Ecological Indicators 121 (2021) 107181

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Temporal-spatial variations in the elemental and stable isotope contents of eelgrass (*Zostera marina* L.) in the Bohai Sea and Yellow Sea, northern China: Sheath as a novel ecological indicator for geochemical research

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ARTICLE INFO

Keywords:
Zostera marina L.
Ecological indicators
Leaf blade
Leaf sheath
Belowground tissues
Elemental and isotope content
Stoichiometry

ABSTRACT

Seagrasses play an important role in the geochemical cycling of elements in coastal ecosystems. Eelgrass (*Zostera marina* L.) is a marine foundation species essential for coastal ecosystem services in the temperate northern hemisphere. The elemental (C, N, and P) and isotope ($\delta^{13}C$ and $\delta^{15}N$) contents of eelgrass tissues were measured at nine sampling sites across northern China. Trends at temporal and spatial scales were analyzed to examine temporal-spatial variations in seagrass characteristics, and relationships between different tissues were also analyzed to identify a valuable ecological indicator. Elemental contents (N and P) of eelgrass were variable and demonstrated marked seasonal variations, while the isotope content ($\delta^{13}C$ and $\delta^{15}N$) demonstrated marked spatial variations. Elemental contents (N and P) in eelgrass tissues showed a clear annual trend with minima in summer and maxima in spring-winter in Swan Lake. Mean N contents (%) of leaf blades and leaf sheaths in Swan Lake were 2.56 ± 0.89 and 2.53 ± 0.96, respectively, and mean P contents of blades and sheaths were 0.31 ± 0.13 and 0.41 ± 0.17, respectively. N and P contents were higher in aboveground than in belowground tissues; due to C stability, C/N and C/P were lower in aboveground than in belowground tissues. Among different eelgrass populations, the ranges of $\delta^{15}N$ isotope ratios in leaf blades and leaf sheaths were 5.57–9.64 and 5.89–9.79, respectively. Elemental (N and P) and isotope ($\delta^{13}C$ and $\delta^{15}N$) contents of leaf sheaths were positively correlated with those of blades; this was evident regardless of the season and site, suggesting that the sheath elemental and stable isotope content could be a valuable indicator of that in leaf blades for seagrass ecosystem geochemical research.

1. Introduction

Plant element contents have a large influence on plant function, including growth and physiological regulation (Sterner and Elser, 2002; Agren, 2008). For example, C is an important component of plant tissues, and N and P can affect many physiological activities such as photosynthesis, and cell growth and division (Elser et al., 2007). In addition, their ratios are a powerful tool for study and assessment of ecological processes (Fourqurean and Zieman, 2002; Agren, 2008). For example, C/N and C/P can used to characterize the utilization efficiency of N and P (Vitousek, 1982; Aerts and Chapin, 2000), and N/P can used to explain plant growth limitation by N or P (Koerselman and Meuleman, 1996). Many studies have been conducted on seagrass elemental contents, with the majority generally focused on seagrass leaves (Atkinson and Smith, 1983; Duarte, 1990; Pérez-Lloréns and Niell, 1993; Enríquez et al., 2004). Furthermore, the internal elemental contents of

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https://doi.org/10.1016/j.ecolind.2020.107181
Received 30 September 2020; Received in revised form 31 October 2020; Accepted 3 November 2020
Available online 28 November 2020
1470-160X/

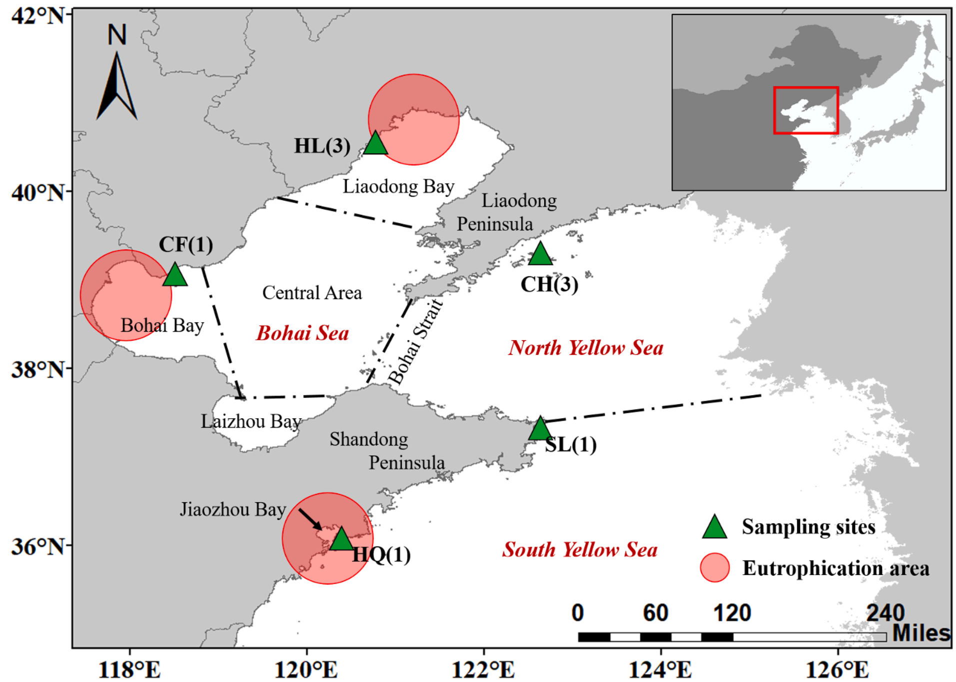

Fig. 1. *Zostera marina* L. sampling sites. Three sampling sites were selected each from Changhai Island (CH) and Huludao (HL), and one site each from Caofeidian (CF), Huiquan Bay (HQ), and Swan Lake (SL). Green triangles represent seagrass sampling sites. Red circles represent coastal waters with serious eutrophication.

plants vary with species, tissue, age, environment, and season (Birch, 1975; Augier et al., 1982; Atkinson and Smith, 1983; Pellikaan and Nienhuis, 1988; Pirć and Wollenweber, 1988; Pérez-Lloréns and Niell, 1989; Borum et al., 1989; Pérez-Lloréns et al., 1991; Lee et al., 2004; Leoni et al., 2008; Hirst et al., 2016; Holmer et al., 2016).

