RESEARCH ARTICLE

Spatial and temporal variation in the elemental and stable isotopic content of the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* from the Illes Balears, Spain

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Abstract Morphology, elemental content and isotopic composition of leaves of the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* were highly variable across the Illes Balears, a Spanish archipelago in the western Mediterranean, and varied seasonally at one site in the study area. The data presented in this paper generally expand the reported ranges of nitrogen, phosphorus, iron and arsenic content and $\delta^{13}C$ and $\delta^{15}N$ for these species. Nitrogen and phosphorus content of *P. oceanica* leaves also showed significant seasonal variability; on an annual basis, *P. oceanica* leaves averaged 1.55% N and 0.14% P at this monitoring site. Both N and P were more concentrated in the leaves in winter than in summer, with winter maxima of 1.76% N and 0.17% P and summer minima of 1.34% N and 0.11% P. There was no significant annual pattern observed in the $\delta^{13}C$ of *P. oceanica* leaves, but there was a repeated 0.6‰ seasonal fluctuation in $\delta^{15}N$. Mean annual $\delta^{15}N$ was 4.0‰; $\delta^{15}N$ was lowest in May and it increased through the summer and autumn to a maximum in November. Over the geographic range of our study area, there were interspecific differences in the carbon, nitrogen and phosphorus content of the two species. *Posidonia oceanica* N:P ratios were distributed around the critical value of 30:1 while the ratios for *C. nodosa* were lower than this value, suggesting *P. oceanica* we collected was not consistently limited by N or P while *C. nodosa* tended toward nitrogen limitation. Nutrient content was significantly correlated to morphological indicators of plant vigor. Fe content of *P. oceanica* leaves varied by a factor of 5×, with a minimum of 31.1 μg g^{-1} and a maximum of 167.7 μg g^{-1}. Arsenic was present in much lower tissue concentrations than Fe, but the As concentrations were more variable; the maximum concentration of 1.60 μg g^{-1} was eight times as high as the minimum of 0.20 μg g^{-1}. There were interspecific differences in $\delta^{13}C$ of the two species; *C. nodosa* was consistently more enriched ($\delta^{13}C = -7.8 \pm 1.7$‰) than *P. oceanica* ($-13.2 \pm 1.2$‰). The $\delta^{13}C$ of both species decreased significantly with increasing water depth. Depth related and regional variability in the $\delta^{13}C$ and $\delta^{15}N$ of both species were marked, suggesting that caution needs to be exercised when applying stable isotopes in food web analyses.

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Introduction

Elemental content and isotopic ratios are increasingly being used to describe nutritional status and nutrient supply in macrophyte communities, but, there can be substantial variation in these values in a seagrass landscape. Elemental content of plants, especially of the essential elements for proper metabolic functioning, is related to the availability of those elements in the environment relative to other elements and to the rate of growth. Spatial gradients in N or P availability are reflected by spatial patterns in the N and P content

of seagrasses along the gradient (Fourqurean et al. 1992, 2005), and nutrient addition experiments have shown that the fertilization will increase the nutrient content of seagrass leaves if the starting composition was below some element-specific critical value (Duarte 1990). Other factors can influence nutrient content as well. For example, N and P content of seagrass leaves increases as light availability decreases (Abal et al. 1994). Herbivory can also influence the bulk nutrient content of a plant's leaves, by removing older, partially senesced leaves (which increases nutrient content; Thayer et al. 1984a) or conversely by preferential grazing on high-nutrient content plant parts (Goecker et al. 2005), which lowers the nutrient content of the remaining leaves.

Given that many factors can influence elemental content of plant tissues, the relationship between plant morphology and nutrient content is not straightforward. One would expect plants living with ample resource supply, in high light and high nutrient environments, to be morphologically robust and have high nutrient content. But, since low light can also lead to high nutrient content, nutrient content itself may be unrelated to the realized size of the plants.

The factors governing the stable isotopic composition of plants are potentially more complex than those influencing elemental content. Any factor that influences the stable isotopic content of the elemental sources will necessarily influence the isotopic composition of plants. Such relationships have led to the hope that isotopic ratios of seagrass and other plant tissues can be used as indicators of the source of N and C for plant growth. However, the fact that factors, which influence elemental uptake rates by the plants, also can alter the isotope ratios of the plants makes the application of information about natural abundance of stable isotopes problematic (Fourqurean et al. 2005). Light availability (Cooper and DeNiro 1989; Abal et al. 1994; Grice et al. 1996; Lepoint et al. 2003) and seasonality (Vizzini and Mazzola 2003; Vizzini et al. 2003) can lead to differential fractionation of the available isotope pools, such that plants grown under low light conditions are isotopically depleted in heavier isotopes compared to plants from high light environments. Owing to the dependence of photosynthesis on light, it is possible that plant vigor could be related to the stable isotope composition of the plants.

The seagrasses of the Illes Balears in the Western Mediterranean grow in very clear, nutrient-poor water and are rooted in carbonate sediments. Plants growing in oligotrophic carbonate environments are often found to be P-limited (Short 1987) as a consequence of the limited mobility of phosphate in carbonate

sediments (de Kanel and Morse 1978). Not all carbonate systems are P-limited, however. Many such systems can be limited by N availability (Ferdie and Fourqurean 2004), and there have been suggestions that the low iron availability in carbonate sediments can also influence seagrass growth (Duarte et al. 1995). Further, interactions between P availability and the uptake of arsenic by marine plants may make As content a proxy for P availability (Fourqurean and Cai 2001). Elemental stoichiometry has proven a useful tool in indicating the relative roles of major plant nutrients in limiting plant growth.

In this paper, we document the spatial variability in shoot morphology; C, N, P, Fe and As concentration and stable isotope ratios (δ^{13}C, δ^{15}N) in seagrasses from the Illes Balears, and examine the intra-annual pattern in these properties for one *P. oceanica* meadow on the island of Mallorca. We were particularly interested in documenting the range of variation in these elemental and isotopic components of the plants, as well as exploring what relationships, if any, existed between plant vigor, as reflected by plant morphology, and the elemental and isotopic content. We aimed to better understand the pattern of variability and the nature of the correlations among these indicator variables to generate hypotheses about causal mechanisms and inform our design of manipulative experiments to test those hypotheses.

