aquatic Organism Research Institute. The precision of each measurement was about ±0.3‰. The C and N isotope ratios are presented as δ values defined as

$$\delta X = \left({R_{sample}}/{R_{standard}} - 1 \right) \times 1000‰ \quad (1)$$

where X is ^{13}C or ^{15}N, R_{sample} is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) for the sample, and $R_{standard}$ is the isotope ratio for the standard. The standards had uniform isotope compositions, were stable, contained large quantities of the relevant isotopes, had specific geological origins, and were straightforward to prepare and analyze. The standards for determining stable C and N isotopes were Pee Dee Belemnite from a cretaceous system in South Carolina, USA, and N from the air, respectively.

Each sample was analyzed once, and one standard was analyzed with every 10 samples. One or two randomly selected samples from every 10 samples were re-analyzed to assess the stability of the instrument and the accuracy of the method.

2.3. Data Processing

Statistical analyses were performed using SPSS 19.0 software (IBM, Armonk, NY, USA). The isotope content of each leaf sample is given as the mean ± standard deviation. One-way analyses of variance were performed using the variable of interest as a single factor. Multiple comparisons were made using the least significant difference method. The samples were divided into groups using the Student–Newman–Keuls test (q-test). The indices were assessed by performing Pearson analyses, and significant differences between variables were identified by performing two-sided tests. The significant level was 0.05.

3. RESULTS

3.1. Macrophyte Species and C and N Isotope Analysis Results for the Macrophyte Tissues

The TC contents of the submerged macrophyte samples were 30.50%–38.28%, the δ^{13}C values were 21.52‰–29.38‰, the TN contents were 1.34%–2.42%, and the δ^{15}N values were 6.86‰–12.54‰. The highest δ^{13}C and δ^{15}N values, 21.52‰ and 12.54‰, for the nine submerged plant species were for *P. crispus* and *Vallisneria spinulosa*, respectively, as shown in Table **1**. The δ^{13}C and δ^{15}N values for the submerged

Table 1: The TC, TN Contents and the Stable Carbon Isotope (δ^{13}C), the Stable Nitrogen Isotope (δ^{15}N) Values of Submerged Macrophytes in Sampling Sites

Genera	Species	TC/%	δ^{13}C/‰	TN/%	δ^{15}N/‰
Myriophyllum	*Myriophyllum spicatum*	36.35±5.60	-26.87±4.82	1.85±1.03	7.43±4.20
Potamogeton	*Potamogeton malaianus*	38.28±5.14	-22.55±1.79	1.34±0.65	10.53±3.85
	Potamogeton crispus	36.36±6.76	-21.52±0.75	2.42±1.15	11.45±1.96
Vallisneria	*Vallisneria spinulosa*	30.50±5.28	-28.44±3.25	2.23±0.61	12.54±3.69
Hydrilla	*Hydrilla verticillata*	36.55±6.88	-23.59±2.82	2.28±0.87	7.32±2.32
Ceratophyllum	*Ceratophyllum demersum*	34.22±7.14	-28.15±2.96	2.26±0.85	9.21±3.69
Ottelia	*Ottelia cordata*	36.06±4.44	-25.48±2.21	1.55±0.59	6.86±1.40
Utricularia	*Utricularia vulgaris*	35.29±5.39	-29.38±6.51	2.18±1.06	9.55±2.22
Najas	*Najas minor*	36.87	-25.64	2.40	7.14
Nymphoides	*Nymphoides peltatum*	40.49±3.89	-28.64±0.69	1.81±0.78	8.61±2.62

and floating plants were not significantly different, but the TC and TN contents were significantly higher for the floating plant *N. peltatum* than for the nine submerged plants. The stable C and N isotope results were in the same ranges for *P. malaianus* as the other macrophytes. The only floating-leaved plant was *N. peltatum*. *P. malaianus* and *N. peltatum* were the dominant macrophyte species at the sampling sites and were widely distributed, so there were sufficient *P. malaianus* and *N. peltatum* samples for analysis. We therefore focused further analysis on these two plant species.

