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Using variation in the chemical and stable isotopic composition of *Zostera noltii* to assess nutrient dynamics in a temperate seagrass meadow

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Abstract

The influence of seasonality in growth and benthic organic matter remineralization on the chemical and isotopic composition of the seagrass *Zostera noltii* was investigated from March to November over the course of two years in a temperate meadow in North Wales, UK. The carbon (C_{org}) and nitrogen (N_{org}) concentrations in new leaf tissue ranged from 25 to 35 mmol C g^{-1} and 2 to 5 mmol N g^{-1}.Their stable isotopic composition ranged from −11.0‰ to −6.9‰ ($\delta^{13}C_{leaf}$) and +3.3‰ to +7.5‰ ($\delta^{15}N_{leaf}$), while the stable isotopic composition of sulphur in the new leaf ($\delta^{34}S_{leaf}$) ranged from −3.0‰ to +6.4‰.The young seagrass tissues had lowest N_{org}, highest C:N, most depleted $\delta^{13}C_{leaf}$, and most enriched $\delta^{15}N_{leaf}$ at the standing biomass maximum (approximately 150–200 g dry weight m^{-2}) in the summer, reflecting the temporal imbalance between inorganic nutrient supply and plant demand imposed by seasonality in the growth rate.The most depleted $\delta^{34}S_{leaf}$ was recorded in the same season.The isotopic composition of the seagrass tissues reflected that of the external inorganic source.The $\delta^{13}C_{leaf}$ correlated ($r^2 \sim 0.4$) with the $\delta^{13}C$ of total dissolved inorganic carbon (DIC) in the surface waters ($\delta^{13}C_{DIC}$ range: −0.4‰ to +1.2‰).The apparent carbon isotope enrichment factor of new leaf relative to the bulk seawater DIC ($\varepsilon_{seagrass\text{-}DIC}$ range: −11.2 to −8.1‰) indicated reliance on direct HCO_3^- uptake, especially early in the growing season (spring).The $\delta^{15}N_{leaf}$ reflected the $\delta^{15}N$ of pore water ammonium ($\delta^{15}N_{NH_4^+}$ range: +6‰ to +10‰; average: $+7.4 \pm 0.8$‰) as the growing season progressed into the summer, indicating utilization of benthic remineralized nitrogen with some degree of limitation in the uptake step despite ample supply (benthic net nitrogen remineralization rate: 8–14 mmol N m^{-2} d^{-1}) relative to conservative, biomass-based estimates of plant demand (1.5–4.0 mmol N m^{-2} d^{-1}) during the growing season. The $\delta^{34}S_{leaf}$ suggested influence on the seagrass plants of the benthic sulphur cycling under anoxic conditions.

1. Introduction

Seagrasses are marine angiosperms that colonize coastal and estuarine sediments, and are amongst the most productive aquatic autotrophic organisms

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on a global scale (Duarte and Chiscano, 1999). Seagrasses play an important role in coastal food webs, carbon sequestration, sediment stabilization, and nutrient cycling (Duarte, 2002). However, recent evidence suggests that seagrass meadows are in decline worldwide due to natural and anthropogenic change (Short and Wyllie-Echeverria, 1996). Managing and ultimately the conservation of seagrass species are necessary under these circumstances, but this goal cannot be attained before a better understanding of seagrasses and their ecosystem function is achieved. The use of stable isotope chemistry at the natural abundance level can help determine the biogeochemical processes in seagrass meadows and how these influence the seagrasses or are, in turn, influenced by the plants. This information, in adding to our knowledge of the natural dynamics in seagrass meadows, can provide valuable advice on potential strategies for maintenance and future management of seagrass habitats.

Seagrass plants obtain nutrient elements from the external waters. Biomass carbon is derived from the dissolved inorganic carbon pool (DIC) of surface waters in the form of dissolved carbon dioxide (CO_2(aq)) and bicarbonate ions (HCO_3^-) (e.g., Beer et al., 2002). Plant nitrogen is sourced from the dissolved inorganic nitrogen pool (DIN) in the form of nitrate (NO_3^-) and dissolved ammonium (NH_4^+) in both surface waters via leaf uptake (e.g., Hemminga et al., 1994) and pore waters in the underlying sediments via root uptake (Thursby and Harlin, 1982; Short and McRoy, 1984; Hemminga et al., 1994). Sulphur is derived from the sulphate (SO_4^{2-}) ions present in seawater (Trust and Fry, 1992).

As seagrass biomass and growth rate change seasonally in a unimodal pattern between summer maximum and winter minimum (Duarte, 1989), the plant demand for nutrient elements changes, while nutrient availability in the external medium can become limiting relative to elevated demand during the period of maximum growth. This can lead to a considerable fluctuation of tissue chemistry over seasonal cycles, which has been demonstrated in the well documented cyclical oscillation of the nitrogen concentration and its molar ratio to carbon in tissues of the temperate *Zostera* species (Pedersen and Borum, 1993; Vermaat and Verhagen, 1996; Plus et al., 2003; Papadimitriou et al., 2005). The decline in the nitrogen concentration of seagrass tissues during the period of fast growth in spring–summer suggests that external inorganic nitrogen and internal recycling of assimilated nitrogen from older tissues cannot satisfy plant demand throughout the year.