Methods

Seagrasses are a common feature of the coastal zone of the Illes Balears in the western Mediterranean sea. The very clear water that surrounds these carbonate islands allows the penetration of seagrasses to over 30 m deep. Three species of seagrasses occur in these islands: *P. oceanica* meadows are the most common seagrass habitat; *C. nodosa* meadows are not uncommon and *Zostera noltii* is more rarely encountered. We made an attempt to collect samples from as wide a range of sites as possible, from just subtidal to sites over 30 m deep; *P. oceanica* was collected from 159 locations and *C. nodosa* was collected from 20 locations (Fig. 1). We repeatedly sampled one site, ca. 300 m off of the shoreline in 7 m of water near Magalluf on the island of Mallorca (Fig. 1) in order to assess the extent of seasonality in the morphology, elemental and isotopic content of *P. oceanica*. At each sampling site, 5 intact shoots of *P. oceanica* or 15 shoots of *C. nodosa* were haphazardly collected from a 10 m^2 area. For this paper, a shoot was defined as a single leaf-bearing branch of the larger clonal plant; it is likely that the shoots that
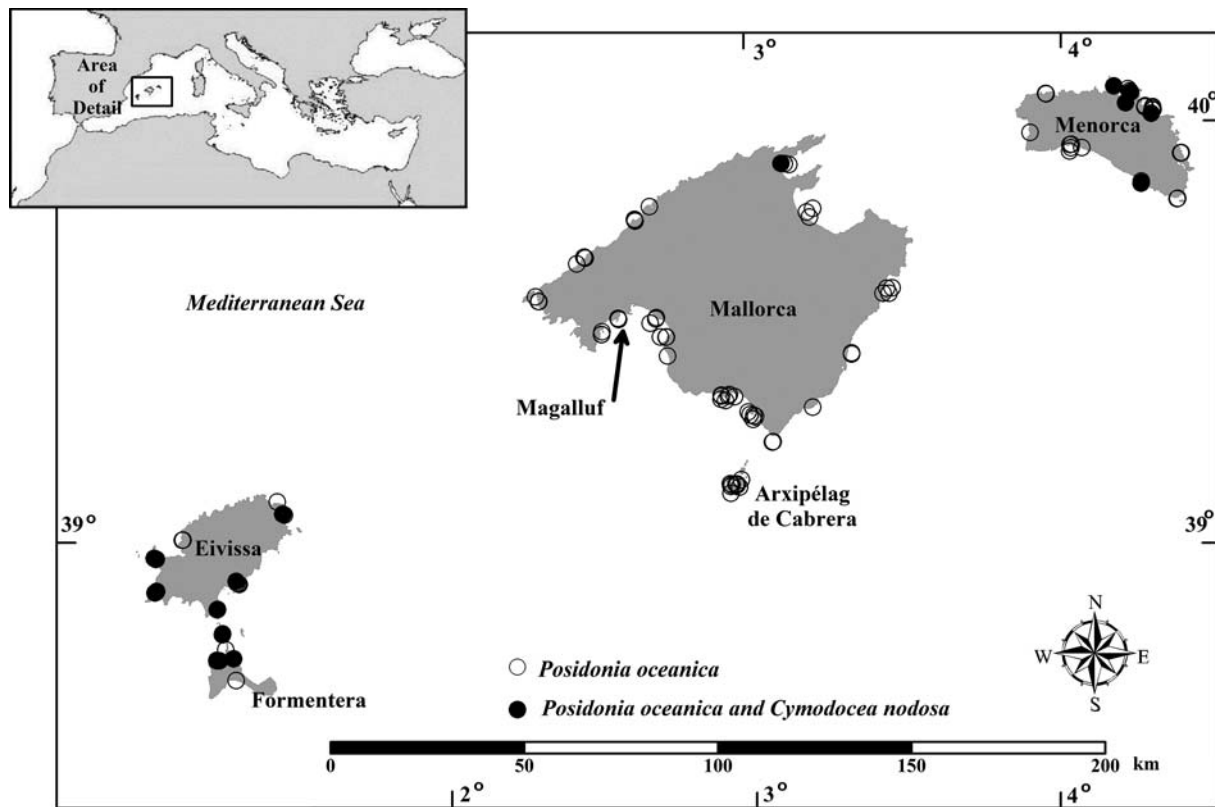
Fig. 1 Map of sampling locations in the Illes Balears, Western Mediterranean. The location of one site (Magalluf) that was sampled repeatedly in a time series is shown. *Open symbols* indicate sites from which only *Posidonia oceanica* was collected; *closed symbols* indicate locations where both *P. oceanica* and *C. nodosa* were collected

composed a single sample were from different genetic individuals, but it was possible that we occasionally sampled multiple shoots from a single clone. These shoots were returned to the lab, where all attached green leaves were cut from the shoots and cleaned of adhering epiphytes by gently scraping with a razor blade. Cleaned leaves were measured to determine leaf and shoot morphology. For each collection of shoots, we calculated mean leaf length, mean leaf width, mean number of leaves per shoot and the mean one-sided leaf area per shoot. All leaves from a site were pooled and dried at 80°C. Dry weight was used to estimate the mean leaf mass per shoot for each sample. Dried leaves were ground to a fine powder using a motorized agate mortar and pestle. Powdered samples were analyzed in duplicate for carbon and nitrogen content using a CHN analyzer (Fisons NA1500). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al. 1992). Iron content was determined on an atomic absorption spectrometer after a concentrated acid digestion. Arsenic content was determined by a HNO_3/H_2O_2 digestion followed by inductively coupled plasma mass

spectrometry (Cai et al. 2000). Elemental content was calculated on a dry weight basis (i.e., mass of element/dry weight of sample); elemental ratios were calculated on a mole:mole basis.

All isotopic analyses were measured using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) procedures. The EA was used to combust the organic material and to reduce the formed gases into N_2 and CO_2, which were measured on a Finnigan MAT Delta C IRMS in a continuous flow mode. The samples' isotopic ratios (R) are reported in the standard delta notation (‰): δ (‰) = $[(R_{sample}/R_{standard})-1] \times 1{,}000$. These results are presented with respect to the international standards of atmospheric nitrogen (AIR, N_2) and Vienna Pee Dee belemnite (V-PDB) for carbon using the secondary standards IAEA N-3 for $\delta^{15}N$ and IAEA CH-6 for $\delta^{13}C$. Analytical reproducibility of the reported δ values, based on sample replicates, was better than ± 0.2‰ for $\delta^{15}N$ and ± 0.08‰ for $\delta^{13}C$.

Statistical methods

Seasonality in the characteristics of *P. oceanica* was addressed by fitting a model of the form:

$$Y = \hat{Y} + \text{amp} \cdot \sin(\text{DOY} + \phi),$$

where $\hat{Y}$ is the mean value of Y, amp was the amplitude of the sine function, DOY was the day of the year transformed into radians (365 days $= 2\pi$ radians), and ϕ was a phase angle that controlled the timing of the peak in the sine wave. Model fitting was done using an iterative least-squares technique, and a model fit was considered significant if the 95% confidence interval for the estimate of the parameter amp did not overlap 0. This sine model has been successfully used to describe seasonality in growth, biomass, elemental and isotopic content of the seagrass *Thalassia testudinum* in south Florida (Fourqurean et al. 2001, 2005).

Relationships among the measured morphological, elemental and isotopic characteristics of samples of the same species across all of the sampled sites were assessed using non-parametric correlations (Spearman's ρ) because many of the variables were not normally distributed.

Results

Seasonality in *Posidonia oceanica*

At our monitoring location in Magalluf (see Fig. 1 for location), the morphology, elemental and isotopic content of *P. oceanica* varied following seasonal patterns over the period 2001–2003 (Fig. 2). Leaf lengths varied by an average of 315 mm on a seasonal basis ($2 \times$ amp from the sine model); they increased from a December minimum of ca. 110 mm to a June maximum of over 400 mm, while leaf width remained constant throughout the year. Conversely, shoots had more than six leaves per shoot in winter, and fewer than five leaves per shoot in summer. As a result of the patterns in leaf length and the number of leaves per shoot, the leaf area per shoot varied by over 150 cm^2 shoot^{-1} on a seasonal basis, from November minima of ca. 75 cm^2 shoot^{-1} to May maxima of ca. 225 cm^2 shoot^{-1}.