3.2. Stable C and N Isotopes in Tissues of Different Macrophyte Species

The mean $\delta^{13}C$ values were higher for the *P. malaianus* stems, roots and leaves than for the *N. peltatum* stems, roots and leaves, but the TC contents were lower for the *P. malaianus* stems, roots and leaves than for the *N. peltatum* stems, roots and leaves. This indicated that the TC content and $\delta^{13}C$ value followed opposite trends in the *P. malaianus* and *N. peltatum* tissues (Figures **2** and **3**). The $\delta^{13}C$ values varied more for *P. malaianus* than for *N. peltatum*. The $\delta^{13}C$ values for *P. malaianus* roots, stems, and leaves were −18.96‰ to −29.94‰, −18.40‰ to −31.51‰, and

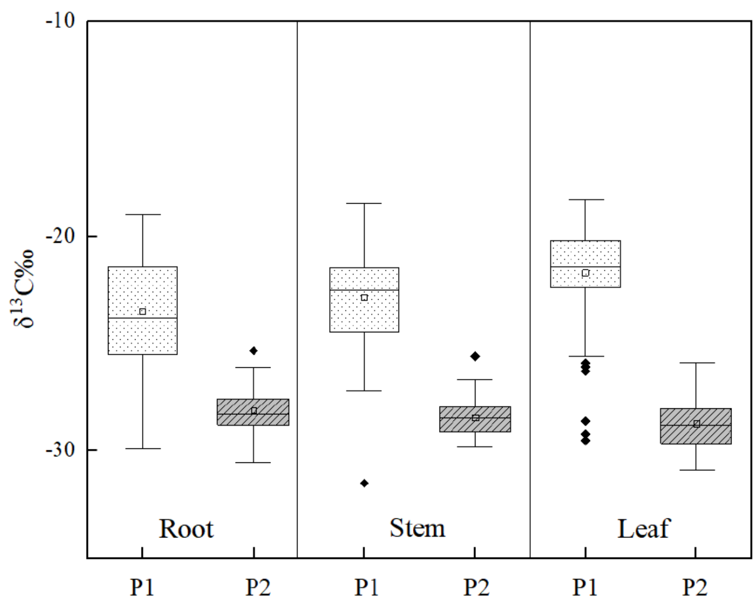

Figure 2: The stable carbon isotope ($\delta^{13}C$) values of *P. malaianus* (P1) and *N. peltatum* (P2) tissues (root, stem and leaf) in Poyang Lake.

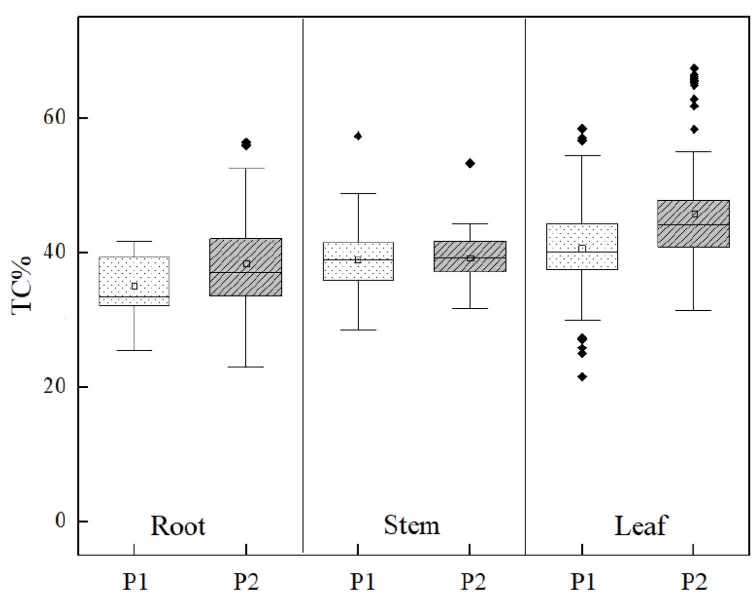

Figure 3: The TC contents of *P. malaianus* (P1) and *N. peltatum* (P2) tissues (root, stem and leaf) in Poyang Lake.