The seasonal shift in the balance between external nutrient supply and plant demand also bears on the stable isotope ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of seagrass tissues. The assimilation of DIC and DIN during autotrophic (primary) production is accompanied by fractionation of their stable isotopes due to faster reaction kinetics of the light (i.e., ^{12}C and ^{14}N) isotope when external nutrient availability is high relative to biological demand (Fogel and Cifuentes, 1993). As a result, the $\delta^{13}C$ and $\delta^{15}N$ in biomass can be different from those of the source inorganic pool, with differences being systematically towards isotopically more depleted biomass. The isotopic composition of biomass will approach that of the source inorganic pool with progressively less isotope fractionation as the nutrient balance shifts towards excess demand relative to supply. Considerable seasonal isotope enrichment and depletion patterns have been recorded in the stable isotope ratio of carbon ($\delta^{13}C$) of seagrass tissues and have been related to coincident changes in plant productivity (Anderson and Fourqurean, 2003; Papadimitriou et al., 2005), as well as changes in the $\delta^{13}C$ of the source inorganic carbon in the external waters (Fourqurean et al., 1997; Papadimitriou et al., 2005). Although a similar pattern can be expected for the stable isotope ratio of nitrogen ($\delta^{15}N$) in seagrass tissues, and temporal variability in seagrass $\delta^{15}N$ has been documented (Anderson and Fourqurean, 2003; Papadimitriou et al., 2005), seagrass $\delta^{15}N$ is often considered mostly a reliable reflection of the $\delta^{15}N$ of the inorganic nitrogen source under natural conditions (McClelland and Valiela, 1998; Papadimitriou et al., 2005).

Isotope fractionation during the uptake and assimilation of inorganic sulphur is minimal (Trust and Fry, 1992), which would imply little or no seasonality in the stable isotope ratio of sulphur ($\delta^{34}S$) of seagrass. Currently there is no published data to test this hypothesis but available data suggest that the $\delta^{34}S$ may vary in seagrass tissues due to seasonal changes in other relevant parameters. The potential for seagrass plants to develop variations in their $\delta^{34}S$ is linked to the influence of sediment anoxia on tissue chemistry (Raven and Scrimgeour, 1997). Microbial oxidation of sedimentary organic matter via SO_4^{2-} reduction under anoxic conditions produces isotopically depleted inorganic sulphur compounds, such as dissolved sulphide (Habicht and

Canfield, 1996). Physiologically, seagrasses are able to maintain a continuous supply of oxygen to rhizomes and roots, which may extend to the rhizosphere (Pedersen et al., 2004; Borum et al., 2005) and can effectively restrict sulphide invasion of plant tissues by immobilization through chemical reaction. However, a combination of parameters, such as low oxygen concentration in the water column and sedimentary iron availability, as well as high organic matter input to the sediments coupled with elevated rate of benthic SO_4^{2-} reduction, can contribute to a set up of unfavourable conditions for dissolved sulphide invasion into the plant, which will be reflected in isotopically depleted $\delta^{34}S$ values in the seagrass tissues (Raven and Scrimgeour, 1997; Chambers et al., 2001; Oakes and Connolly, 2004).

Zostera noltii Hornem. is a small seagrass species of the intertidal zone of Europe and Africa (Auby and Labourg, 1996), with pronounced unimodal biomass seasonality (Perez-Llorens and Niell, 1993; Vermaat and Verhagen, 1996; Plus et al., 2003). In this paper, we report seasonal chemical and isotopic measurements of carbon and nitrogen, as well as isotopic measurements of sulphur in *Z. noltii*, which were made during a wide scope programme investigating the response of a number of European seagrass species to changing environmental conditions. To understand the controls on element cycling and the factors that influence the elemental and isotopic composition of the seagrass plants, we have made additional measurements of the potential sources of carbon and nitrogen in the water column and sediment pore waters. Our aims were (i) to determine the extent to which the seasonal shift in the balance between external nutrient supply and plant demand controls the stable isotope ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of seagrass tissues; (ii) to document any seasonal change in the stable isotopic composition of sulphur ($\delta^{34}S$); and (iii) to assess whether seasonal changes in the location of the dominant DIN uptake, i.e., leaf or root and rhizome, had any effect on $\delta^{15}N$.

2. Study site and sampling

Sampling was undertaken in a *Z. noltii* meadow located in Inland Sea, a shallow (mean depth: 0.75 m) artificial tidal lagoon formed by road and rail embankments in the channel that separates Holy Island from the island of Anglesey in North Wales, UK (Fig. 1). The lagoon receives negligible freshwater discharge and exchanges seawater with the Irish Sea through culverts in the embankments (Hill, 1994). This mode of communication with the open sea results in a unique tidal regime within the lagoon; the overall tidal range is 1.2 m compared to 5.0 m during spring tides outside the lagoon, the diurnal tidal range is 0.1–0.6 m, and the shore zones that are tidal on neap tides (neap tidal zone) are continuously submerged on spring tides, and vice versa (Jones, 1978).