Nitrogen and phosphorus content of *P. oceanica* leaves also showed significant seasonal variability at Magalluf (Fig. 3), while carbon content did not vary seasonally. On an annual basis, *P. oceanica* leaves averaged 1.55% N and 0.14% P at this monitoring site. Both N and P were more concentrated in the leaves in winter than in summer, with winter maxima of 1.76% N and 0.17% P and summer minima of 1.34% N and 0.11% P. There was no significant annual pattern observed in the δ^{13}C of *P. oceanica* leaves because of isotopically light δ^{13}C values measured in November in both 2001 and 2002. There was a repeated 0.6‰ sea-
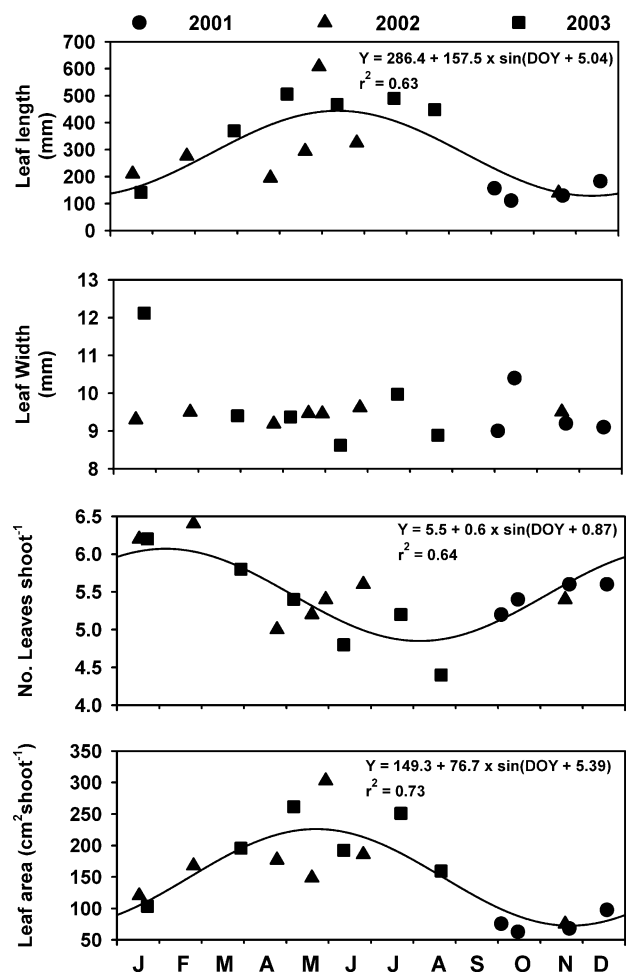

Fig. 2 *Posidonia oceanica*. Annual pattern in seagrass morphology at the Magalluf monitoring site. *Lines* represent the significant fit of the sine model to the data

sonal fluctuation in δ^{15}N (Fig. 4). Mean annual δ^{15}N was 4.0‰; δ^{15}N was lowest in May and it increased through the summer and autumn to a maximum in November.

Spatial variability in *Posidonia oceanica* and *Cymodocea nodosa*

Posidonia oceanica samples were collected from 159 locations distributed from the island of Formentera in the south to Menorca in the north (Fig. 1). *Cymodocea nodosa* was more rarely encountered, and was collected from 20 locations in the same geographic range. *Posidonia oceanica* samples were collected as deep as 30.8 m, but since we did not make an attempt to document the deep edges of the samples meadows it is certain to occur at depths greater than 30.8 m (c.f. Marbà et al. 2002). *Cymodocea nodosa* was collected as deep as 26.0 m, and likely occurs at deeper locations.
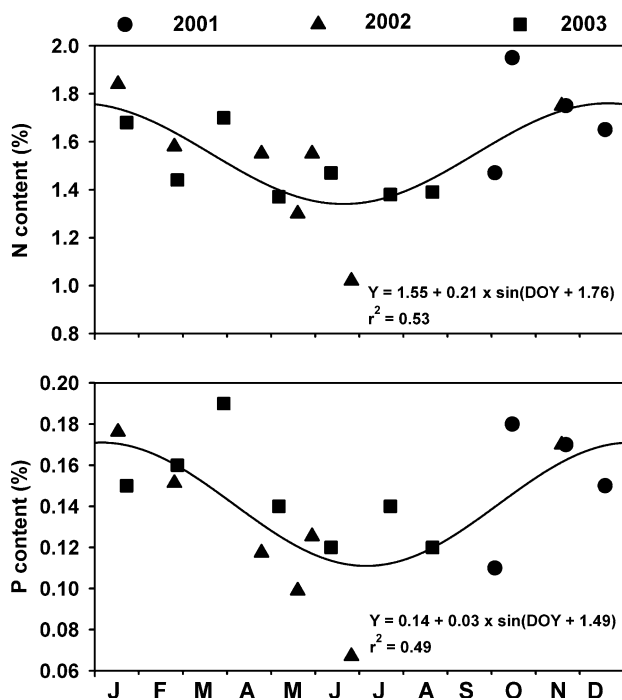
Fig. 3 *Posidonia oceanica*. Annual pattern in the nitrogen and phosphorus content of green leaves from the Magalluf monitoring site. *Lines* represent the significant fit of the sine model to the data
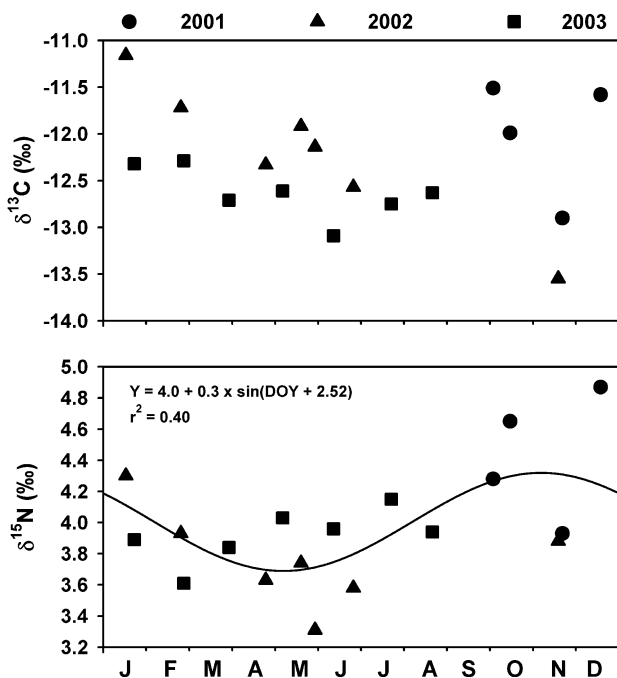

Fig. 4 *Posidonia oceanica*. Annual pattern in the stable carbon and nitrogen isotope ratios in green leaves from the Magalluf monitoring site. *Lines* represent the significant fit of the sine model to the data

The morphology of *P. oceanica* and *C. nodosa* are very different, and there was substantial variation in the shoot morphology within a species across the sampled geographic range (Table 1). *Posidonia oceanica* averaged 5.4 ± 0.9 (±1 SD) leaves per shoot, with a range from an average of 3.6 to 8.4 leaves per shoot at a sampling site. The leaves of this species are long and wide compared to other seagrasses, with a mean leaf length of 302.0 mm. Sites had average leaf lengths that ranged from 89.1 to 814.7 mm. The average leaf area per shoot ranged from 45.3 to 359.2 cm^2 $shoot^{-1}$. The mass per unit area of *P. oceanica* leaves was 4.38 ± 0.81 mg cm^{-2}, so the variation in leaf mass per shoot followed the pattern set by the leaf area per shoot. *Cymodocea nodosa* shoots were much smaller than those of *P. oceanica*. They had between 2.2 and 4.0 leaves per shoot, with a mean leaf length of 108.2 ± 33.2 mm and a mean width of 2.6 ± 0.5 mm. Leaf area per shoot for *C. nodosa* averaged 8.7 ± 4.3 cm^2 $shoot^{-1}$, or about 20% of the value for *P. oceanica*. The mass per unit area of *C. nodosa* was 4.64 ± 0.72 mg cm^{-2}, similar to the value for the larger *P. oceanica*.