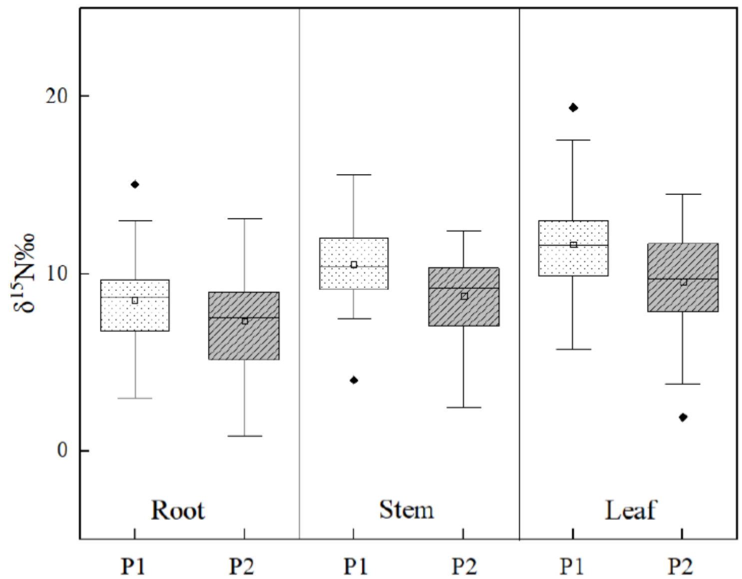

Figure 4: The nitrogen isotope ($\delta^{15}N$) values of *P. malaianus* (P1) and *N. peltatum* (P2) tissues (root, stem and leaf) in Poyang Lake.

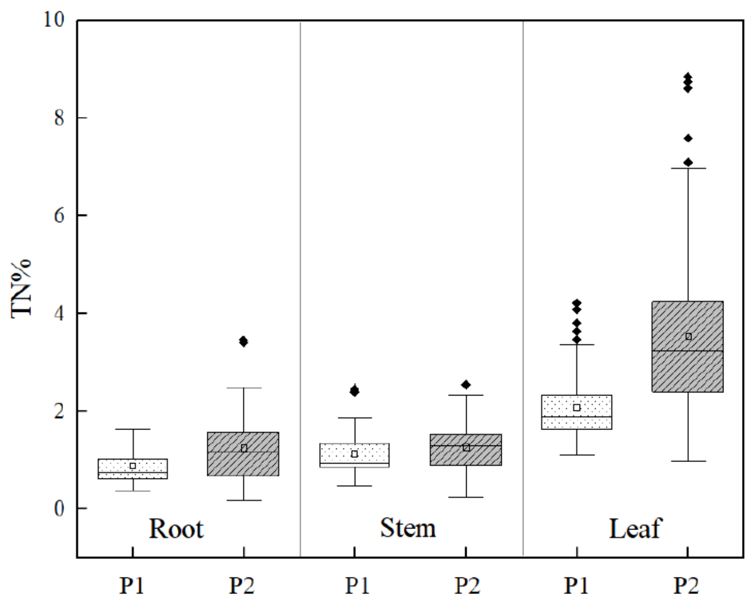

Figure 5: The TN contents of *P. malaianus* (P1) and *N. peltatum* (P2) tissues (root, stem and leaf) in Poyang Lake.

−18.23‰ to −29.54‰, respectively, and the $\delta^{13}C$ values for *N. peltatum* roots, leaves, and stems were much lower and more stable, being −25.32‰ to −30.56‰, −25.58‰ to −29.82‰, and −25.86‰ to −30.89‰, respectively. This would have been related to the sources of C to which the aquatic macrophytes were exposed.

The $\delta^{15}N$ values were higher for the *P. malaianus* stems, roots, and leaves than for the *N. peltatum* stems, roots, and leaves but the TN contents were lower for the *P. malaianus* stems, roots, and leaves than for the *N. peltatum* stems, roots, and leaves (Figures **4** and **5**). This indicated that the TN contents and $\delta^{13}C$ values for *P. malaianus* and *N. peltatum* followed opposite trends. The TC and TN contents followed the same trends (Figures **3** and **5**), indicating that C and N uptake by macrophyte tissues are related. A similar positive correlation between the C and N contents of plant leaves was found by Suresh *et al*. (2008).

The $\delta^{15}N$ values for *P. malaianus* and *N. peltatum* varied widely (2.98‰ to 15.08‰, 3.97‰ to 15.61‰, and 5.71‰ to 19.44‰ for *P. malaianus* roots, stems, and leaves, respectively, and 0.86‰ to 13.13‰, 2.45‰ to 12.40‰, and 1.92‰ to 14.52‰ for *N. peltatum* roots, stems, and leaves, respectively), indicating that there

Table 2: The TC Contents and the Stable Carbon Isotope ($\delta^{13}C$) Values of Macrophytes in Poyang Lake Throughout the Year (N Represents the Number of Samples)