The sampling in the meadow was conducted over the course of three years. A pilot study was undertaken in July 2001, while intensive sampling was conducted in the following two years at one of the stations of the pilot study, which was located in the neap tidal zone at approximately 0.6 m depth. Only the measurements at this station from the 2001 study are included here. In 2002, a contiguous matrix of 36 plots, each of 2 m^2, were marked out and sampled monthly during the growing season between April and September 2002. During neap

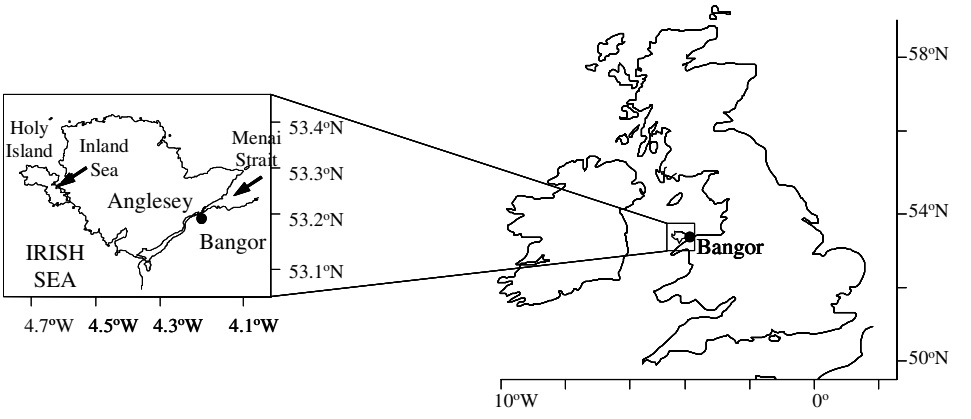

Fig. 1. Study area in North Wales, UK, magnified in left panel.

tides, the plots were partially covered with seawater pools; the depth difference amongst the plots was in the order of 5–10 cm. Each monthly data set consisted of an average of the measurements made in each of three plots. At the end of the sampling period in 2002, approximately half of the plots remained unsampled. These were used to undertake weekly sampling during a period of rapid DIN depletion in the seawater in March 2003, as well as once in April, May, August, and November of the same year in order to extend measurements into the late autumn. During this period, sediment cores were taken from the meadow to determine the microbial remineralization rate of dissolved ammonium in the rhizosphere and the stable isotope ratio of nitrogen in the dissolved ammonium in the pore water through laboratory-controlled anoxic incubations. In order to obtain suitably concentrated seawater samples for isotopic analysis of nitrogen in dissolved nitrate, additional sampling was undertaken during March 2003 and 2004 in the Menai Strait, the narrow 20 km long tidal conduit of seawater from the Irish Sea, which separates the island of Anglesey from North Wales (Fig. 1).

3. Methods

3.1. Seagrass tissues

Biomass measurements were conducted in 2001 and 2002 on material collected with 10 cm (ID) corers inserted to 10 cm depth below the sediment surface. Back at the laboratory, the live seagrass tissues were separated, rinsed with seawater, and divided into leaves, rhizomes, and roots, all of which were weighed after drying at 60 °C for 24 h. The dried tissues were retained for chemical and isotopic measurements. Seagrass tissue chemistry was investigated in new, actively growing leaf of *Z. noltii* (for definition, see Pedersen and Borum, 1993) with measurements of the carbon (C_{org}) and nitrogen (N_{org}) concentrations, as well as their respective stable isotope ratio ($\delta^{13}C_{leaf}$ and $\delta^{15}N_{leaf}$) as described in Kennedy et al. (2004). The same set of measurements was conducted on additional new leaf samples obtained weekly in March 2003, and once in April, May, and November 2003. Sampling for the determination of the stable isotope ratio of sulphur in new leaf ($\delta^{34}S_{leaf}$) occurred in each of three plots on each occasion in 2002 but was restricted to the period between April and August due to sample size constraints. For this set of analyses, 8–10 mg of new leaf material was weighed into a tin boat along with 5 mg vanadium pentoxide (Fry et al., 2002).

The organic carbon (C_{org}) and nitrogen (N_{org}) concentrations of seagrass tissues were determined on a EUROPA Scientific ROBOPREP C/N Analyzer. The precision was better than 10% for C_{org} and N_{org} based on triplicate sample measurements. The isotopic measurements on seagrass tissue, as well as in materials described subsequently, were all conducted on a PDZ-EUROPA GEO 20/20 mass spectrometer for carbon, a SIRA VG II mass spectrometer for nitrogen, and a ThermoFinnigan elemental analyser + Delta XL continuous flow mass spectrometer system for sulphur. The isotopic measurements are reported in the δ notation relative to standards Vienna Pee Dee Belemnite for carbon, air for nitrogen, and Canyon Diablo troilite for sulphur, i.e., $\delta_{sample} = 1000\ [(R_{sample}/R_{standard}) - 1]$, where $R = {}^{13}C/{}^{12}C$, $R = {}^{15}N/{}^{14}N$, and $R = {}^{34}S/{}^{32}S$. The precision of the carbon and nitrogen isotopic measurements was better than 0.1‰, while the precision of the sulphur isotopic measurements was better than 0.4‰, all based on internal standards.