The elemental content of both species was variable (Table 2). Nitrogen content of *P. oceanica* varied by a factor of 3×, from a minimum of 0.96% to a maximum of 2.95%, while the P content varied by a factor of 4x, from 0.06 to 0.27%. Carbon content was more consistent, with a mean of 37.8 ± 1.9% (±1 SD, n = 159). C:N varied widely, from 16 to 47.7, with values relatively evenly distributed around the median value of 28 (Fig. 5). Note that C:N values were generally above the "Seagrass Redfield Ratio" (SRR) of 18–20 derived from the central tendencies of literature vales on global seagrass elemental content (Atkinson and Smith 1983; Duarte 1990). C:P also varied greatly, from 366.5 to 1,800.5, and most of the values were significantly above the SRR of 500–550. N:P too was variable, from 18.2 to 57.7; the median N:P of 29.2 was within the range of SRR values. The distribution of *P. oceanica* N:P was skewed toward values higher than the SRR of 25–30, indicating potential P limitation for many meadows, but there were also values below the SRR, indicating potential N limitation.

The C:N and C:P of *C. nodosa* were generally lower than those of *P. oceanica* (Table 2; Fig. 5). C:N ranged from 17.6 to 31.2; most of the values were higher than the SRR. C:P vales ranged from 229.4 to 754.8, and the median of 568.9 was slightly higher than the SRR. N:P ranged from 7.4 to 34.8, and the data were bimodally distributed with more values below the SRR, indicating potential N limitation, than values above the SRR indicating potential P limitation.

Table 1 Morphological characteristics of seagrass shoots (ss) from the study area

	Leaves/shoot	Leaf length (mm)	Leaf width (mm)	Leaf area (cm² shoot⁻¹)	Leaf mass (g shoot⁻¹)
Posidonia oceanica					
n	158	158	158	158	158
Minimum	3.6	89.1	7.6	45.3	0.17
Maximum	8.4	814.7	12.1	359.2	1.73
Mean	5.4	302	9.2	148.4	0.57
Median	5.4	237	9.2	124.5	0.66
SD	0.9	167.9	0.7	77.8	0.36
Cymodocea nodosa					
n	20	19	19	19	19
Minimum	2.2	53.2	1.7	2	0.012
Maximum	4	189.2	3.4	16.3	0.072
Mean	2.9	108.2	2.6	8.7	0.039
Median	2.9	110.3	2.7	7.7	0.037
SD	0.5	33.2	0.5	4.3	0.017

Leaf area is computed as the one-sided leaf area. Values are the average for all shoots collected at a site for a species

Table 2 Summary of the elemental and stable isotopic composition determinations for *P. oceanica* and *C. nodosa* from the Illes Balears, Spain

|	C content (%)	N content (%)	P content (%)	C:N	C:P	N:P	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Fe content (µg g⁻¹)	As content (µg g⁻¹)
P. oceanica										
n	159	159	159	159	159	159	156	156	145	36
Minimum	33.1	0.96	0.06	16	366.5	18.2	–15.9	1.6	31.1	0.2
Maximum	43.8	2.95	0.27	47.7	1801	57.8	–9.6	7.1	167.7	1.6
Mean	37.8	1.63	0.12	28.4	878.7	30.9	–13.2	3.7	72.7	0.69
Median	37.7	1.54	0.11	28	828.3	29.2	–13.3	3.7	67.6	0.61
SD	1.9	0.39	0.04	6	276.3	7.2	1.2	1.1	26.4	0.36
C. nodosa										
n	20	20	20	20	20	20	20	20		
Minimum	35	1.47	0.12	17.6	229.4	7.4	–9.9	0.4	ND	ND
Maximum	40.1	2.62	0.45	31.2	754.8	34.8	–5.3	6.2	ND	ND
Mean	38.2	1.91	0.19	23.8	558.9	24	–7.8	3	ND	ND
Median	38.7	1.97	0.17	22.8	568.9	24.1	–7.6	3.1	ND	ND
SD	1.7	0.3	0.07	3.4	133.4	6.6	1.7	1.5		

ND No data

The stable carbon isotope ratios of both *P. oceanica* and *C. nodosa* leaves were quite variable, and *C. nodosa* leaves had heavier isotope ratios than *P. oceanica* (Table 2; Fig. 6). For *P. oceanica*, $\delta^{13}C$ ranged from –15.9 to –9.6‰, with a median of –13.3‰. The observations were normally distributed around the median. There was very little overlap in the distributions of the $\delta^{13}C$ of leaves of the two species; $\delta^{13}C$ for *C. nodosa* ranged from –9.9 to –5.3‰, with a median of –7.6‰. There were also very broad ranges of the $\delta^{15}N$ measured for leaves of both species (Table 2; Fig. 6). For *P. oceanica*, $\delta^{15}N$ ranged from 1.6 to 7.1‰, with a median of 3.7‰. $\delta^{15}N$ for *C. nodosa* ranged between 0.4 and 6.2‰, with a median of 3.1‰.

Fe and As content of *P. oceanica* leaves was also variable (Table 2; Fig. 7). Fe content varied by a factor of 5×, with a minimum of 31.1 µg g⁻¹ and a maximum

of 167.7 µg g⁻¹. The median was 72.7 µg g⁻¹, and the distribution of Fe content was skewed by a few high values. Arsenic was present in much lower tissue concentrations than Fe, but the As concentrations were more variable; the maximum concentration of 1.60 µg g⁻¹ was eight times as high as the minimum of 0.20 µg g⁻¹. The distribution of As content had a median of 0.61 µg g⁻¹, and was skewed to the right by high values. We did not collect data on the Fe and As content of *C. nodosa* samples.

