

## $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variability in *Posidonia oceanica* associated with seasonality and plant fraction

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### Abstract

The carbon and nitrogen isotopic compositions of fractions of the seagrass *Posidonia oceanica* (L.) Delile in a Mediterranean shallow environment (Stagnone di Marsala, western Sicily) were investigated seasonally throughout 1998. The stable isotope ratios of seagrass leaves (intermediate and adult), rhizomes, leaf litter and aegagropiles were compared over 1 year in order to distinguish between seasonal and plant part variability. Significant differences in the isotopic composition tested using ANOVA were observed as a function of both plant fraction and season. There was an overall trend towards less discrimination against  $^{13}\text{C}$  in summer (average  $\delta^{13}\text{C} \sim -10\text{‰}$ ) than in winter (average  $\delta^{13}\text{C} \sim -13\text{‰}$ ). In contrast, greater  $\delta^{15}\text{N}$  enrichment was observed in winter (average  $\sim 5\text{‰}$ ) than in summer (average  $\sim 3\text{‰}$ ). The possibility of a link between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  seasonal variability and seagrass physiology such as plant carbon balance and reserve dynamics is proposed to explain the observed patterns. The demonstration of seasonal and plant fraction variability in *P. oceanica* isotopic composition provides a broader perspective to seagrass ecological and physiological studies. Overall, the observed variability can be up to 4 and 5‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, a range that largely exceeds that associated with the transition between consecutive trophic levels (1 and 3.5‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively). Our results corroborate the need for, at least, seasonal sampling designs to obtain a good understanding of organic matter fluxes into food webs and highlight the limitations in drawing general conclusions about food webs where a low seasonal sampling effort has been applied. They also suggest that stable carbon and nitrogen isotope ratios deserve attention as a possible valuable approach in seagrass ecophysiological studies.

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## 1. Introduction

Since the late 1970s, food web investigations in seagrass-dominated ecosystems have benefited from the use of carbon and nitrogen stable isotopic compositions (Fry et al., 1977, 1987; Dauby, 1989; Boon and Bunn, 1994; Jennings et al., 1997; Lepoint et al., 2000; Pinnegar and Polunin, 2000). Isotopic signatures are informative because their changes associated with the transition between two consecutive trophic levels can be predicted from the initial isotopic signal in the primary producer (Michener and Schell, 1994).

Despite its essential role in evaluating the potential diet of a particular consumer, little attention has been paid so far to the natural isotopic variability of marine macrophytes, the scarcity of information being specially noticeable in seagrasses. The stable isotopic composition of marine phanerogames is highly variable, owing to physiological and environmental factors that are usually linked to each other (Hemminga and Mateo, 1996). Specifically, the most important factors inducing variation in seagrass isotopic signatures appear to be, in order of decreasing importance, interspecific variability, the isotopic signal of the sources for synthesis (i.e. dissolved inorganic carbon and the various assimilable forms of nitrogen and sulphur), irradiance, and temperature, the last two being particularly determinant for carbon isotope ratios and strongly associated with seasonality. To our knowledge, this study represents the first attempt to assess the seasonal variability of  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  in different plant parts and compartments of the seagrass *Posidonia oceanica* (L.) Delile. Our ultimate goal is to contribute to increasing the accuracy of food web stable isotopic investigations in this key Mediterranean seagrass.

## 2. Materials and methods

Whole *P. oceanica* plants and plant detritus were collected in January, April, July and November 1998 in the Stagnone di Marsala coastal sound (37°52'N; 12°28'E), a semi-enclosed basin of approximately 2000 ha in western Sicily, Italy, with an average depth of 1.5 m. Two openings (in the northern and southern parts) allow water exchange. Water temperature and salinity present a wide annual range (11.8–28.6 °C; 33.1–45.5 PSU, respectively; Sarà et al., 1999). The basin is oligotrophic, with chlorophyll-a values of around  $1.0\text{ }\mu\text{g l}^{-1}$  (Sarà et al., 1999). *P. oceanica* and *Cymodocea nodosa* colonize the sandy-muddy bottom of the central-southern and northern areas of the basin, respectively (Scilipoti, 1998). No significant continental water and organic matter inputs to the basin have been observed (Sarà et al., 1999).