Tissue	Object	*P. malaianus*		*N. peltatum*	
		TC/%	$\delta^{13}C$/‰	TC/%	$\delta^{13}C$/‰
Roots	Sample number *N*	16	16	47	47
	Mean ± SD	35.01±4.49	-23.49±2.87	38.4±7.23	-28.11±1.02
	Minimum	25.38	-29.94	23.05	-30.56
	Maximum	41.84	-18.96	56.32	-25.32
Stems	Sample number N	49	49	36	36
	Mean ± SD	38.92±5.13	-22.84±2.52	39.23±3.81	-28.45±0.87
	Minimum	28.50	-31.51	31.63	-29.82
	Maximum	57.36	-18.40	53.30	-25.58
Leaves	Sample number *N*	113	113	87	87
	Mean ± SD	40.55±6.54	-21.68±2.31	45.79±8.89	-28.73±1.08
	Minimum	21.46	-29.54	31.35	-30.89
	Maximum	58.45	-18.23	67.38	-25.86

Table 3: The TN Contents and the Stable Nitrogen Isotope ($\delta^{15}N$) Values of Macrophytes in Poyang Lake Throughout the Year (N Represents the Number of Samples)

Tissue	Object	*P. malaianus*		*N. peltatum*	
		TN/%	$\delta^{15}N$/‰	TN/%	$\delta^{15}N$/‰
Roots	Sample number *N*	16	16	47	47
	Mean ± SD	0.88±0.38	8.51±2.94	1.25±0.78	7.36±2.51
	Minimum	0.37	2.98	0.18	0.86
	Maximum	1.65	15.08	3.47	13.13
Stems	Sample number *N*	49	49	36	36
	Mean ± SD	1.14±0.47	10.50±2.12	1.27±0.55	8.76±2.42
	Minimum	0.47	3.97	0.25	2.45
	Maximum	2.49	15.61	2.54	12.40
Leaves	Sample number *N*	113	113	87	87
	Mean ± SD	2.08±0.66	11.66±2.46	3.55±1.72	9.57±2.69
	Minimum	1.09	5.71	0.97	1.92
	Maximum	4.23	19.44	8.85	14.52

were various sources of N to biota in the sampling area. This also indicated that there are various sources of N pollution and therefore eutrophication in Poyang Lake. Wang (2014) analyzed the sources of N pollution in Poyang Lake.

3.3. Pearson Correlations between C and N Signatures for *P. Malaianus* and *N. Peltatum* Roots, Stems, and Leaves

There were no significant correlations between the TC contents, $\delta^{13}C$ values, TN contents, and $\delta^{15}N$ values for the *P. malaianus* roots. The $\delta^{13}C$ and $\delta^{15}N$ values for the *P. malaianus* stems significantly positively correlated, but the TC contents and TN contents did not strongly correlate with each other or the $\delta^{13}C$ and $\delta^{15}N$ values. The TC contents, $\delta^{13}C$ values, and TN contents of the *P. malaianus* leaves significantly positively correlated, and the $\delta^{13}C$ and $\delta^{15}N$ values significantly positively correlated, but the other values did not strongly correlate. The TC contents, $\delta^{13}C$ values, TN contents, and $\delta^{15}N$ values for the *N. peltatum* roots positively correlated. The TC contents and δ^{15}N values for the *N. peltatum* roots significantly negatively correlated but the other factors did not strongly correlate. The TC contents and δ^{13}C values for the *N. peltatum* stems significantly positively correlated and the TC contents, δ^{13}C values, and TN contents positively correlated, but the other factors did not strongly correlate. The TC contents, δ^{13}C values, and TN contents for the *N. peltatum* leaves significantly positively correlated (*P<0.05*) and the TC contents and δ^{15}N values for the *N. peltatum* leaves significantly negatively correlated (*P<0.05*). The TC contents, δ^{13}C values, TN contents, and δ^{15}N values more strongly correlated for the *P. malaianus* and *N. peltatum* leaves than for the roots and stems (Tables **4** and **5**).

The correlations between the *P. malaianus* and *N. peltatum* leaf tissue isotope values, element contents of the plants, and aquatic environment characteristics are rather stable. This has led to researchers around the world studying leaves (O'Leary *et al*., 1981; Huang *et al*., 2003; Herzschuh *et al*., 2010). Leaves have the advantages of being stable and giving repeatable analytical results. Leaves also indicate correlations between macrophytes and lake water well and reflect macrophyte growth characteristics well.