3.2. Water column

Surface seawater from the meadow was sampled for $\delta^{13}C_{DIC}$, nitrate plus nitrite (hereafter, NO_3^-), and NH_4^+ determinations between April and September 2002. Further surface seawater samples for $\delta^{13}C_{DIC}$ measurements were obtained from the water column overlying the meadow monthly in March, April, May, August, and November 2003, while NO_3^- was determined weekly during the spring bloom in March 2003, as well as in April and November 2003. Owing to the requirement for relatively high concentration for the determination of the isotope ratio of NO_3^--nitrogen ($\delta^{15}N_{NO_3^-}$), additional weekly sampling for NO_3^- was conducted for this purpose in the adjacent Menai Strait (Fig. 1) in March 2003 and in March 2004. The samples for $\delta^{13}C_{DIC}$ measurements were collected in 250 ml glass bottles; they were immediately poisoned with 1 ml saturated $HgCl_2$ solution in the field, and 10 ml sub-samples were flame-sealed under N_2 atmosphere in glass ampoules. Several sub-samples taken in March, May, August, and November 2003 were analysed in lieu of internal standard ($n = 18$, 5, 7, and 4, respectively) and are, therefore, taken to serve as reasonable indicators of analytical precision. The DIC was extracted as CO_2 by cryogenic gas distillation after reaction with 85%

H_3PO_4 in vacuum, followed by $\delta^{13}C_{DIC}$ determination. The samples for NO_3^- and NH_4^+ measurements were collected in 10 l aspirators and were stored filtered through pre-combusted (3 h at 500 °C) 47 mm GF/F filters (WHATMAN) in acid-washed polythene bottles at −20 °C until analysis. The NO_3^- concentration was determined colourimetrically (Grasshoff et al., 1983) on a LACHAT Instruments Quickchem FIA 8000 series autoanalyzer, while NH_4^+ was determined with the fluorimetric method of Holmes et al. (1999) using a HITACHI F2000 fluorescence spectrophotometer. The $\delta^{15}N_{NO_3^-}$ was determined in duplicate at the pre-bloom maximum concentration of the solute in the Menai Strait in early March 2004 using the method described in Sigman et al. (1997).

3.3. Sediments

Aspects of the benthic geochemistry of nitrogen were investigated in sediment cores taken with 14.2 cm internal diameter corers from within the meadow in 2003 and processed immediately upon return to the laboratory. Approximately 10 cm long sediment cores were collected in March ($n = 1$), May ($n = 3$), and August 2003 ($n = 3$). After manually removing seagrass shoots and visible infaunal organisms (mainly polychaete worms), the topmost 5 cm of the sediment of each core was separated, as remineralization is most active in the uppermost sediment, and manually homogenized. The homogenized sediment was equally divided among 50 ml acid-washed centrifuge tubes, which were incubated in anoxic mud in a temperature-controlled water bath for 10–15 days. The temperature during the anoxic incubations was set to that of the surface seawater at the time of collection. To obtain time series measurements of benthic NH_4^+ remineralization, one tube from each core was removed after specific time periods, the pore water was extracted by centrifugation at 3000 rpm for 15 min, and sub-samples were filtered through syringe filters (GF/D, 0.45 μm, WHATMAN) into acid-washed polypropylene scintillation vials that were kept frozen at −20 °C for NH_4^+ measurements. The net anoxic remineralization rate of NH_4^+ was calculated from the linear regression slope of the concentration change with time ($r = 0.933$–0.998, $p = 0.001$–0.023) for average surface sediment porosity and density of dry solids (see below), and is reported in areal units integrated over the 5 cm depth interval. The linear regression also provided the 95% standard error of the remineralization rate. The anoxic incubations provided the elevated pore water NH_4^+ concentrations (at $t > 0$) that allowed determination of the stable isotope ratio of NH_4^+-nitrogen ($\delta^{15}N_{NH_4^+}$) in small sample volumes, especially on final time points when triplicate sub-samples were possible, using the method in Holmes et al. (1998). In order to further compare the $\delta^{15}N_{NH_4^+}$ measurements obtained during the anoxic incubations, a set of triplicate cores was collected in November 2003. Upon return to the laboratory, the pore waters from the upper 5 cm of the sediments were extracted and were pooled together, followed by immediate determination of the NH_4^+ concentration, as well as $\delta^{15}N_{NH_4^+}$ in triplicate sub-samples of the pooled pore water sample. In the same vein, the stable isotope ratio of sedimentary nitrogen was also determined in the total sediment fraction (<500 μm grain size) in May, August, and November 2003, with samples prepared as described in Kennedy et al. (2004).

Further investigation into the benthic NH_4^+ pools was carried out in May 2003 with determination of the amount of reversibly adsorbed NH_4^+ in the surface sediment. For this purpose, the sediment pellets were collected after pore water extraction and were prepared for the extraction of exchangeable NH_4^+ with 2N KCl using the method of Mackin and Aller (1984). Estimates of the total NH_4^+ remineralization rate were calculated by adjusting the net rate measured in all incubated cores for the effect of reversible adsorption as in Dennison et al. (1987). In order to characterize the sedimentary setting, the porosity (ϕ) of the sediments was determined from the weight loss on heating at 50 °C for several days. Further, the density of dry solids was determined by volume displacement and weight difference in a 0.5 ml graduated volumetric cylinder using distilled water at constant temperature and known water density.