Samples were randomly collected in triplicate over an area of ca. 400 m<sup>2</sup>. Each replicate consisted of a composite sample of 10 whole plants and 5 or more aegagropiles in order to minimize within-meadow variability (Fry et al., 1987). Leaf litter was collected from three 50 cm × 50 cm squares approximately 10 m apart. All samples were frozen at –20 °C within 24 h after sampling. Plants were fractionated in intermediate and adult leaves (according to Giraud, 1979), and rhizomes (including the attached dead leaf sheaths). Special care was taken to use always the same length of living rhizome (the top 4 cm) to minimize isotopic variability derived from the use of sections of rhizome of different age or bearing a different rhizome to sheath ratio. Among the plant detritus, leaf litter and aegagropiles (round to

ovoid packed dead sheath fibers of up to 10 cm diameter; Boudouresque and Meinesz, 1982) were considered separately. All samples were first rinsed to remove sediment particles then acidified in 1N HCl for varying times, depending on the plant fraction, to remove carbonates. They were then rinsed with distilled water, oven dried at 60 °C to constant weight and ground to a fine powder.

Isotopic analysis was performed using a Finnigan Delta S IRMS in continuous flow configuration. Overall analytical error was always less than 0.2‰ for both carbon and nitrogen isotope ratios. Isotopic data is reported in common delta ( $\delta$ ) units referred to VPDB and atmospheric nitrogen standards for carbon and nitrogen, respectively.

Variability in isotopic composition associated with plant fraction and seasonality was analysed using ANOVA (mixed design, GMAV-1997 statistical package, University of Sydney, Australia; Underwood, 1997). Four levels were considered for both plant fraction (intermediate and adult leaf, rhizome and leaf litter) and season (spring, summer, autumn and winter). Times (two for each season) were treated as random (two levels) and nested in season. Three replicates were randomly effected in each time. Heterogeneity of variances was tested using Cochran's test prior to analysis of variance and appropriate means compared using Student–Newman–Keuls (SNK) tests. Due to the probable high residence time of the material in the aegagropiles this plant fraction was not considered in the analysis outlined above.

### 3. Results and discussion

The mean carbon and nitrogen isotopic compositions obtained in this study (Table 1) were of the same order as those reported in previous studies (−16.4 to −8.3‰ for  $\delta^{13}\text{C}$  and 2.8 to 3.5‰ for  $\delta^{15}\text{N}$ ; McMillan et al., 1980; Cooper and De Niro, 1989; Dauby, 1989; Jennings et al., 1997; Lepoint et al., 2000; Pinnegar and Polunin, 2000). Substantial significant differences were found associated with both seasonality and plant fraction (Table 2).

Although differences in  $\delta^{13}\text{C}$  between intermediate and adult leaves were only significant in spring (SNK test:  $P \leq 0.01$ ), the pattern strongly suggests that intermediate leaves are more enriched in  $^{13}\text{C}$  than adult leaves (Fig. 1, top). This observation is coherent with current theory, which demonstrates that for constant carbon availability, a higher carbon demand for photosynthesis (as would be expected in younger leaves due to their higher photosynthetic

Table 1

Annual means (January, April, July and November 1998) of carbon and nitrogen stable isotopic composition of different components of *Posidonia oceanica* in the study area (S.D.: standard deviation)

	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
	Mean	±S.D.	Minimum	Maximum	Mean	±S.D.	Minimum	Maximum
Intermediate leaf	−10.3	1.1	−12.2	−8.8	3.0	1.4	1.0	5.6
Adult leaf	−11.5	1.6	−15.2	−9.3	2.8	1.3	1.0	5.9
Leaf litter	−10.6	1.1	−12.7	−9.0	3.8	2.1	2.3	9.9
Rhizome	−12.1	1.2	−14.6	−10.8	5.5	1.7	2.9	8.3
Aegagropilae	−13.6	0.7	−14.3	−12.9	3.5	0.9	2.5	4.1

Table 2  
Summary of ANOVA results

Source of variation	d.f.	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
		MS	F	P	MS	F	P
Fraction	3	17.0	25.1	***	0.2	14.2	***
Season	3	21.7	6.9	*	0.1	18.9	**
Time (seas)	4	3.2	8.2	***	0.0	1.0	NS
Fraction $\times$ season	9	4.5	6.7	**	0.1	4.1	*
Fraction $\times$ time (seas)	12	0.7	1.8	NS	0.0	2.5	*
Residual	64	0.4			0.0		
Cochran's C				NS			NS

NS: non-significant difference ( $P \geq 0.05$ ). d.f.: degrees of freedom; MS: mean squares; F: Fischer's F; P: significance level.