The morphology of shoots of *P. oceanica* was correlated with elemental and stable isotopic composition of the leaves in the 159 samples collected from the study area (Table 3). There was a positive correlation between the area of shoots and the thickness of the leaves, as assessed by the mass per unit area. The leaf area per shoot was positively related to the C:N and

Fig. 5 Frequency
distributions of the ratios of
the elements of carbon,
nitrogen and phosphorus in
the seagrass leaf samples
analyzed for this paper,
separated by species. The
''seagrass Redfield Ratios''
that indicate a balance in the
availability of the elements
are shown (see text)
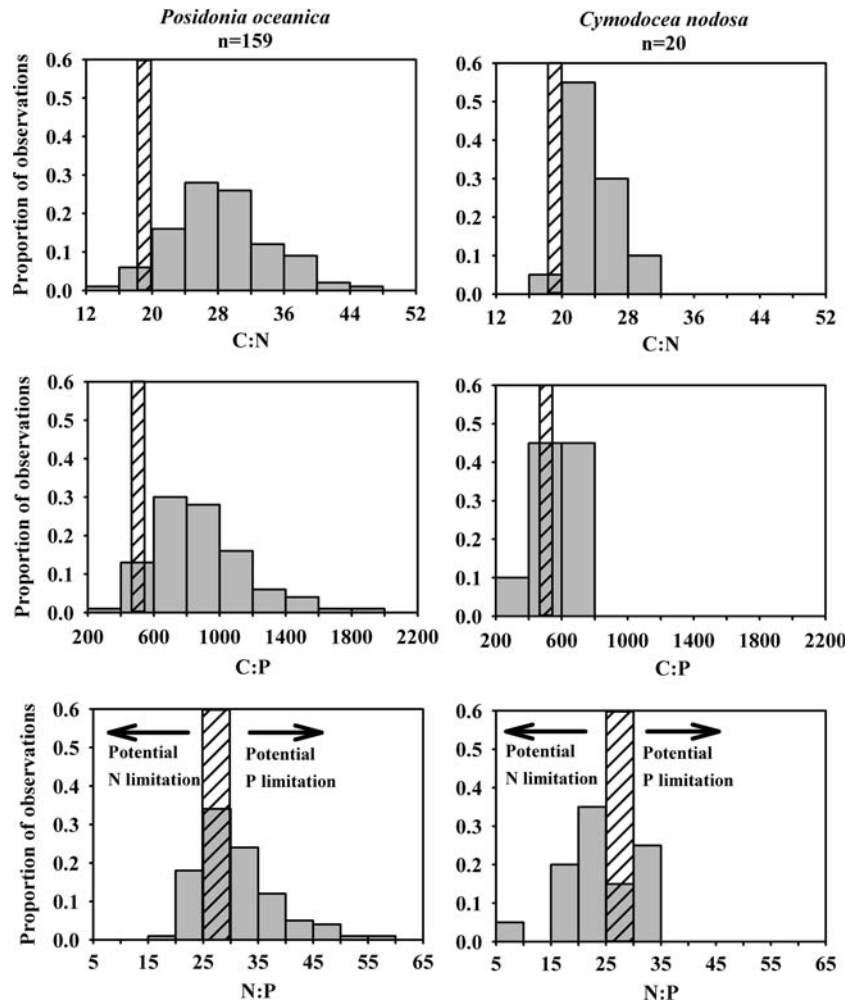
N:P of the leaves, indicating that larger shoots had lower concentrations of N and P in their tissues than smaller shoots; similarly, As and Fe concentrations were higher in the leaves of smaller shoots. The mass per unit area, consequently, was also positively correlated with C:N and C:P and negatively correlated with Fe concentration. In addition, the mass per unit area of *P. oceanica* was positively correlated with N:P, indicating that thicker leaves were related with higher availability of N relative to P. Mass per unit area was also positively correlated to the $\delta^{13}C$ and $\delta^{15}N$ of the leaves. Positive correlations between N:P and $\delta^{15}N$ indicated that plants with higher N content relative to P were isotopically enriched in ^{15}N compared to plants with lower N content. Plants with isotopically heavy $\delta^{15}N$ were also characterized by isotopically heavy $\delta^{13}C$. Further, the concentrations of Fe and As in leaves were positively correlated.

The water depth was correlated to plant morphology (mass per unit area) as well as stable isotopic and elemental content of *P. oceanica* (Table 3). Phospho-

rus content of the leaves increased (C:P decreased) with the depth of plant collection. Further, there was a strong pattern of isotopically lighter $\delta^{13}C$ with depth of collection (Table 3; Fig. 8). The $\delta^{13}C$ decreased at a rate of 0.09‰ m^{-1}.

Morphology of *C. nodosa* shoots was not as intricately related to the elemental and isotopic content of the leaves as was the case for *P. oceanica* (Table 4). The leaf area of the shoots was not correlated with any of the elemental or isotopic variables. Mass per unit area was positively correlated with C:N, indicating that thicker leaves were characterized by lower N concentrations for *C. nodosa* as well as for *P. oceanica*. In contrast to the positive relationship between mass per unit area and N:P in *P. oceanica*, there was a negative correlation in *C. nodosa*. The N:P of *C. nodosa* leaves was positively correlated with $\delta^{13}C$ and negatively correlated with $\delta^{15}N$. Phosphorus content increased relative to N content as water depth increased. In a manner similar to *P. oceanica*, the $\delta^{13}C$ of *C. nodosa* decreased by 0.13‰ m^{-1} with depth (Fig. 8).

Fig. 6 Frequency distributions of the stable isotopic ratios $\delta^{13}C$ and $\delta^{15}N$ in the seagrass leaf samples analyzed for this paper, separated by species
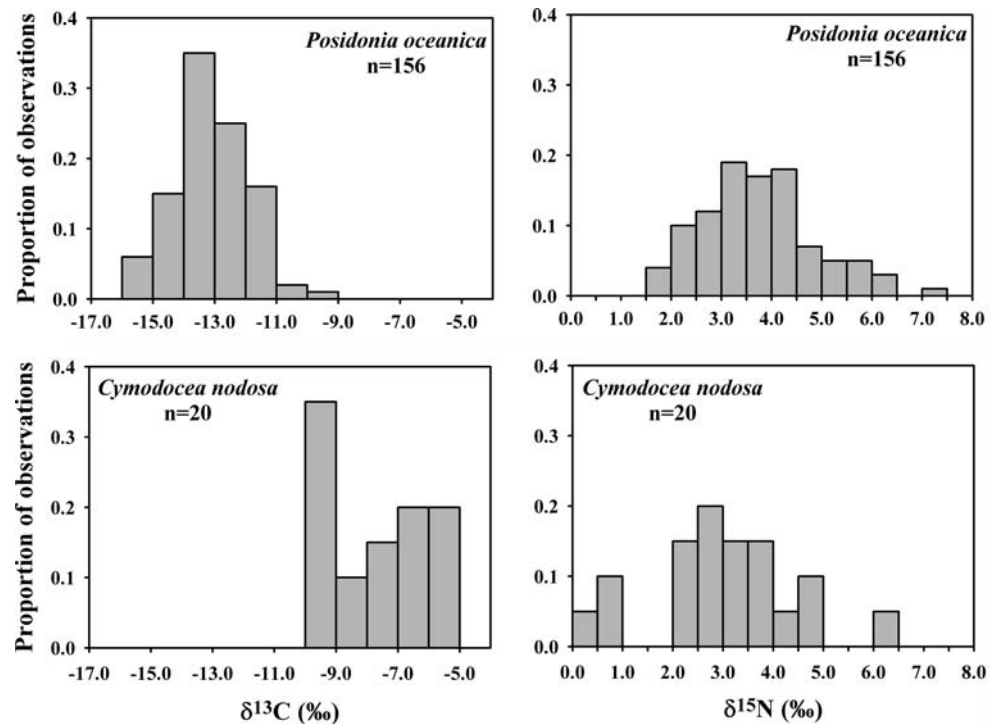