Plant stable isotope contents can be utilized for study and assessment of ecological processes. For example, stable isotopes can be used to identify nutrient sources and processing within ecosystems (Dawson et al. 2002), and to provide information for food web analysis and the study of energy flow among trophic levels (Peterson and Fry 1987). Many studies have reported that seagrasses are a critically important food source for many aquatic life forms, such as dugongs, manatees, sea turtles, and waterfowl (Short et al., 2016), with most of the species eating the living leaves directly. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis is globally utilized to quantify food web structure in seagrass areas (Mukhtar et al., 2016). However, the stable isotope contents of primary producers are complex due to spatial and temporal variations in stable isotope contents (Campbell and Fourqurean, 2009).

Leaves were the representative tissue for studying the elemental and stable isotope contents in seagrasses. However, seagrass leaf blades can host large amounts of epiphytic algae, which can make significant contributions to the productivity of seagrass communities (Jernakoff et al., 1996; Borowitzka et al., 2006; Lavery et al., 2007). In addition, fine sediment particles can settle on seagrass blades, especially if already covered by epiphytes, which are highly efficient at trapping sediment (Hendriks et al., 2008; Pereira et al., 2009; Hamisi et al., 2013; Brodersen et al., 2017). Consequently, to measure the elemental and stable isotope contents, removal of blade attachments (epiphytes and fine sediment particles) by researchers is time consuming and has associated labor costs.

Eelgrass (*Zostera marina* L.) is a marine foundation species essential for coastal ecosystem services across the northern hemisphere. As a dominant species in northern China, it is distributed in the Bohai Sea and Yellow Sea, northern China (Zheng et al., 2013; Liu et al., 2019; Xu et al., 2020a; Zhou et al., 2014). The Bohai Sea is one of the most polluted sea areas in China (Zhou et al., 2020). The elemental and isotope content of eelgrass can be used to characterize nutritional status and environmental conditions of eelgrass beds (Udy et al., 1999; Leoni et al., 2008; Darnell et al., 2017; Yang et al., 2018). Yang et al. (2018) evaluated four seagrass species (*Zostera marina*, *Z. japonica*, *Z. caespitosa*, and *Phyllospadix iwatensis*) as early warning indicators of N overloading in the coastal waters of Rongcheng, in the South Yellow Sea, and concluded that the leaf C/N ratios of *Z. marina* were sensitive to N discharge, indicating that eelgrass may provide information for eutrophic evaluation. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis revealed that eelgrass was the main food source for the overwintering whooper swan *Cygnus cygnus* in Swan Lake, northern China (unpublished data). Most of the studies on eelgrass elemental and isotope content have been generally focused on the leaf (Duarte, 1990; Udy et al., 1999; Leoni et al., 2008; Darnell et al., 2017; Yang et al., 2018). Large amounts of epiphytes and fine sediment particles are attached to leaf blades, while there are almost no epiphytes or other material attached to the leaf sheath (Hwang et al., 2019; Xu et al., 2020a, 2020b).

In this study, we investigated temporal-spatial variations in the elemental and stable isotope content of eelgrass at nine sampling sites across northern China, and we explored the elemental and stable isotope content relationships between sheaths and blades, and between the rhizomes and roots. We were particularly interested in documenting the temporal variation in properties (C, N, P, $\delta^{13}C$, and $\delta^{15}N$ contents) among different eelgrass tissues within Swan Lake. We hypothesized that elemental contents demonstrate marked temporal variations in Swan Lake, and elemental and isotope contents show marked spatial variations across northern China. We additionally hypothesized that there are significant correlations in eelgrass properties (C, N, P, $\delta^{13}C$, and $\delta^{15}N$ contents) between blades and sheaths. Comparisons of eelgrass properties (C, N, P, $\delta^{13}C$, and $\delta^{15}N$ contents) at both broad (among all sites) and local (within sites) scales allowed us to examine the extent of temporal and spatial variations in the properties, and their effect on the isotope and elemental composition of eelgrass. Furthermore, we explored the suitability of eelgrass sheathes as a predictor of elemental and stable isotope contents in leaves, with potential utilization as a novel ecological indicator in geochemical research.

Table 1
Environmental conditions at the nine sampling sites and *Zostera marina* L. sampling dates.