3.4. Stable C and N Isotopes in Tissues of the Same Macrophyte Species

The results for the roots, stems, and leaves of *P. malaianus* and *N. peltatum* were investigated by performing analyses of variance. Significant differences were found between the δ^{13}C values, δ^{15}N values, TC contents, and TN contents of the different *P. malaianus* tissues (F=6.52, *P*<0.01 for δ^{13}C; F=13.67, *P*<0.01 for δ^{15}N; F=6.35, *P*<0.01 for the TC content; F=59.91, *P*<0.01 for the TN content), as shown in Figures **7** and **9**. The δ^{13}C values, δ^{15}N values, TC contents, and TN contents in the tissue samples increased in the order root<stem<leaf. The δ^{13}C values for roots and leaves

Table 4: Pearson Correlation Among TC, δ^{13}C, TN and δ^{15}N Signatures of *P. Malaianus* Tissues

Tissues	***P. malaianus***	δ^{13}C	TN	δ^{15}N
Roots	TC	-0.222(*P*=0.409)	-0.067(*P*=0.807)	0.156(*P*=0.536)
	δ^{13}C	1	0.143(*P*=0.598)	0.114(*P*=0.674)
	TN		1	0.079(*P*=0.771)
Stems	TC	0.113(*P*=0.438)	-0.084(*P*=0.565)	-0.093(*P*=0.524)
	δ^{13}C	1	0.043(*P*=0.772)	0.565**(*P*<0.01)
	TN		1	0.281(*P*=0.05)
Leaves	TC	0.260**(*P*<0.01)	0.497**(*P*<0.01)	-0.033(*P*=0.725)
	δ^{13}C	1	-0.005(*P*=0.961)	0.465**(*P*<0.01)
	TN		1	-0.072(*P*=0.446)

* *P*< 0.05;** *P* < 0.01.

Table 5: Pearson Correlation Among TC, δ^{13}C, TN and δ^{15}N Signatures of *N. Peltatum* Tissues

Tissues	***N. peltatum***	δ^{13}C	TN	δ^{15}N
Roots	TC	0.477**(*P*<0.01)	0.424**(*P*<0.01)	-0.354*(*P*=0.015)
	δ^{13}C	1	0.521**(*P*<0.01)	-0.205(*P*=0.167)
	TN		1	-0.112(*P*=0.453)
Stems	TC	0.488**(*P*<0.01)	0.422*(*P*=0.010)	-0.086(*P*=0.618)
	δ^{13}C	1	0.634*(*P*=0.029)	-0.125(*P*=0.468)
	TN		1	0.066(*P*=0.701)
Leaves	TC	0.268*(*P*=0.012)	0.758**(*P*<0.01)	-0.377**(*P*<0.01)
	δ^{13}C	1	0.191(*P*=0.076)	-0.059(*P*=0.589)
	TN		1	-0.085(*P*=0.436)

* *P*< 0.05;** *P* < 0.01.