Fig. 7 Frequency distributions of the elemental content for iron (Fe) and arsenic (As) in the *P. oceanica* leaf samples analyzed for this paper
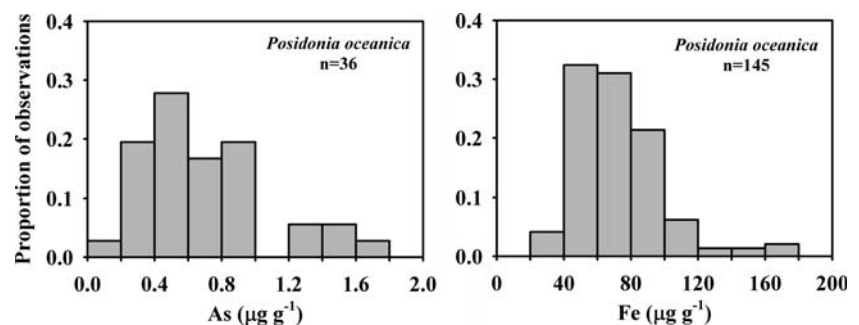

Table 3 *Posidonia oceanica*

	Area/shoot	Mass/area	C:N	C:P	N:P	$\delta^{13}C$	$\delta^{15}N$	Fe	As	Depth
Area/shoot		0.254	0.356	0.220	–0.043	0.039	–0.015	–0.216	–0.482	–0.032
Mass/area	0.001		0.293	0.444	0.297	0.272	0.189	–0.190	–0.302	–0.427
C:N	<0.001	<0.001		0.704	0.066	–0.068	–0.146	–0.033	0.034	–0.142
C:P	<0.006	<0.001	<0.001		0.720	–0.084	0.040	–0.007	0.108	–0.218
N:P	0.591	<0.001	0.409	<0.001		–0.082	0.206	0.033	0.147	–0.144
$\delta^{13}C$	0.629	0.001	0.398	0.296	0.309		0.247	0.164	–0.050	–0.594
$\delta^{15}N$	0.853	0.018	0.070	0.623	0.010	0.002		0.047	0.337	–0.027
Fe	0.009	0.022	0.696	0.937	0.690	0.051	0.574		0.518	0.025
As	0.003	0.073	0.844	0.531	0.391	0.771	0.044	0.001		0.214
Depth	0.690	<0.001	0.075	0.006	0.071	<0.001	0.735	0.762	0.209	

Correlations (non-parametric Spearman's ρ) among aspects of plant morphology, elemental content, stable isotope ratios and water depth. Correlation coefficients (ρ) are above the diagonal, and the P values for the pairwise comparisons are below the diagonal. Significant ($P \leq 0.05$) correlations are italicized

There was a noisy, but statistically significant, relationship between the latitude of sample collection of *P. oceanica* leaves and the $\delta^{15}N$ (Fig. 9). $\delta^{15}N$ was lower in the south around the island of Formentera, but increased northward at a rate of 1.3‰ per degree of latitude (linear regression, slope = 1.3 ± 0.2‰ per
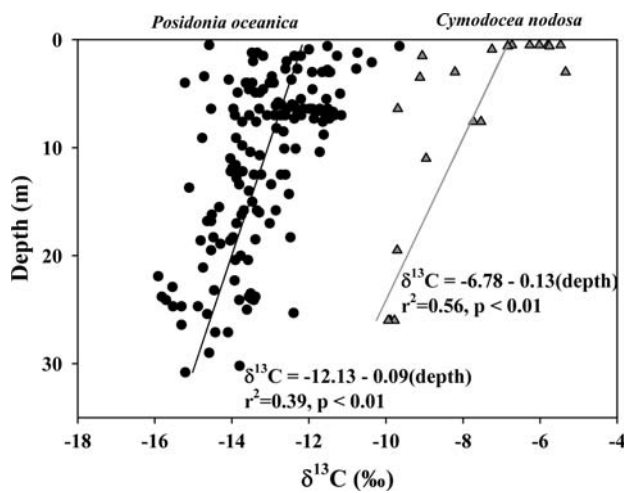
Fig. 8 The stable carbon isotope ratios of both *P. oceanica* and *C. nodosa* leaves decreased with depth in the water column

Fig. 9 *Posidonia oceanica*. The stable nitrogen isotope ratio of leaves increased from the south to the north in the study area

degree of latitude, $P < 0.001$). There was also a significant but weak relationship between latitude and the N:P of *P. oceanica*; N:P increased at a rate of 7.2 ± 1.4 per degree of latitude (linear regression, N:P $= -250.6 + 7.2 \cdot$ latitude in degrees, $r^2 = 0.15$, $P < 0.001$) but there were sizable residuals around this relationship. We did not have enough data points to test for patterns in the isotopic and elemental content of *C. nodosa* with latitude.

Discussion

Morphology, elemental content and isotopic composition of leaves of the seagrasses *P. oceanica* and *C. nodosa* were highly variable across the Illes Balears, and varied seasonally at one site in the study area. The data presented in this paper generally expand the reported ranges for these species (e.g., Papadimitriou et al. 2005; Vizzini et al. 2003). This documentation of
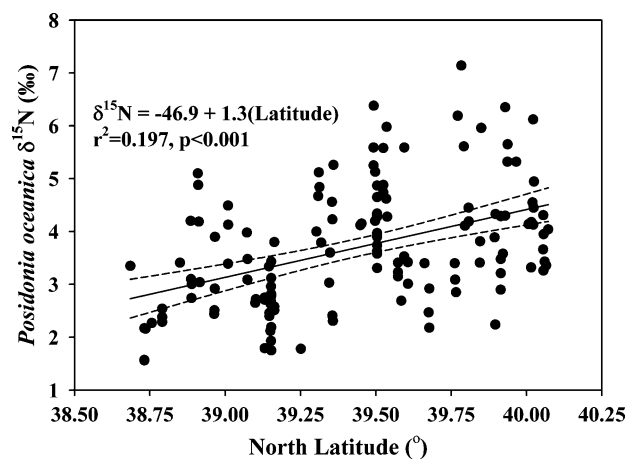
variability for these species joins an increasing number of recent papers that have documented similar spatial and temporal variation in other species (e.g., *Zostera marina* Papadimitriou et al. 2005; *T. testudinum* Fourqurean et al. 2005).