Sampling sites and locations			Environmental conditions	Seagrass sampling dates
Yellow Sea	Swan Lake 37°21′N, 122°34′E		A marine lagoon. Salinity: 31.3–33.7 psu; annual average water temperature: 16.13 ± 7.76 °C; proportion of sand in sediment (0.063–2.0 mm): 81.02% ± 1.57%; average depth: <1.5 m; irregular semidiurnal mixed tides (tidal range of ~ 0.9 m); sampling site was exposed on most low tides (Xu et al., 2018). Annual concentrations of NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} were 2.14 ± 1.29, 1.31 ± 1.53, 0.15 ± 0.10, and 0.28 ± 0.17 μmol L^{-1}, respectively (Zhang et al., 2014; Zhou et al., 2015).	April 2015 to October 2016
	Huiquan Bay 36° 3′N, 120°20′E		An open bay. Salinity: 31.6–32.7 psu; annual average water temperature: 17.94 ± 7.80 °C; proportion of sand in sediment: 87.64% ± 4.70%; regular semidiurnal tides (tidal range of ~ 2.78 m); sampling site was exposed on most low tides (Xu et al., 2018). Annual concentrations of NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} were 3.81 ± 2.16, 5.87 ± 3.06, 0.68 ± 0.59, and 0.18 ± 0.09 μmol L^{-1}, respectively (Liu, 2012).	Jun 26 2016
	Changhai Island, Dalian		Salinity: 30.71–32.40 psu (Liang, 2019); annual average water temperature: 10.9 ± 6.5 °C (Zhang et al., 2016); regular semidiurnal tides (tidal range of ~ 2.5–3.0 m). Concentration ranges of NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} in 2017 were 0.065–1.839, 0.032–0.613, 0.355–4.645, and 0.065–0.355 μmol L^{-1}, respectively (Wang, 2018).	
		Xidatan Bay 39°13′N, 122°42′E	An open bay. Proportion of sand in sediment: 95.93% ± 0.91%; sampling site was exposed on most low tides.	May 17 2019
		Haxian Island 39°14′N, 122°30′E	An open bay. Proportion of sand in sediment: 68.83% ± 19.83%; sampling site was exposed on most low tides.	May 18 2019
		Linyang Bay 39°16′N, 122°35′E	An open bay. Proportion of sand in sediment: 98.8% ± 0.90%; sampling site was predominantly submerged (Subtidal zone).	May 19 2019
Bohai Sea	Huludao		Salinity: 29.95–32.25 psu; annual average water temperature: ~15 °C (Ji et al., 2015); irregular semidiurnal tides (tidal range of ~ 2.06 m (Wang, 1991); sampling site was exposed on most low tides. Ranges of concentrations of inorganic N and PO_4^{3-} in May 2016 were 0.094–0.242, and 1.858–5.097 μmol L^{-1}, respectively (Li et al., 2016).	
		Juehua Island 40°30′N, 120°46′E	An open bay. Proportion of sand in sediment: 87.12% ± 3.19%.	Jun 20 2019
		Xingcheng 40°32′N, 120°44′E	An open bay. Proportion of sand in sediment: 84.22% ± 1.02%.	Jun 21 2019
		Xiaohaishan Island 40°24′N, 120°36′E	An open bay. Proportion of sand in sediment: 86.91% ± 0.82%.	Jun 22 2019
	Caofeidian 39° 3′N, 118°42′E		An open bay. Salinity: 25.0–34.8 psu (Du et al., 2018); the annual average water temperature: ~12.3 °C (Du et al., 2018); proportion of sand in sediment: 93.28% ± 4.52%; irregular semidiurnal tides (tidal range of ~ 1.54 m); sampling site was exposed on most low tides. Concentrations of NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} and SiO_3^{2-} in Bohai Bay in the early summer of 2016 were 1.31 ± 1.24, 5.87 ± 5.70, 0.49 ± 0.38, 0.07 ± 0.08, and 5.45 ± 3.01 μmol L^{-1}, respectively (Zhang et al., 2018).	Jun 11 2019

*Modified after Xu et al., 2020b.

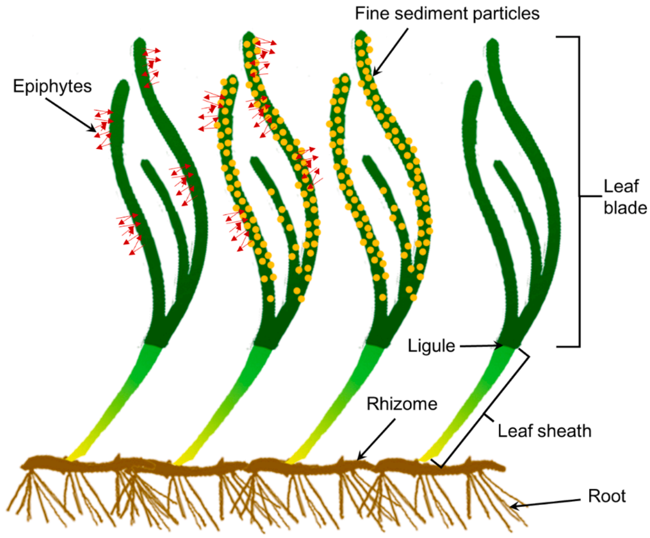

Fig. 2. Aboveground (leaf blade and leaf sheath) and belowground (root and rhizome) tissues of *Zostera marina* L. Epiphytes and fine sediment particles are attached to the leaf blade.

2. Material and methods

2.1. Study sites and seagrass sampling

The Bohai Sea is a semi-enclosed marginal sea on the northern coast of China; it is connected to Yellow Sea by the Bohai Strait. It is often divided into three major bays: Liaodong Bay, Bohai Bay, and Laizhou Bay, with the remaining area referred to as the Central Area (Fig. 1). With the development of industry and agriculture, construction of ports, and increased urbanization, the impact of human activities on the marine environment is increasing. The Bohai Sea has become one of the most polluted sea areas in China. In particular, seawater eutrophication is one of the main threats to the coastal ecological balance (Yang et al., 2007). The heavily eutrophic sea areas are mainly concentrated in Liaodong Bay and Bohai Bay (Yu et al., 2013).

The Yellow Sea is located between China and the Korean Peninsula. According to the physical geography characteristics, it is often divided into two parts, the North Yellow Sea and South Yellow Sea. The Yellow Sea is slightly less polluted than the Bohai Seas due to lower sewerage and higher water exchange rate. However, there are several serious ecological threats (e.g. heavy metal pollution and eutrophication) in some local waters, especially in Jiaozhou Bay (Fig. 1) (Zhu, 2007; Wang et al., 2010).

According to eutrophication status and seagrass distribution, nine sampling sites were selected in the Bohai Sea and Yellow Sea, northern China (Fig. 1). To explore temporal variations in the elemental content among different tissues (leaf blade, leaf sheath, rhizome, and root) of *Z. marina*, eelgrass samples from Swan Lake, a national nature reserve for the whooper swan *C. cygnus* in the South Yellow Sea (Table 1), were collected. Three eelgrass samples (30 × 30 cm quadrat) were randomly collected for each sampling event at a sampling region (Region 1; 37°21′2.50″, 122°34′41.28″). A total of fifteen sampling events were conducted from April 2015 to October 2016. In addition, to examine local spatial variations in elemental contents and their ratios at Swan Lake, we selected another sampling region (Region 2; 37°20′37.91″ N, 122°34′0.14″ E) for sampling in June 2015.