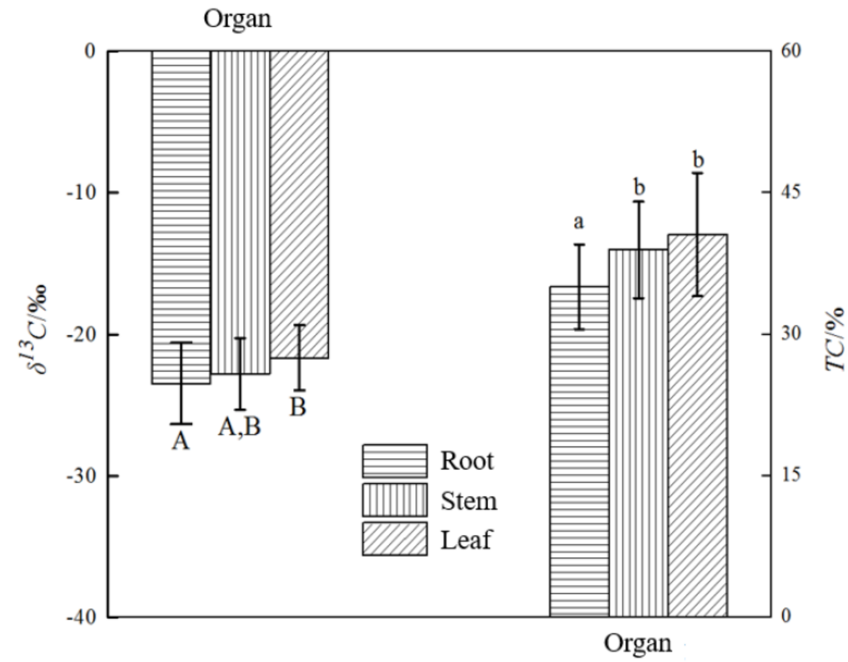

Figure 6: The stable carbon isotope ($\delta^{13}C$) values and the TC contents of *P. malaianus* tissues in Poyang Lake (Different letters represent statistically significant difference (P<0.05), the same as follows).

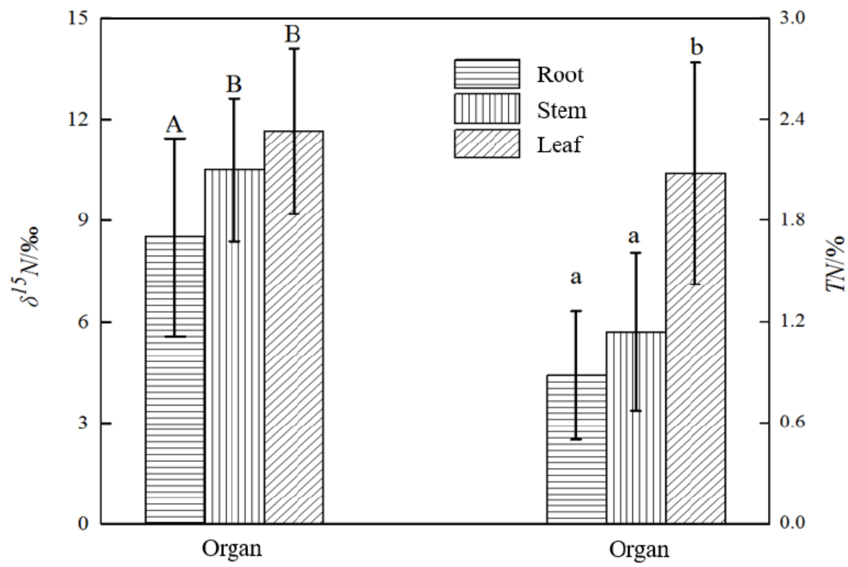

Figure 7: The stable nitrogen isotope ($\delta^{15}N$) values and the TN contents of *P. malaianus* tissues in Poyang Lake.

were significantly different. The $\delta^{15}N$ values for the roots, stems, and leaves were also significantly different. The TC contents and $\delta^{13}C$ values and the TN contents and $\delta^{15}N$ values for the various *P. malaianus* tissues positively correlated, indicating that the relationships between the *P. malaianus* stems and leaves were relatively close. The $\delta^{13}C$ values for *N. peltatum* tissues decreased in the order root>stem>leaf, but the other results followed the same trends as the results for *P. malaianus*.

4. DISCUSSION

Macrophytes play important roles in shallow aquatic ecosystems. The characteristic isotope contents of macrophyte tissues can be used to assess environmental conditions and the roles of macrophytes in food webs (Wang *et al*. 2009; Wang *et al*. 2015). We found significantly higher TC contents for floating plants than for submerged plants. The $\delta^{13}C$ ranges were larger for the *P. malaianus* (submerged plant) tissues than for *N. peltatum* (floating-leaved plant) tissues. The