Temporal patterns at Magalluf

The canopy structure of the *P. oceanica* bed at the Magalluf monitoring site varied greatly on a seasonal basis. Given that there is little seasonality in the shoot density in *P. oceanica* beds (Marbà et al. 1996), a threefold increase in the leaf area per shoot from November lows to May highs seems the source for a dramatic seasonal change in structural complexity, and therefore the habitat value, of the those beds. We note that our meadow showed more seasonal variability in *P. oceanica* morphology than documented for meadows in the Adriatic, but the timing of the seasonal patterns was similar (Guidetti et al. 2002). The decline

Table 4 *Cymodocea nodosa*

	Area/shoot	Mass/area	C:N	C:P	N:P	δ^{13}C	δ^{15}N	Depth
Area/shoot		–0.416	–0.354	–0.349	–0.028	0.289	–0.177	–0.198
Mass/area	0.077		*0.625*	–0.154	*–0.544*	–0.451	0.109	0.378
C:N	0.137	*0.004*		–0.141	*–0.650*	–0.217	0.233	0.136
C:P	0.143	0.528	0.552		*0.805*	*0.465*	*–0.559*	*–0.634*
N:P	0.909	*0.016*	*0.002*	*<0.001*		*0.513*	*–0.549*	*–0.579*
δ^{13}C	0.229	0.053	0.359	*0.039*	*0.021*		–0.423	*–0.821*
δ^{15}N	0.468	0.658	0.323	*0.010*	*0.012*	0.063		0.425
Depth	0.417	0.111	0.567	*0.003*	*0.007*	*<0.001*	0.062	

Correlations (non-parametric Spearman's ρ) among aspects of plant morphology, elemental content, stable isotope ratios and water depth. Correlation coefficients (ρ) are above the diagonal, and the P values for the pairwise comparisons are below the diagonal. Significant ($P \leq 0.05$) correlations are italicized

in the size of the shoots beginning in summer, despite maximum light availability in this season, suggests that factors other than light availability are driving the changes in plant morphology. Factors such as reallocation of carbon reserves for autumn flowering (Gobert et al. 2005), internal phenological rhythms (Marbá et al. 1996), increases in sediment metabolism with increasing temperatures (Holmer et al. 2003a) that could lead to anoxic conditions (Greve et al. 2003), or seasonal patterns of herbivory may be responsible.

The concentration of nutrients in plant tissues is determined by the supply of the nutrients relative to the demands for growth of the plants. Spring decreases in the concentrations of N and P in *P. oceanica* leaves have been documented at other locations in the Mediterranean (Delgado 1986; Pirc and Wollenwebber 1988; Alcoverro et al. 1995; Lepoint et al. 2002; Invers et al. 2004), and for other species in both temperate (Fourqurean et al. 1997) and tropical environments (Fourqurean et al. 2005). It is likely that these spring decreases are a result of dilution of the internal pool resulting from the increased demand for nutrients to support growth during favorable periods (Alcoverro et al. 1995; Fourqurean et al. 1997). Relatively high concentrations of N and P in *P. oceanica* leaves would suggest that the supply of both nutrients was sufficient in the winter.

The seasonal pattern in $\delta^{15}N$ of *P. oceanica* leaves we observed at Magalluf (spring and summer minima, autumn and winter maxima, Fig. 4) was similar to the pattern observed in a shallow *P. oceanica* meadow in Sicily (Vizzini et al. 2003). Increases in N demand during summer periods of high growth have been hypothesized to cause a decrease in discrimination against ^{15}N during uptake in *T. testudinum*, leading to maximum $\delta^{15}N$ ratios during times of lowest leaf N content (Fourqurean et al. 2005). However, the seasonal pattern in $\delta^{15}N$ at Magalluf and in Sicily (Vizzini et al. 2003) exhibited minimum $\delta^{15}N$ ratios at the time of peak growth. Perhaps at our Magalluf site and in Sicily, there is always excess N available to support seagrass growth so there is no chance for decreased fractionation during summer. Alternatively, at these two oligotrophic sites, there may not be seasonal differences in the relative availability of nitrogen for plant growth, and the seasonal signals may be a consequence of either seasonal changes in the $\delta^{15}N$ of the source DIN, or a seasonal difference in the relative importance of different components of the DIN with different $\delta^{15}N$ ratios. The lack of a relationship between $\delta^{15}N$ and depth, and therefore light availability, on a regional basis (Table 3) also suggests that there is no differential fractionation of the available N pool

caused by differences in photosynthetic rate for *P. oceanica* in the western Mediterranean. The seasonal pattern we observed in the $\delta^{15}N$ of *P. oceanica* leaves is most likely a result of a change in the isotopic signature of the source DIN on a seasonal basis at this site and elsewhere in the western Mediterranean. Determining the causes of the seasonal patterns in $\delta^{15}N$ will require careful manipulative experimentation.

Despite the known importance of light intensity in determining the $\delta^{13}C$ of seagrass leaves (Cooper and DeNiro 1989; Abal et al. 1994; Grice et al. 1996), we did not observe a significant seasonality in the $\delta^{13}C$ in *P. oceanica* leaves at Magalluf (Fig. 4) as has been observed elsewhere for this species (Vizzini and Mazzola 2003) and other seagrass species (Fourqurean et al. 1997, 2005; Papadimitriou et al. 2005). This lack of seasonality may be explained by anomalously low $\delta^{13}C$ values recorded in November 2001 and 2002. Had those November values fallen in line with the seasonal pattern suggested by the rest of the data, there would have been a seasonal pattern of isotopically more depleted $\delta^{13}C$ in summer months, a pattern opposite to what would be expected if maximum photosynthetic rates in summer led to decreased isotopic discrimination. Perhaps the lack of a summertime maximum in the $\delta^{13}C$ in *P. oceanica* leaves from Magalluf may be a consequence of the ability of this species to utilize bicarbonate via carbonic anhydrase to supply DIC for photosynthesis (Invers et al. 1999). Inter-year differences in plant phenology may explain the variability in autumn $\delta^{13}C$ of *P. oceanica*. For example, floral structures reach maximum development in autumn in this species, and the elaboration of the floral structures requires translocation of C from leaves to the flowers (Gobert et al. 2005). At Magalluf, there was substantial interannual variation in flowering during the years that we collected samples with an unusually intense flowering event in autumn 2003 (Diaz-Almela et al. 2006).

Spatial variability across the Illes Balears

The large variability measured for the elemental content of *P. oceanica* and *C. nodosa* across the study area indicates that environmental conditions varied broadly in seagrass meadows of the Illes Balears. There were interspecific differences in the carbon, nitrogen and phosphorus content of the two species. *Posidonia oceanica*, the more robust species, generally had more structural carbon than *C. nodosa*, as evidenced by the elevated C:N and C:P ratios of the species (Fig. 5). *Posidonia oceanica* ratios were generally far above the SRR of ca. 550:30:1 of seagrasses on a global scale (Atkinson and Smith 1983; Duarte 1990) while the

ratios for *C. nodosa* were closer to the SRR. The N:P ratios of the two species also suggests a difference in the element most often in limiting supply for the two species. The median N:P for *P. oceanica* matched the SRR, indicating that most often the *P. oceanica* we collected was not limited by N or P; when N:P deviated from the SRR it was generally higher than the SRR, indicating a prevalence of P limitation. This is to be expected, given the behavior of phosphate in carbonate sediments (Short 1987). The weak but significant increase in N:P of *P. oceanica* with latitude suggests that the tendency toward P limitation in *P. oceanica* beds increased from south to north across the archipelago. In contrast, *C. nodosa* leaves generally displayed N:P lower than the SRR, suggesting prevalence of N limitation in seagrass beds dominated by *C. nodosa*, despite the underlying carbonate sediments. It is becoming clear that factors other than the mineralogy of the sediments determines which nutrient will limit seagrass growth (Ferdie and Fourqurean 2004); the apparent qualitative difference in N versus P limitation for these sympatric seagrass species deserves experimental investigation.