4. Results

4.1. Biomass and growth rate

The biomass of *Z. noltii* was low in the beginning of April 2002 and appeared to have increased exponentially to a maximum by the beginning of July (Table 1), i.e., in the course of approximately 3 months. Comparable values of high biomass had been measured during the pilot study a year earlier (July 2001; Table 1). The average growth rate,

Table 1
Areal live biomass (mean $\pm 1\sigma$) based on dry weight (B, in g m^{-2}), carbon content (B_C, in mmol C m^{-2}) and nitrogen content (B_N, in mmol N m^{-2}) measurements

	B	B_C	B_N
Above-ground tissues			
July 2001	59.0 ± 27.5	1860.2 ± 865.3	103.6 ± 46.0
April 2002	0.4	15.0	2.4
May 2002	5.4 ± 2.4	121.2 ± 60.0	16.5 ± 8.2
June 2002	28.9 ± 8.2	983.6 ± 283.2	103.4 ± 31.4
July 2002	88.8 ± 24.3	2731.6 ± 762.2	281.0 ± 78.6
August 2002	70.0 ± 12.7	1817.8 ± 413.5	148.8 ± 36.2
Below-ground tissues			
July 2001	112.9 ± 78.7	2260.4 ± 1243.3	62.5 ± 33.5
April 2002	2.2 ± 1.0	55.5 ± 25.0	4.0 ± 1.9
May 2002	24.2 ± 10.2	435.3 ± 187.9	30.7 ± 13.3
June 2002	36.4 ± 16.4	958.9 ± 440.7	44.2 ± 21.9
July 2002	110.9 ± 95.4	2812.3 ± 2445.9	97.9 ± 85.1
August 2002	61.8 ± 24.0	1384.6 ± 659.4	35.2 ± 17.8

$\mu = [\ln(B_{Jul}) - \ln(B_{Apr})]\Delta t^{-1}$ (Burkhardt et al., 1999), with B = areal biomass and $\Delta t \cong 100$ days, was calculated to be 0.05 d^{-1} for the above-ground tissues, 0.04 d^{-1} for the below-ground tissues, with $\mu \cong 0.045$ d^{-1} based on the total biomass estimates. Using the product of the biomass estimates and the carbon concentration measured in above- and below-ground tissues (Table 1), the increase in organic carbon during the growth phase ($\Delta B_C \Delta t^{-1}$) was equivalent to 18–27 mmol C m^{-2} d^{-1} in the above-ground tissues, 22–28 mmol C m^{-2} d^{-1} in the below ground-tissues, with a total of 40–55 mmol C m^{-2} d^{-1}. Similarly, the increase in organic nitrogen during this period ($\Delta B_N \Delta t^{-1}$) was equivalent to 1–3 mmol N m^{-2} d^{-1} in the above-ground tissues, 0.5–1 mmol N m^{-2} d^{-1} in the below-ground tissues, with a total of 1.5–4 mmol N m^{-2} d^{-1}. These values are net rates because they do not include the carbon and nitrogen respired and lost as dissolved organic matter or detrital tissue during the growth period.

4.2. Organic carbon and nitrogen concentration in new leaf

The concentration of organic carbon (C_{org}) varied temporally between 25 and 35 mmol g^{-1} (Fig. 2a), with the highest concentrations recorded in early March or April, and the lowest concentrations recorded during May. While this temporal decline in new leaf C_{org} appears to be continuous in 2003, the trend in 2002 was not monotonic; after an initial decrease until May, concentrations increased again in June before a gradual decrease until August–September of that year (Fig. 2). The average ($\pm 1\sigma$) of all available C_{org} measurements was 29.2 ± 3.5 mmol g^{-1} ($n = 16$; equivalent to 35.1 ± 4.2% on a dry weight basis).

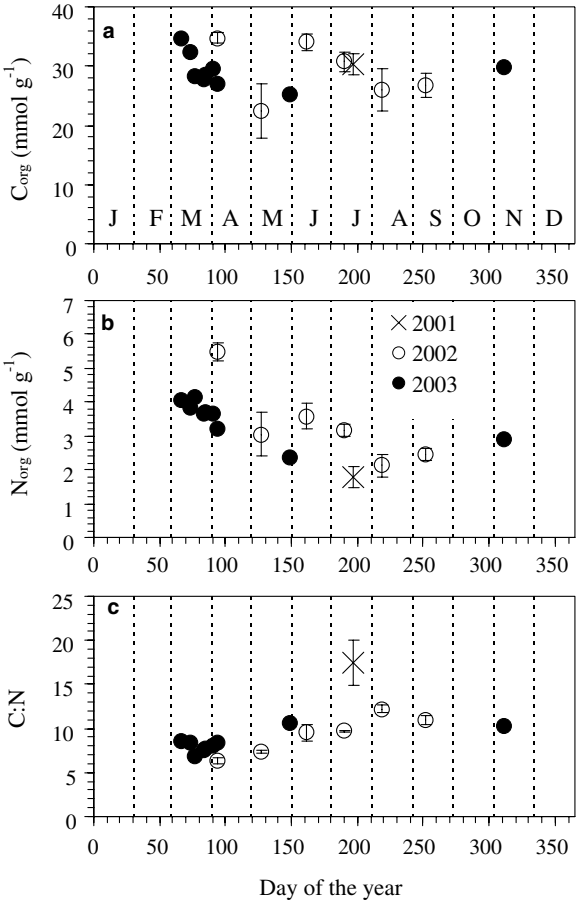

Fig. 2. The concentration of (a) organic carbon ($C_{org,}$), (b) organic nitrogen ($N_{org,}$), and (c) the molar C_{org} to N_{org} ratio (C:N) in new leaf of *Z. noltii*.