To explore spatial variations in the elemental content among different tissues of eelgrass, eelgrass samples were also collected in Huiquan Bay, located at the mouth of Jiaozhou Bay in the South Yellow Sea, Changhai Island (three sampling sites) in the North Yellow Sea, Huludao (three sampling sites) in Liaodong Bay, and Caofeidian in Bohai Bay (Fig. 1; Table 1). One sampling event was conducted for each sampling site with three samples replicates; sampling dates are shown in Table 1.

In addition, to explore spatial variations in stable isotope contents among eelgrass leaf blades, leaf sheaths, and belowground tissues (rhizome and root), samples were collected from Swan Lake, Huiquan Bay, Caofeidian, Xingcheng, and Linyang Bay on June 21, June 6, June 1, June 27, and June 18, 2020, respectively. All eelgrass samples were placed in plastic bags and transported to the laboratory in darkness while maintained at a low temperature.

2.2. Analyses

Eelgrass samples were rinsed with freshwater to remove any salt and sediment. For eelgrass elemental and stable isotope content analysis, all epiphytes and other material (Fig. 2) on the shoots were removed with a scalpel. Fresh eelgrass tissues for elemental content analysis were separated into leaf blade, leaf sheath, rhizome, and root (Fig. 2), and samples collected in June 2020 for stable isotope content analysis were separated into leaf blade, leaf sheath, and belowground tissues (rhizome and root). The ligule is located between the leaf sheath and leaf blade, this is the intersection point of the top of the sheath and where the leaf blade emerges (Gaeckle et al., 2006). Eelgrass tissues were dried at 60 °C to a constant weight, and ground and sieved for elemental (C, N, and P) and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) measurements.

The C and N contents of different tissues of *Z. marina* were analyzed using a VarioEL III CHONS analyzer (Elementar Analysensysteme GmbH, Germany), and the P content was determined by means of a modified method after Solórzano and Sharp (1980) for particulate total P determination (Zhou et al., 2003). The elemental content of C, N, and P in eelgrass was calculated on a dry weight basis; all elemental ratios were calculated on a molar basis.

C and N isotope ratios of eelgrass tissues were determined using an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, USA) coupled with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific).

The samples' isotopic ratios were reported in the standard delta notation (δ):

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

The values were reported relative to the international standards of N_2 and Vienna Pee Dee belemnite for C. Analytical precisions of the reported δ values, based on sample replicates, were ±0.08‰ for C and ±0.2‰ for N.

2.3. Statistical analysis

Results are presented as means ± SD. Because the homogeneity of variance was not significantly different ($p < 0.05$), spatiotemporal variations in elemental and stable isotope content, and differences in stable isotope content among tissues and sites were statistically analyzed using Scheirer-Ray-Hare test. Two-sided *t*-test was used to identify specific treatment differences. Simple linear regression was used to test the significance of the relationships between leaf sheaths and leaf blades, between rhizomes and roots regarding elemental content and their ratios, and between leaf sheaths and leaf blades regarding stable isotope content. The simple linear relationship can be expressed using Eq. 1:

$\boldsymbol{y} = \boldsymbol{a}\,\mathbf{x} - \boldsymbol{b}$ (1)where **y** and **x** represent the elemental contents or their ratios of the sheath/rhizome and blade/root, respectively; and **a** and **b** represent regression coefficients. Homogeneity of variance was tested using Levene's test (Zar, 1999). R v.4.0.2 for Windows 8.1 was used for data analyses. Simple linear regression analyses were considered significant at a probability level of $p < 0.05$.