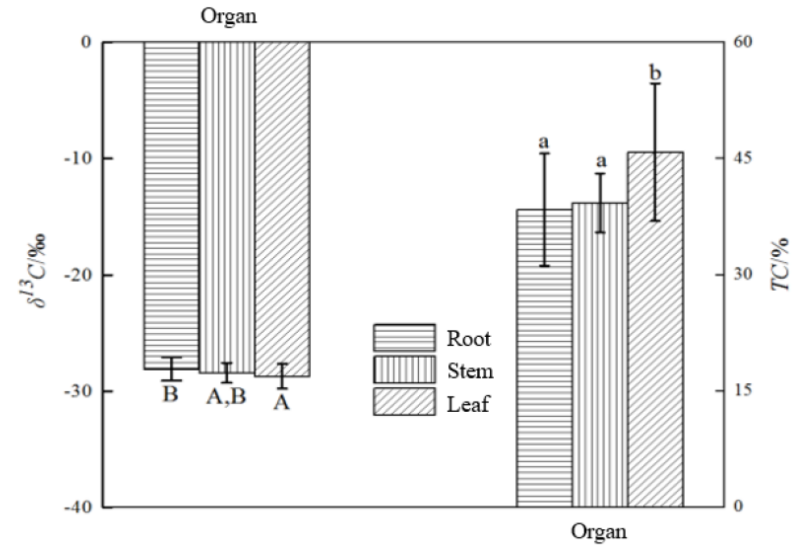

Figure 8: The stable carbon isotope ($\delta^{13}C$) values and the TC contents of *N. peltatum* tissues in Poyang Lake.

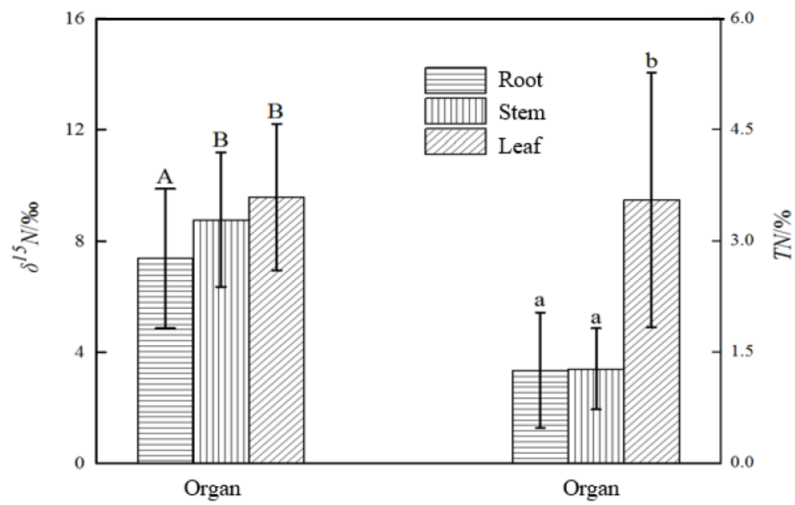

Figure 9: The stable nitrogen isotope ($\delta^{15}N$) values and the TN contents of *N. peltatum* tissues in Poyang Lake.

$\delta^{15}N$ ranges for the roots, stems, and leaves were also larger for *P. malaianus* than for *N. peltatum.*

C used for photosynthesis by submerged plants is supplied by the water body. The inorganic C sources in water include CO_2, HCO_3^-, and H_2CO_3. The CO_2 diffusion rate into water is low, so CO_2 contributes only ~0.5% of the total inorganic C in water but HCO_3^- contributes ~89%. The main source of C to submerged plants for photosynthesis is therefore HCO_3^-, which is relatively enriched in ^{13}C (James *et al*., 1996; Madsen *et al*., 1995; Lin *et al*., 2001). The C fraction is affected by the pH, temperature, and other factors (Benedict *et al*., 1980; Madsen *et al*., 1993; Liang *et al*., 2018; Chen *et al*., 2022). The C content of water is relatively low and varies widely (Liu *et al*. 2020). C used for photosynthesis by floating plants is mainly supplied by the air above the water. C sources are abundant and stable in air and are enriched in ^{12}C. The $\delta^{13}C$ values for *P. malaianus* were therefore high and variable but the $\delta^{13}C$ values for *N. peltatum* were low and stable. The $\delta^{15}N$ values for roots, stems, and leaves were significantly higher for *P. malaianus* than for *N. peltatum*, and the TN contents were lower for *P. malaianus* than for *N. peltatum.* The TN contents and $\delta^{15}N$ values for *P. malaianus* and *N. peltatum* followed opposite trends because *P. malaianus* and *N. peltatum* have different growth periods and different N absorption patterns and the N contents of the water in the lake would have been different at different places. For example, the $\delta^{15}N$ values for nitrate fertilizer, ammonia fertilizer, rain, and domestic sewage are −2.5‰ to 2.0‰, −4.0‰ to 2.0‰, −8.0‰ to 3.0‰, and 5.0‰ to 10.0‰, respectively (Lin *et al*., 2012; Cao *et al*., 1991). If a macrophyte absorbs N from more scarce sources, the $\delta^{15}N$ value will be smaller and otherwise the $\delta^{15}N$ value will be larger.