The concentration of organic nitrogen (N_{org}) also varied temporally between 2–2.5 mmol g^{-1} and 4–5 mmol g^{-1} (Fig. 2b). The highest N_{org} was invariably measured at the same time as the highest C_{org} concentration (early March or April), with a decline thereafter towards the lowest N_{org} in both years of sampling. The temporally more extended data set from the year 2002 suggests that the attainment of the lowest N_{org} occurred around the end of summer (August). The average ($\pm 1\sigma$) of all available N_{org} measurements was 3.3 ± 0.9 mmol g^{1} ($n = 16$; equivalent to 4.8 ± 1.2% on a dry weight basis). The molar C_{org} to N_{org} ratio (C:N) in new leaf exhibited a clear temporal (seasonal) pattern,

increasing by a factor of 2, from the lowest values of 6–7 in early March and April to the highest value of 12 during late summer (Fig. 2c), with an overall average ($\pm 1\sigma$) value of 9.4 ± 2.7 ($n = 16$).

4.3. The isotopic composition of carbon, nitrogen, and sulphur in new leaf

The stable isotope ratio of organic carbon, nitrogen, and sulphur ($\delta^{13}C_{leaf}$, $\delta^{15}N_{leaf}$, and $\delta^{34}S_{leaf}$, respectively) exhibited a seasonal pattern of enrichment and depletion in the heavy isotope. The $\delta^{13}C_{leaf}$ ranged from $-11.0 \pm 0.2‰$ to $-6.9 \pm 0.1‰$ ($n = 3$), with the highest isotopic enrichment recorded in March–April followed by a tendency towards isotopic depletion that was highest in August (Fig. 3a). The $\delta^{15}N_{leaf}$ ranged from $+3.3 \pm 0.6‰$ ($n = 3$) to $+7.5‰$, with a tendency for isotopic depletion in March–April followed by isotopic enrichment thereafter (Fig. 3b). The overall average $\delta^{13}C_{leaf}$ and $\delta^{15}N_{leaf}$ were $-8.9 \pm 1.1‰$ and $+5.7 \pm 1.1‰$, respectively ($n = 16$). The $\delta^{34}S_{leaf}$, averaged monthly over 3 plots, ranged over 13‰. The initial sampling in April gave the highest $\delta^{34}S_{leaf}$ of $+10.5 \pm 0.9‰$, which steadily decreased through $+6.4 \pm 3.0‰$ in May and $+3.4 \pm 3.2‰$ in June to a distinct minimum value of $-3.0 \pm 0.8‰$ in July. The $\delta^{34}S_{leaf}$ began a return to more positive values in August ($+4.0 \pm 5.4‰$).

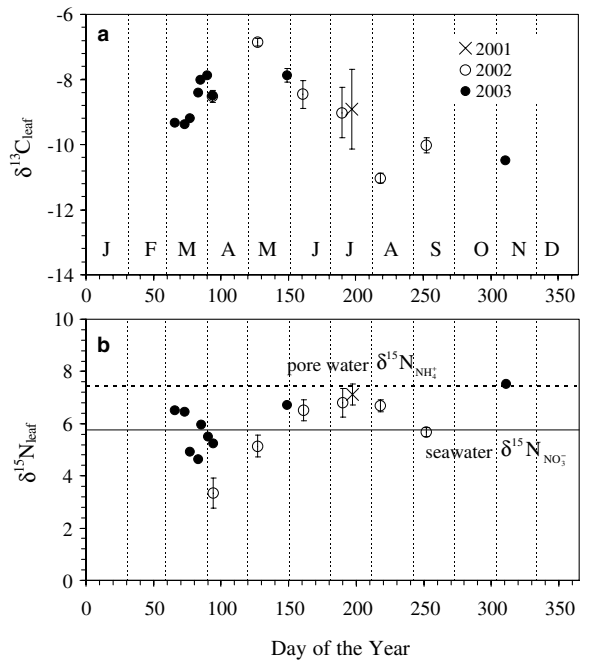

Fig. 3. Stable isotope ratio of (a) carbon ($\delta^{13}C_{leaf}$), and (b) nitrogen ($\delta^{15}N_{leaf}$) in new leaf of *Z. noltii* plants.