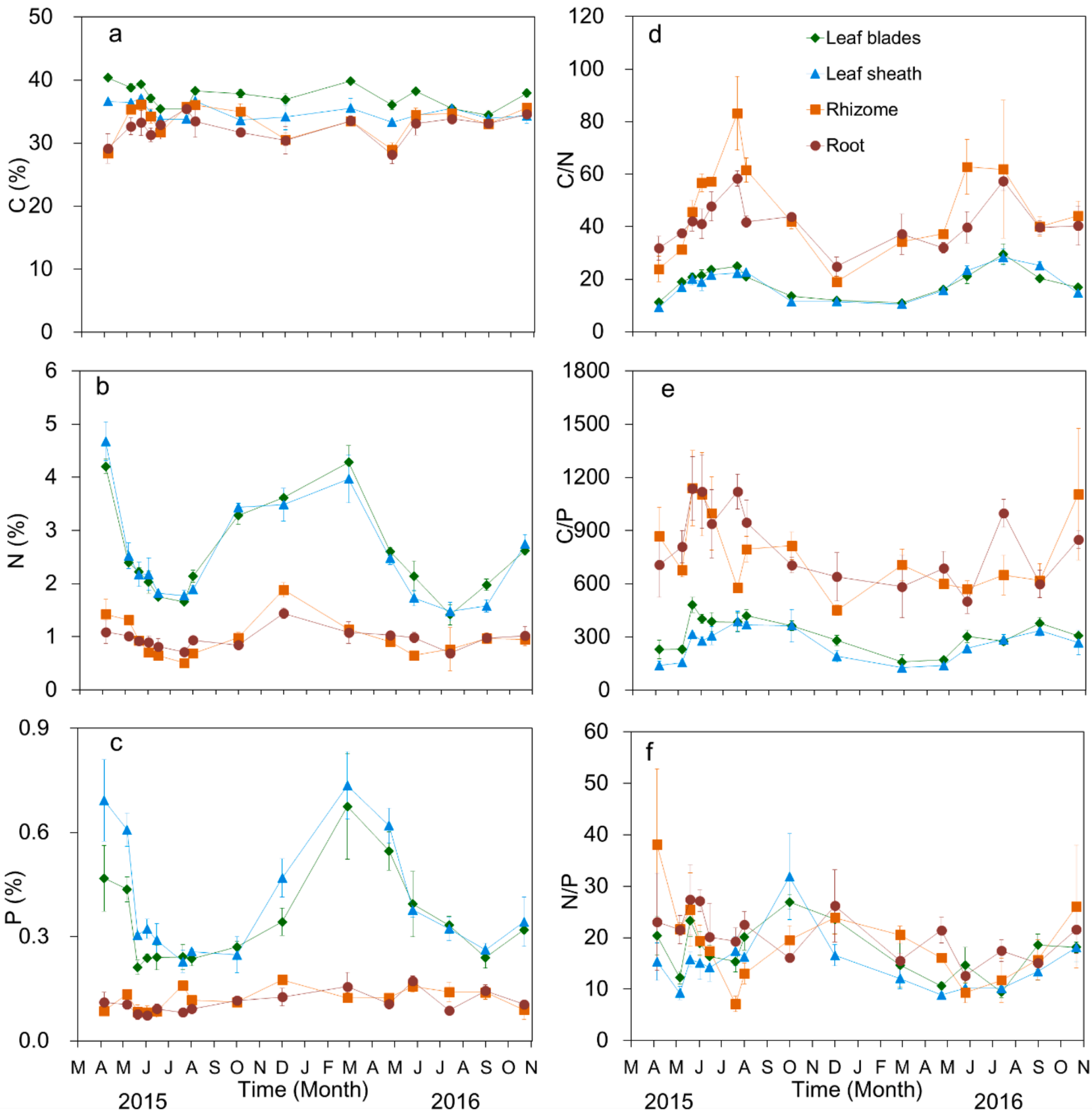

Fig. 3. Temporal changes in the content of C (a), N (b), and P (c), and the ratios of C/N (d), C/P (e), and N/P (f) of eelgrass tissues (leaf blade, leaf sheath, rhizome, and root) at Swan Lake. Values are means ± SD.

3. Results

3.1. *Variations in elemental contents and their ratios in different eelgrass tissues at Swan Lake*

3.1.1. *Temporal variation*

The annual pattern of C in eelgrass tissues was much more variable than that for N and P. Values fluctuated between 28.15% and 40.37% of dry weight (Fig. 3a), and average C contents of leaf blades, leaf sheaths, rhizomes, and roots were 37.43% ± 1.79%, 34.92% ± 1.28%, 33.56% ± 2.54%, and 32.44% ± 1.98%, respectively.

N contents of eelgrass tissues showed a clear annual trend with minima in summer and maxima in spring-winter (Fig. 3b). Values fluctuated between 0.51% and 4.68% of dry weight, with greater fluctuations in the aboveground than in the belowground tissues; the values of aboveground tissues were in general higher than those of belowground tissues ($p < 0.05$). The N content of leaf blades declined gradually from 4.20% ± 0.13% (April 2015) to 1.66% ± 0.03% (July 2015), and then increased to 4.28% ± 0.32% (February 2016), and that of sheaths showed a similar seasonal pattern.

The P content in aboveground tissues showed a clear seasonal trend similar to N content, but there was no obvious seasonal trend for belowground tissues (Fig. 3c). Values of aboveground tissues fluctuated between 0.21% and 0.74% of dry weight, with greater values in spring, and lower values in summer-autumn. The P contents were generally higher in aboveground than in belowground tissues ($p < 0.05$).

Given that the elemental contents (N and P) were higher in aboveground than in belowground tissues, coupled with C stability, the C/N and C/P were generally lower in aboveground than in belowground tissues ($p < 0.05$). The C/N ratios of eelgrass tissues showed a clear seasonal trend, peaking in summer and being lowest in winter-spring (Fig. 3d). The C/N ratios of leaf blades fluctuated between 10.91 and 29.56, with an annual mean of 17.45 ± 5.61. The C/P ratios of leaf blades ranged from 158.64 to 481.80, with an annual mean of 318.88 ±

Table 2
Ratios of the leaf blade-to-leaf sheath of eelgrass in the two regions at Swan Lake (SL) in elemental contents and its ratios.

Sampling Region	R (Leaf blade/Leaf sheath)					
	C	N	P	C/N	C/P	N/P
Region 1	1.090 ± 0.004[a]	0.912 ± 0.033	0.886 ± 0.009	1.196 ± 0.048	1.230 ± 0.008	1.029 ± 0.048
Region 2	1.046 ± 0.002[b]	0.985 ± 0.056	0.736 ± 0.103	1.064 ± 0.063	1.442 ± 0.213	1.363 ± 0.271

*Different letters indicate significant difference among treatments ($p < 0.05$).

109.16 (Fig. 3e). The blade N/P ratios ranged from 9.41 to 26.91 (Fig. 3f), with an annual mean of 18.41 ± 5.07, and all values were lower than 25 except for that in Oct 2015 (26.91).

3.1.2. Comparison between sampling regions

The leaf blade-to-leaf sheath ratios in elemental contents and their ratios of eelgrass in the two regions at Swan Lake are shown in Table 2. Most values were within the range of 0.8 – 1.4, and there were no significant differences in those values between the two regions, except carbon content, indicating that the relationships (ratios) between leaf blade and leaf sheath in elemental contents and their ratios were not affected by sampling region.

3.2. Spatial variation in elemental and stable isotope contents across northern China

3.2.1. Elemental contents

Elemental contents and their ratios differed significantly among sites (Fig. 4; $p < 0.001$), and the values were also significantly different among tissues (Fig. 4; $p < 0.001$). C, N, and P contents in aboveground tissues were generally higher than those in belowground tissues (Fig. 4). Therefore, elemental contents and their ratios were variable and demonstrated marked spatial variations ($p < 0.001$); they also revealed marked variations among different tissues ($p < 0.001$). In addition, the ranges of C/N, C/P, and N/P ratios in eelgrass leaf blades were 18.32–38.96, 193.38–473.99, and 10.54–24.60, respectively.