The large variation in stable isotopic signatures measured for both species underscores the necessity for rigorous sampling regimes for stable isotope-based food web studies. Natural variation in the δ^{15}N of *P. oceanica* had a range of 1.6–7.1‰ (Table 2; Fig. 6), a difference as large as that expected over two trophic levels of a food web (Minagawa and Wada 1984). Further, the existence of the latitudinal pattern of a 1.3‰ increase in the δ^{15}N with latitude (Fig. 9) suggests regional differences in either source nitrogen, as could be caused by anthropogenic N sources, or nitrogen processing within the ecosystem. However, as pointed out in Fourqurean et al. (2005), the complicated and interrelated factors that determine the δ^{15}N of seagrass leaves indicate that caution should be used when interpreting the causes of spatial variability without well-designed manipulative experiments to confirm the mechanisms.

There were no consistent differences between the δ^{15}N of the seagrasses *P. oceanica* and *C. nodosa* across the study area; however, there were interspecific differences in the δ^{13}C (Fig. 6), indicating that δ^{13}C should be a useful tool for assessing the relative importance of primary production from these two species to local food webs. The interspecific δ^{13}C differences did not seem to be related to differences in light availability; both species showed the expected decline in δ^{13}C with increasing depth and therefore decreasing light availability (Fig. 8). We can think of no reason for there to be large systematic differences in

the δ^{13}C of the available CO_2 pool for the two species; it is likely that interspecific differences in the degree of bicarbonate uptake (Invers et al. 1999) are responsible for the interspecific difference in the δ^{13}C of the two species.

Shoots of *P. oceanica* varied by over an order of magnitude in size in the study area (Table 1), and this size variation was negatively correlated with nutrient content (Table 3). Large shoots had lower concentrations of N and P in their tissues, perhaps because large shoots have the ability to grow at a rate that results in local depletion of N and P supply. Although across the entire spatial and temporal range of our samples there was no simple correlation between depth of collection and nutrient content (Table 3), others have shown that shoot size is positively related to the amount of light reaching the canopy (Alcoverro et al. 1995) and that high light availability can lead to lower tissue nutrient concentrations as a result of demand exceeding local supply (Alcoverro et al. 1995; Fourqurean et al. 1997, 2005). In contrast with *P. oceanica*, there was no relationship between nutrient content and size of the shoots of *C. nodosa* across the range of samples (Table 4), but the sample size for *C. nodosa* (20 observations) was quite small.

Besides the mass or area of seagrass leaves, the mass per unit area is a measure of the robustness of the leaves. In *P. oceanica*, the mass/area was positively correlated with shoot size (Table 3), indicating that larger plants had thicker leaves than smaller plants. More robust shoots of *P. oceanica* (ones with high leaf mass/area) were characterized by isotopically-enriched δ^{13}C and δ^{15}N of leaves. This may indicate that more robust plants discriminate less against the heavier isotope because they draw down the available pools further than less robust plants. Alternatively, both decreases in leaf mass/area and stable isotopic ratios of both *P. oceanica* and *C. nodosa* may be a result of concomitant changes in light availability and wave energy with depth. Shallow water plants may have more robust leaves in response to the higher physical energy of water motion compared to deeper plants; there was a negative correlation between leaf mass/area and depth for *P. oceanica* (Table 3). Decreases in light with depth lead to a 0.09‰ m^{-1} decrease in δ^{13}C of *P. oceanica* and a 0.13‰ m^{-1} decrease in δ^{13}C of *C. nodosa* (Fig. 8); the δ^{13}C differences between our shallow and deep plants were ca. 3‰ in *P. oceanica* and ca. 4‰ for *C. nodosa*. Such large differences in the stable isotopic composition could easily obscure the analysis of isotope-based food web studies, since these depth-related differences introduce signals much greater than the ca. 1‰ δ^{13}C fractionation associated

with each trophic level (Fry and Sherr 1984). Other studies (e.g., Cooper and DeNiro 1989; Lepoint et al. 2003) have shown a decrease in the $\delta^{13}C$ of seagrass leaves with increasing depth or decreasing light; such changes may be a result of high photosynthetic activity decreasing the available pool of CO_2 and thereby decreasing fractionation (Cooper and DeNiro 1989; Abal et al. 1994) or, as Lepoint et al. (2003) suggests, the plants may shift inorganic carbon sources to bicarbonate in high light environments.

There have been few studies of the Fe content of seagrasses despite evidence that this element may limit seagrass growth on carbonate sediments (Duarte et al. 1995), and even fewer of the As content. Iron content of *P. oceanica* (Table 2; Fig. 7) was an order of magnitude lower than reported for *T. testudinum* from Florida (Chambers et al. 2001) or for *Z. marina* (Thayer et al. 1984b). Indeed, our current data are only 20% of those previously reported from continental Spain but they are similar to values reported for other carbonate sediment areas (Duarte et al. 1995). It has been shown that increased sediment iron content of carbonate sediments can increase the availability of phosphorus to seagrasses growing in tropical systems (Chambers et al. 2001); we therefore had expected to see a correlation between tissue Fe content and the N:P of *P. oceanica*, but no such correlation was detected (Table 3). The lack of correlation may be because the ambient Fe concentrations were so low such that the amount of orthophosphate sorbed onto FeOOH particles was inconsequential. Arsenic concentrations of *P. oceanica* (Table 2; Fig. 7) were roughly half those recorded for *T. testudinum* from the Gulf of Mexico (Fourqurean and Cai 2001). The concentrations of Fe and As were negatively correlated with shoot size in *P. oceanica* (Table 3). One could interpret this as evidence of limitation, as other seagrasses growing in carbonate sediments have been shown to be Fe limited (Duarte et al. 1995). On the other hand, as As is a well known toxic element, the negative correlation between plant size and As could be an indication of a negative impact of As on seagrasses. Also, owing to the chemical similarity of arsenate and phosphate, As content could also be an indicator of severity of P limitation in this carbonate environment (Fourqurean and Cai 2001). Determination of the causes of these correlations calls for controlled experimentation before we can reach conclusions about their ecological significance.

It is becoming quite clear that ecosystem-scale studies that aim to understand nutrient processing or food webs using stoichiometric and stable isotopic data must thoroughly understand the nature of spatial and temporal variation of these plant characteristics. The

variability we documented was not random: there were clear seasonal patterns, regional spatial patterns and patterns associated with depth. The presence of these patterns suggests that the variability is responding to environmental forcing, so that an understanding of the patterns of change in morphology, elemental content or stable isotopic content provides a tool for understanding underlying environmental conditions influencing seagrass-dominated ecosystems.

The seagrass beds surrounding the Illes Balears are remarkable in their extent and the depth of penetration into the Mediterranean Sea. Despite the long history of human development and significant agriculture and urban development, water clarity around the islands is sufficient to allow both *P. oceanica* and *C. nodosa* to grow to depths of 45 m (Bethoux and Copin-Montegut 1986; Pasqualini et al. 1998; Marbà et al. 2002). However, recent environmental perturbations in the region are threatening to cause change to the distribution of these species. Urbanization continues, and runoff of nutrients, sediments and toxic chemicals threaten to cause loss of seagrass beds here, as elsewhere in the world. Aquaculture is also increasing in the Mediterranean; and fish farms are often placed in close proximity to seagrass meadows and has the potential to cause the loss of seagrasses through eutrophication or organic matter loading (Cancemi et al. 2003; Holmer et al. 2003b). This study provides a useful baseline to check the effects of such developments. In addition, the elemental and isotopic composition of seagrass leaves are proven useful indicators of eutrophication, urban runoff and changing light environments provided that natural causes of variation are understood.

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