4.4. Sediment characteristics

The sediments within the meadow had the black colour and distinct sulphide odour characteristic of anoxic conditions below a sub-mm thin dark brown surface layer. The sediment porosity (ϕ) and density of dry solids (ρ_s) were 0.63 ± 0.07 ($n = 7$) and 2.24 ± 0.11 g ml^{-1} ($n = 6$), respectively. The isotopic composition of sedimentary nitrogen ($\delta^{15}N_{sed}$) was invariant, averaging $+5.8 \pm 0.3‰$ ($n = 9$). The extractable ammonium ($[NH_4^+]_{extr}$) was found to be very low at $1.1 \times 10^{-4} \pm 0.6 \times 10^{-4}$ mmol N g^{-1} ($n = 6$), which represents less than 0.1% of the total sedimentary nitrogen pool (0.012–0.014 mmol N g^{-1}; unpubl. data) but $50 \pm 50\%$ (on wet sediment basis) of the contemporaneous dissolved ammonium reservoir in the pore waters ($[NH_4^+]_{pw}$), leading to a variable solid-solution partition coefficient $K_D = [NH_4^+]_{extr}/[NH_4^+]_{pw} = 0.4 \pm 0.3$ ml g^{-1}. Based on the porosity and dry density measurements, the dimensionless sorption constant of ammonium in these sediments was calculated to be $K = K_D[\rho_s(1-\phi)\phi^{-1}] = 0.4 \pm 0.4$, which is similar to the K values obtained in *Z. marina* beds and other marine sediments (e.g., Dennison et al., 1987).

4.5. Dissolved inorganic carbon and nitrogen in the surface seawater

The isotopic composition of total dissolved inorganic carbon ($\delta^{13}C_{DIC}$) in the surface seawater ranged from $-0.38 \pm 0.06‰$ (November 2003, $n = 4$) to $+1.24 \pm 0.05‰$ (May 2002, $n = 2$) (Fig. 4a). A seasonal pattern of ^{13}C enrichment of the DIC pool was apparent; the highest isotopic enrichment was observed in May, with lower (i.e., more negative) $\delta^{13}C_{DIC}$ at either side of the spring maximum.

The concentration of dissolved nitrate in the water column ranged from 0 in the summer to 8.8 μM in early March in the meadow, and from 6.6 to 16.0 μM during March–April in the Menai Strait. The concentration of dissolved ammonium in the water column varied between 0.2 and 1.4 μM between May and July in the meadow (Fig. 4b). The detailed dataset available from the Menai Strait (Fig. 4b; Al-Azri, 2002) indicates that the highest dissolved nitrate concentrations in the region occur early in the year (February–March) and are followed by a rapid decline to undetectable

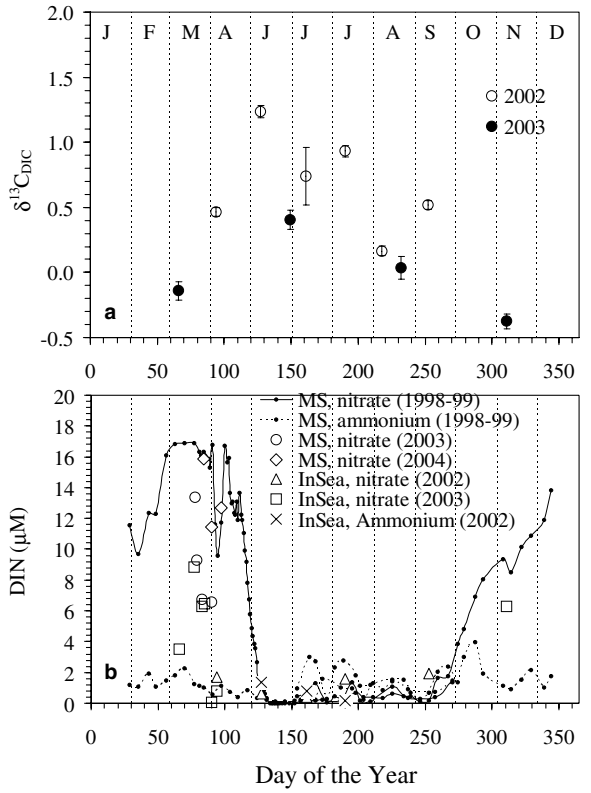

Fig. 4. (a) Stable isotope ratio of total dissolved inorganic carbon ($\delta^{13}C_{DIC}$) in the seawater overlying the *Z. noltii* meadow. Error bars represent 1σ of replicate measurements (error bars are smaller than symbol when not shown). (b) Dissolved inorganic nitrogen (DIN) in the water column in the Menai Strait (MS) and in Inland Sea (InSea). The data for 1998 and 1999 were taken from Al-Azri (2002).

levels during the summer, with an increase again in September–October towards the seasonal maximum. It is noted that in both locations the decline in the concentration of dissolved nitrate occurred earlier in 2003–2004 than in 1999. No clear seasonal trend is apparent in the dissolved ammonium in the water column. The stable isotope ratio of nitrogen in dissolved nitrate ($\delta^{15}N_{NO_3^-}$) at the concentration maximum (16 μM; March 2004) in the Menai Strait was $+5.8 \pm 0.2‰$ ($n = 2$).

4.6. Dissolved ammonium in the sediments

The concentration of pore water ammonium (NH_4^+) in the surficial sediments was variable on small spatial scales within the meadow without a systematic temporal trend. Based on the $t = 0$ measurements during the anoxic incubations that represent in situ conditions, the NH_4^+ concentration was 300 μM in March, ranged from 11 to 250 μM in May and from 130 to 430 μM in August, while a concentration of 160 μM was measured in November. These concentrations are lower than those reportedly associated with detrimental effects on *Z. marina* of excessive pore water ammonium (500–1600 μM; Touchette and Burkholder, 2000). The stable isotope ratio of $NH_4^+ - N$ could not be measured in the $t = 0$ samples due to sample volume constraints, but a $\delta^{15}N_{NH_4^+} = +7.5 \pm 0.5‰$ ($n = 3$) was determined in the surface pore waters in November.