3.2.2. Stable isotope contents

The ranges of $\delta^{13}C$ isotope ratios in eelgrass leaf blades and leaf sheaths were (−12.33‰)–(−8.56‰) and (−11.32‰)–(−7.81‰), respectively. The $\delta^{15}N$ isotope ratio ranges of leaf blades and leaf sheaths were 5.57‰–9.64‰ and 5.89‰–9.79‰, respectively. Although there were no significant differences in stable isotope contents ($\delta^{13}C$ and $\delta^{15}N$ isotope ratios) among different tissues at the five sampling sites (Fig. 5; $p > 0.05$), they differed significantly among sites (Fig. 5; $p < 0.001$). Therefore, stable isotope contents demonstrated marked spatial variations ($p < 0.001$).

3.3. Relationships of elemental and isotope contents among different eelgrass tissues across northern China

Although elemental contents demonstrated marked temporal variations at Swan Lake, elemental contents (C, N, and P) and their ratios (C/N, C/P, and N/P) showed positive correlations between eelgrass leaf sheaths and leaf blades, and between rhizomes and roots (Fig. 6, $p < 0.01$). In particular, the R^2 values for the N, P, and C/N relationships between sheaths and blades were 0.89, 0.76, and 0.82, respectively.

Similarly, although elemental and isotope contents demonstrated marked spatial variations across northern China, there were positive correlations between leaf sheaths and blades (Figs. 7 and 8, $p < 0.01$). The R^2 values for the N, P, and C/N relationship between sheaths and blades at the nine sampling sites were 0.71, 0.75, and 0.65, respectively. In particular, the R^2 value for the isotope content relationship between sheaths and blades was approximately 0.94.

4. Discussion

The elemental and isotope content of eelgrass displayed marked temporal and spatial variations at both local and broad spatial scales across northern China, respectively. Despite these temporal and spatial variations in seagrass tissues, there were significant relationships between elemental and isotope contents among seagrass tissues. This study

Fig. 4. Elemental contents and their ratios in eelgrass tissues at the nine sampling sites. CF: Caofeidian, HQ: Huiquan Bay, HX: Haxian Island, JH: Juehua Island, LY: Linyang Bay, SL: Swan Lake, XC: Xingcheng, XD: Xidatan Bay, XH: Xiaohaishan Island.

Fig. 5. $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of eelgrass tissues at five sampling sites. CF: Caofeidian, HQ: Huiquan Bay, LY: Linyang Bay, SL: Swan Lake, XC: Xingcheng.

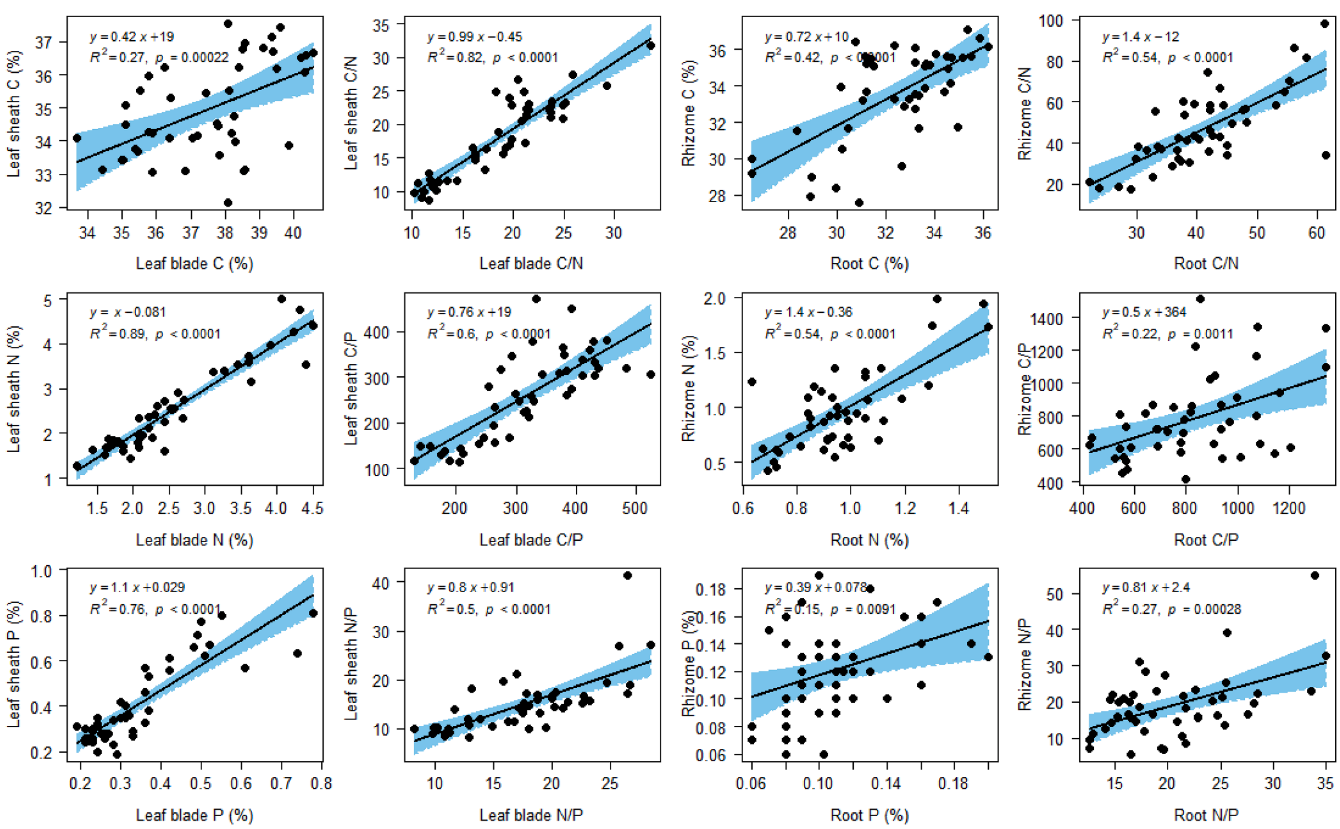

Fig. 6. Regressions for nutrient content (C, N, and P) and ratios (C/N, C/P, and N/P) between eelgrass leaf sheaths and leaf blades, and between rhizomes and roots at Swan Lake. Equations, R^2, and probabilities for each regression line are given in insets. Linear regression and 95% confidence interval of the regression are indicated.