\*  $P \leq 0.05$ .

\*\*  $P \leq 0.01$ .

\*\*\*  $P \leq 0.001$ .

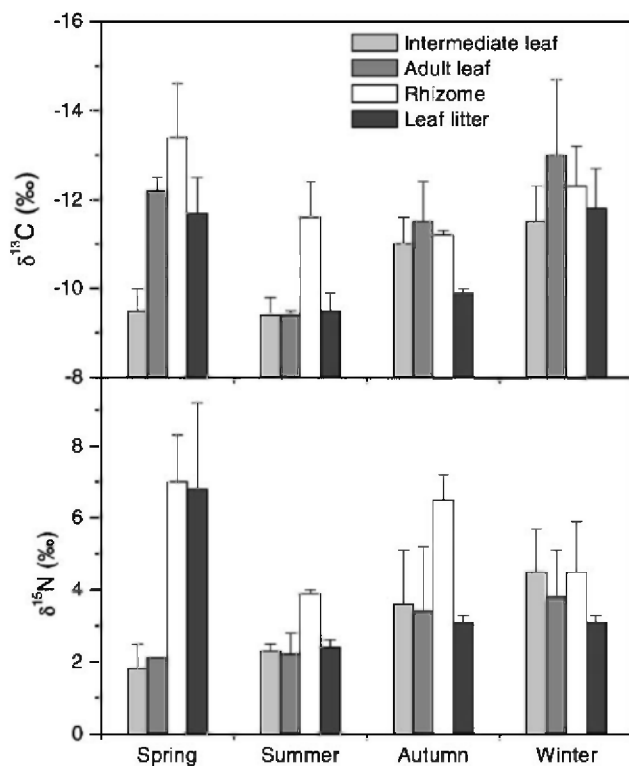


Fig. 1. Seasonal changes in carbon and nitrogen isotopic composition of *P. oceanica* leaves (intermediate and adult), rhizomes and leaf litter. Error bars represent the standard deviations of triplicate composite samples.

capacity; e.g. [Alcoverro et al., 2001](#)) results in lower discrimination against  $^{13}\text{C}$  ([Farquhar et al., 1989](#); [Maberly et al., 1992](#)). The same rationale can be applied to the general seasonal pattern observed. Maximum and minimum  $\delta^{13}\text{C}$  values were obtained in summer and winter, respectively, while spring and autumn yielded intermediate values. Again, a higher carbon demand during the summer period, owing to maximum irradiance levels reaching the plant, would result in lower discrimination against  $^{13}\text{C}$  (i.e. in an increase in  $\delta^{13}\text{C}$  values). This relationship between irradiance and seagrass carbon isotopic composition has already been suggested as being associated with experimental changes in light in various seagrass species ([Grice et al., 1996](#)), with depth gradient ([Durako and Hall, 1992](#)) and with interannual changes in water transparency ([Mateo et al., 2000](#)), the last two in *P. oceanica*.

Rhizomes were significantly richer in  $^{13}\text{C}$  in summer and autumn than in winter and spring. Similar patterns have been described for *Zostera marina* ([Cooper, 1989](#)). *P. oceanica* is a sucrose-storing plant that accumulates up to eight times more sucrose in the rhizomes than in the leaves during summer and early autumn ([Alcoverro et al., 2001](#)). In spring and winter, soluble carbohydrate concentration in *P. oceanica* below ground organs is similar to that in the leaves. Soluble carbohydrates in general, and sucrose in particular, are more enriched in  $^{13}\text{C}$  than is total plant material (e.g. [Duranceau et al., 1999](#)). The seasonal pattern in reserve allocation in *P. oceanica* thus seems consistent with the  $\delta^{13}\text{C}$  seasonal variability observed in the rhizomes. The significant interaction plant fraction  $\times$  season revealed by ANOVA suggests that  $^{13}\text{C}$  distribution within the plant varies with season. Again, this observation may be explained in large part by the annual pattern of reserve distribution.