The isotope characteristics of the different organs of the two species were analyzed further. The δ^{13}C values for the *P. malaianus* tissues increased in the order roots<stems<leaves. The roots and leaves of macrophytes are separated but connected by the stems, and the δ^{13}C values for roots and leaves were significantly different. The δ^{13}C values for the stems were not significantly different from the δ^{13}C values for the roots and leaves. The TC contents and δ^{13}C values for the *P. malaianus* tissues positively correlated. The reason may be that *P. malaianus* used inorganic C supplied by the water (in which ^{13}C would have been enriched) for photosynthesis and produced carbohydrates (mainly sucrose) through the Calvin cycle (Anthony, 1993; Madsen & Breinholt, 1995). The carbohydrates would have been transported to other organs through vascular bundles. Assimilates generated by the leaves are generally transported preferentially to the closest organs in the plant making demands (Anthony *et al*., 1993; Graham *et al*., 2000). The amount of assimilates acquired by plant organs decreases in the order leaves>stems>roots (Minchin & Thorpe, 1996), which explained the TC contents and δ^{13}C values for the *P. malaianus* tissues described above.

The roots, stems, and leaves of submerged plants can all absorb nutrients from the water or sediment, so many factors and complicated mechanisms would have affected the δ^{15}N values for the *P. malaianus* tissues presented above. The dominant cause was probably the different abilities of the different tissues to absorb nutrients. Roots can absorb N from sediment and water, while N is absorbed less effectively by stems than roots and even less effectively by leaves. The TC and TN contents of the *P. malaianus* tissues followed similar trends and both increased in the order roots<stems<leaves. This would have been because the C and N contents of plant tissues positively correlate (Suresh *et al*., 2008).

The TC contents and δ^{13}C values for the *N. peltatum* tissues negatively correlated. This would have been because *N. peltatum* leaves float and absorb C for photosynthesis from the air above the water (which is enriched in ^{12}C, LaZerte and Szalados, 1982; Wu, 2012). Synthetic assimilates are generally distributed to the different plant organs following the principle of contiguity. This resulted in the highest TC content and the lowest δ^{13}C value being for the *N. peltatum* leaves.

5. SUMMARY

1. Stable C & N isotopes of macrophytes have increasingly been employed as important indicators to detect environmental changes in aquatic ecosystems. It is a first study to systemically investigate and explore the distribution of the δ^{13}C and δ^{15}N values in different plant species and different tissues of dominant euhydrophytes in Poyang Lake, the largest shallow lake of China and the Ramsar freshwater wetland of world.

2. The δ^{13}C and δ^{15}N values for the stems, roots, and leaves were higher for *P. malaianus* than for *N. peltatum* in Poyang Lake. The ranges were higher for *P. malaianus* than for *N. peltatum* tissues. The δ^{15}N ranges for the *P. malaianus* and *N. peltatum* tissues had similar wide ranges. The TC and TN contents were lower for *P. malaianus* than for *N. peltatum*.

3. The δ^{13}C and δ^{15}N values for the different tissues of *P. malaianus* and *N. peltatum* were significantly different in Poyang Lake. The δ^{13}C values increased in the order root<stem<leaf for *P. malaianus* and decreased in the order root>stem>leaf for *N. peltatum*. The δ^{15}N values increased in the order root<stem<leaf. The δ^{13}C values for the *P. malaianus* and *N. peltatum* roots and leaves were significantly different. The δ^{15}N values for the *P. malaianus* and *N. peltatum* roots, stems, and leaves were significantly different. The TC and TN contents of the *P. malaianus* and *N. peltatum* tissues increased in the order root<stem<leaf.

In the future, it is necessary to establish the list and database of stable carbon & nitrogen isotopes signatures for other ecotypes of aquatic plants in Poyang Lake wetland, and also need to analyze the differences of δ^{13}C and δ^{15}N values in the tissues or components (*e.g*. cellulose) of the aquatic plants. In addition, the amphibious freshwater plants such as *P. malaianus, N. peltatum* and *M. spicatum* should be expected to study in Poyang Lake and the difference of both δ^{13}C and δ^{15}N values need to be analyzed in two growth types of these amphibious plants among different seasons. The species-specific pattern of stable isotopes signatures is also needed to uncover in different lakes and to provide the accurate signs for detecting the ecological changes of the lakes.

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DISCLOSURE STATEMENT

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