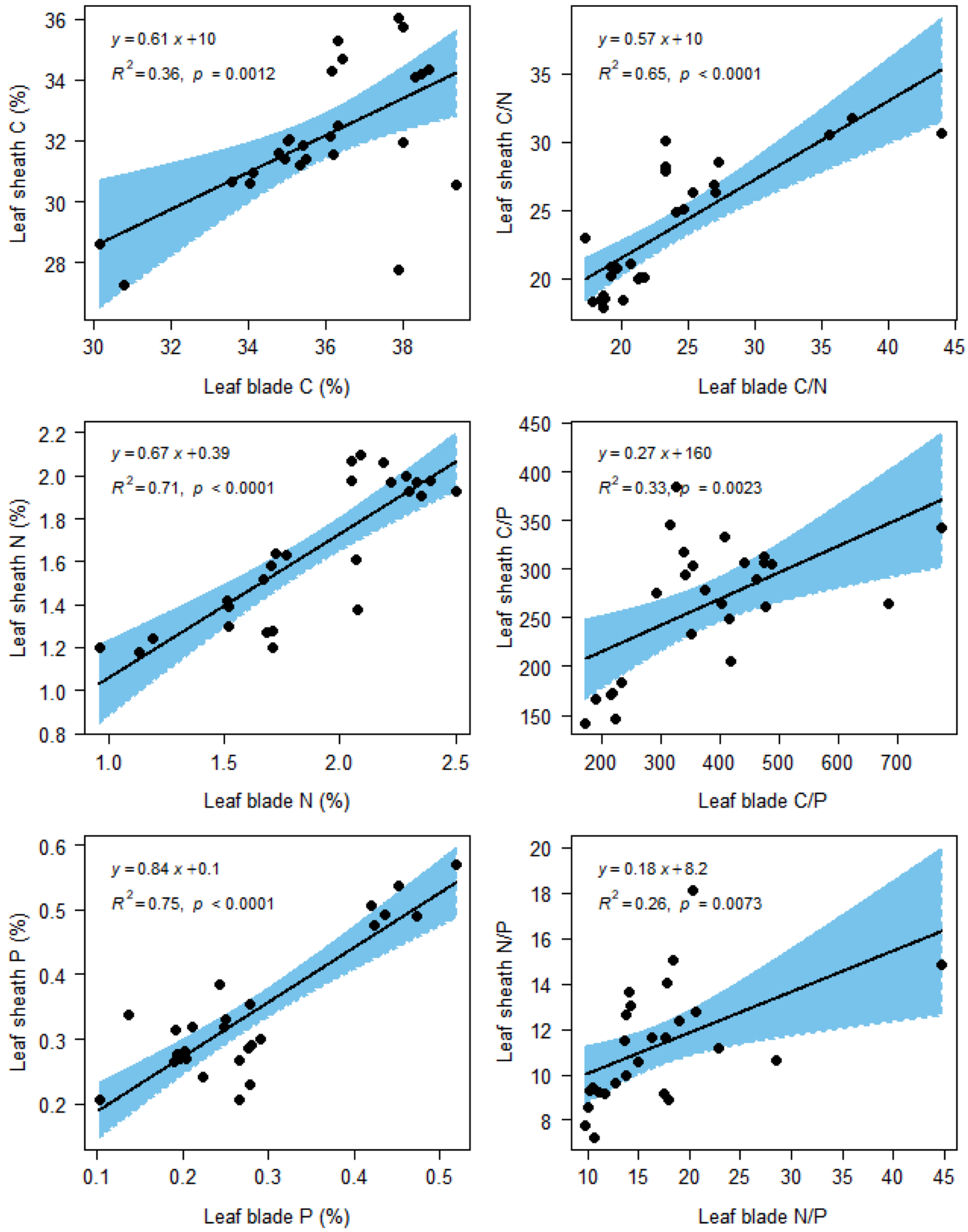

Fig. 7. Regressions for elemental content (C, N, and P) and ratios (C/N, C/P, and N/P) between eelgrass leaf sheaths and leaf blades at nine sampling sites. Equations, R^2, and probabilities for each regression line are given in insets. Linear regression and 95% confidence interval of the regression are indicated.

provides a novel and practical method, using eelgrass sheaths instead of leaf blades with attachments, to evaluate element and isotope data for coastal monitoring efforts and food web analyses.

The marked temporal variations at local spatial scales mainly resulted from seasonal seagrass growth. We documented temporal variation in elemental contents (C, N, and P) and their ratios in different tissues of *Z. marina* in Swan Lake. The patterns of seasonality in elemental contents (N and P) of aboveground tissues were higher in winter and lower in summer for *Z. marina*, consistent with other seagrass species (e.g., *Zostera noltii*, Pérez-Lloréns and Niell, 1993; *Posidonia oceanica*, Alcoverro et al., 2000). High elemental contents before the main growing season is the result of active nutrient uptake with rapid protein synthesis before growth (Brock et al., 1983; Pérez-Lloréns and Niell, 1993). Low elemental contents during summer can be explained by dilution processes (Stocker, 1980), resulting from a faster utilization than uptake in summer. In addition, the elemental contents (C, N, and P) of aboveground tissues at the nine sampling sites were generally higher than those of belowground tissues (Borum et al., 1989; Papadimitriou et al., 2005), suggesting that the major proportion of N and P accumulated in aboveground tissues, and the distribution of nutrients, was similar to the patterns reported for related species (e.g., *Z. tasmanica*, Bulthuis et al., 1992; *Zostera noltii*, Pérez-Lloréns and Niell, 1993).