No important isotopic effects associated with litter decay have so far been reported over a short to medium time period ([Thayer et al., 1978](#); [Fry et al., 1987](#)). Except in autumn and winter, when the  $\delta^{13}\text{C}$  of leaf litter was significantly lower than that of living leaves (SNK test:  $P \leq 0.01$ ), no significant differences were observed, supporting the above hypothesis. Leaf fall and export dynamics should be taken into account when interpreting leaf litter seasonal isotopic composition data. The substantial depletion in  $^{13}\text{C}$  in aegagropiles compared with the other fractions may be ascribed to the long period of decay this material undergoes (years and, probably, decades). Aegagropiles are formed almost exclusively by recalcitrant fibres from *P. oceanica* leaf sheaths. Highly decay-resistant cellulose and lignin are thought to be the major components of this material (as is the case for old wood, [MacFarlane et al., 1999](#)). The  $^{13}\text{C}$  depletion of these two compounds with respect to the bulk organic matter (particularly in the case of lignin, e.g. [Von Fischer and Tieszen, 1995](#); [MacFarlane et al., 1999](#)) would be reflected in the  $^{13}\text{C}/^{12}\text{C}$  ratio observed.

When nitrogen isotopic composition variability is used in applications other than as a metabolic or geochemical tracer, its interpretation remains controversial and poorly explored (e.g. [Handley and Scrimgeour, 1997](#); [Handley et al., 1998](#)). Although many clear patterns have been found in the  $\delta^{15}\text{N}$  values of plants, the causal processes are not yet understood. This is a result of the large number of potential interacting factors that affect the nitrogen stable isotope signature of plant tissues. These factors include the rapidly changing sediment-N isotopic signal (affected by simultaneously occurring multiple N sources or sediment bacterial activity), symbiotic associations and the conceptually complex nature of plant nitrogen metabolism ([Shearer and Kohl, 1986](#); [Handley and Scrimgeour, 1997](#); [Handley et al., 1998](#)).

Despite these interpretational difficulties, as in the case of  $\delta^{13}\text{C}$  values, our  $\delta^{15}\text{N}$  seasonal data (Fig. 1, bottom) strongly suggest a direct link with nitrogen reserve dynamics. As in most terrestrial plants (Chapin et al., 1990), *P. oceanica* nitrogen is mainly stored as soluble proteins and free aminoacids (largely asparagine), the total nitrogen concentration being two to four times higher in the rhizomes than in the leaves (Invers, 2002). The accumulated nitrogen can represent up to 8% of the total dry weight of the rhizome (Lawrence et al., 1989; Mateo and Romero, 1997). Nitrogen reserves begin to be remobilised in spring, starting with those stored in the leaves. Accordingly, leaf nitrogen reserve (LNR) minimum is attained earlier in the summer than that for rhizome nitrogen reserves (RNR). In autumn, both LNRs and RNRs begin to load up again until the winter maximum is attained (Invers, 2002; Invers et al., submitted). Because proteins and aminoacids are more enriched in  $^{15}\text{N}$  than the bulk material, changes in  $^{15}\text{N}$  natural abundance in the different plant parts may roughly mirror the relative changes in the concentration of nitrogen-storing compounds in *P. oceanica*, as has been proposed for trees (Nasholm, 1994).

In our study, LNRs appear to have already been remobilised in spring while RNRs seem to remain intact, as the presence of much lower  $\delta^{15}\text{N}$  values in the leaves than in the rhizomes would suggest. In summer, both plant parts reach their minima, as indicated by the minimum  $\delta^{15}\text{N}$  values. Then, a gradual increase in  $\delta^{15}\text{N}$  values (and thus in nitrogen reserves) takes place towards winter, except for RNRs, which present lower values in winter than in autumn. However, this discrepancy may be attributed to the high interannual variability that has been repeatedly reported for *P. oceanica* nutritional status and carbon balance (e.g. Alcoverro et al., 2001).

Finally, it should be emphasised that, owing to the scarcity of information on  $^{15}\text{N}$ –plant nitrogen relations and the lack of statistical significance in some of the variability observed, this discussion is necessarily of a speculative nature.

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