The elemental and isotope content of eelgrass were also significantly influenced by many environmental factors with spatial variations. Atkinson and Smith (1983) found that plants living in nutrient-rich environments usually had lower C/N and C/P ratios than those living

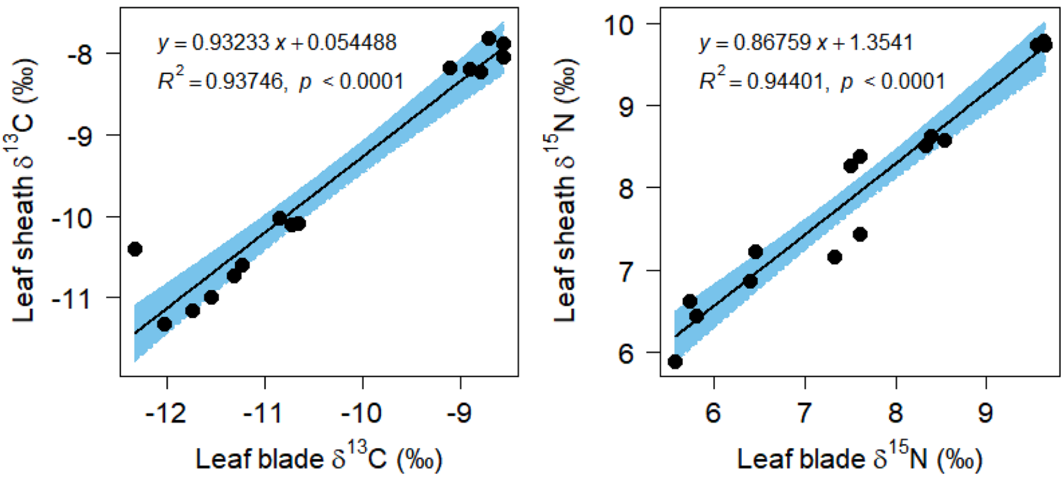

Fig. 8. Relationship of $\delta^{13}C$ and $\delta^{15}N$ isotope ratios between leaf sheaths and leaf blades at five sampling sites: Swan Lake, Huiquan Bay, Linyang Bay, Xingcheng, and Caofeidian. Equations, R^2, and probabilities for each regression line are given in insets. Linear regression and 95% confidence interval of the regression are indicated.

in low-nutrient environments, indicating that seagrass growing in eutrophic waters have higher elemental contents than in other waters. Yang et al. (2018) reported that *Z. marina* could be a highly valuable early warning environmental indicator on a global scale. Similarly, the stable isotope contents of plants were influenced by many environmental factors. C source, irradiance, and temperature are considered major factors controlling the stable C isotope content of seagrass material (Durako and Hall 1992, Abal et al. 1994, Grice et al. 1996, Hemminga and Mateo 1996). In the present study, the isotope content of eelgrass displayed marked spatial variations at broad spatial scales across northern China, indicating that it is important to document background variation in food web analysis.

Leaf sheaths and blades in grass species play distinct roles; the sheath strengthens the culm while the blade is the main site of photosynthesis (Toriba et al., 2019). However, there are positive metrological relationships between the blade and sheath. For example, there is a positive relationship between leaf sheath length and leaf growth, indicating that sheath length reliably reflects leaf growth (Tesařová et al., 1992; Gaeckle et al., 2006; Xu et al., 2020a, 2020b). Cha-Um et al. (2010) reported a positive relationship between the leaf blade and sheath in proline levels. Meling-López et al. (2016) reported that there were a strong correlation between eelgrass length structures (leaves and sheaths) and its biological and morphometric variables. The present study demonstrated significant correlations in elemental and isotope contents between eelgrass leaf blades and sheaths at both broad (among all sites) and local (within Swan Lake) scales, indicating that the correlations are not restricted by time and space. Therefore, eelgrass sheaths, with almost no attached epiphytes or other material, could be used as an ecological indicator of elemental and stable isotope contents in leaves. These correlations may be partly explained by the fact that the inside part of sheath comprises undifferentiated leaf.

5. Conclusion

Given the large amounts of attachments on leaf blades, which is time-consuming and labor-cost to clean them from leaf blades (Hwang et al., 2019; Xu et al., 2020a, 2020b), we propose the idea of replacing blades with sheaths in elemental and stable isotope content analysis. Although the elemental and stable isotope content of eelgrass in northern China displays considerable temporal-spatial variations at both local and broad spatial scales, there was always a significantly simple relationship between sheaths and blades that was not influenced by time and space. Therefore, eelgrass sheaths could be used as an ecological indicator of leaf blade elemental and stable isotope contents. In addition, given the large temporal variations in eelgrass elemental contents, we recommend synchronous sampling events, at broad spatial scales, for seagrass elemental content analysis, to minimize temporal variations. Overall, our findings might be applied to other seagrass species with sheaths, and also provide information for geochemical research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Yicheng Li, Hongrong Wang, and Jingtai Wang for their help in the field survey. This research was supported by the National Key R&D Program of China (2019YFD0901301), the National Science & Technology Basic Work Program (2015FY110600), the Key Research Project of Frontier Sciences of CAS (QYZDB-SSW-DQC041-1), the International Partners Program of the Chinese Academy of Sciences (133137KYSB20180069) and the Taishan Scholars Program (Distinguished Taishan Scholars).

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