During the anoxic incubation the NH_4^+ concentration increased linearly with time to values in excess of 3000 μM, depending on the incubation period and temperature (Fig. 5a). These concentration changes yielded a net NH_4^+ remineralization rate ($R_{NH_4^+,rem}$) that ranged from 8 mmol m^{-2} d^{-1} at 7 °C to 11–14 mmol m^{-2} d^{-1} at 16 and 22 °C (Table 2). Concurrently at $t > 0$, the $\delta^{15}N_{NH_4^+}$ varied between +6.0‰ and +10.0‰ but did not exhibit a

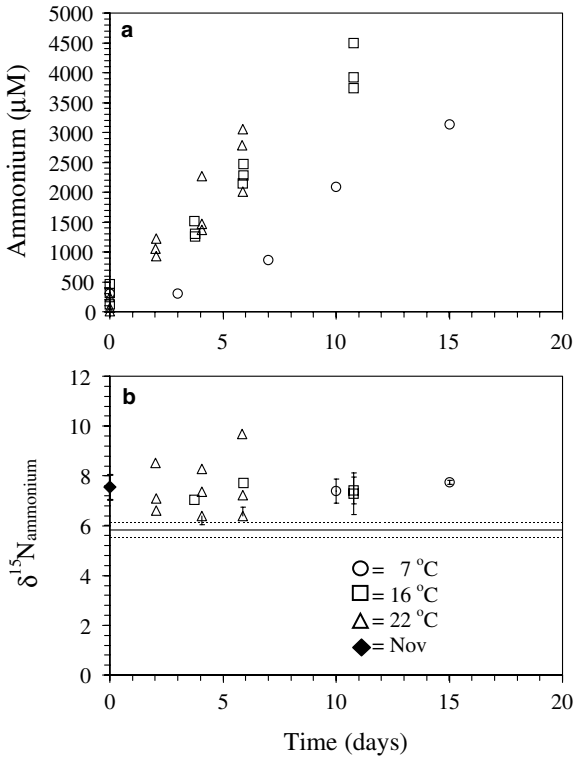

Fig. 5. Time series of pore water measurements of (a) the concentration of dissolved ammonium, and (b) the stable isotope ratio of ammonium nitrogen during anoxic incubation of surficial sediment from the *Z. noltii* meadow. The solid line in (b) indicates the mean stable isotope ratio of nitrogen in the sediment solids, while the dashed lines indicate $\pm 1\sigma$ of this value.

Table 2
Anoxic remineralization rate of sedimentary nitrogen as ammonium ($R_{NH_4^+,rem}$) and its isotopic composition ($\delta^{15}N_{NH_4^+}$) during anoxic incubation at different temperatures (θ in °C) of surface sediments from the *Z. noltii* meadow

Month	θ	$R_{NH_4^+,rem}$	$R_{NH_4^+,rem}$ [a]	$\delta^{15}N_{NH_4^+}$
March	7.0	7.8 ± 0.2	10.6 ± 6.7	$+7.6 \pm 0.5$
		12.2 ± 0.3	16.5 ± 10.4	$+7.4 \pm 0.4$
May	16.0	11.4 ± 0.3	15.5 ± 9.7	$+7.4 \pm 0.6$
		11.3 ± 0.3	15.2 ± 9.6	
		14.1 ± 0.4	19.0 ± 12.0	$+9.0 \pm 1.0$
August	22.0	8.3 ± 0.2	11.2 ± 7.1	$+6.4 \pm 0.4$
		13.5 ± 0.3	18.3 ± 11.5	$+7.3 \pm 0.1$

The remineralization rate is given in mmol m^{-2} d^{-1}, and the isotopic composition (in per mil) reported is the average of the $\delta^{15}N_{NH_4^+}$ measurements available per incubation.
[a] Adjusted for ammonium adsorption.

systematic variation with time (Fig. 5b) or NH_4^+ concentration. Furthermore, it was significantly more enriched in ^{15}N than the $\delta^{15}N_{sed}$ at all times, i.e., ANOVA $p \leqslant 0.006$ based on the comparison of the means of $\delta^{15}N_{sed}$ and $\delta^{15}N_{NH_4^+}$ from each core. Based on all available observations, the mean pore water $\delta^{15}N_{NH_4^+}$ during the anoxic incubations was $+7.4 \pm 0.8‰$ ($n = 16$), which is identical to the $\delta^{15}N_{NH_4^+}$ measured in November 2003 but is analytically discernible from the $\delta^{15}N_{NO_3^-}$ measured at the nitrate concentration maximum in seawater in early spring.

5. Discussion

The current measurements of the chemical and isotopic parameters in *Z. noltii* new leaf revealed maximum C:N and $\delta^{15}N_{leaf}$, and minimum $\delta^{13}C_{leaf}$ and $\delta^{34}S_{leaf}$ coincident with high standing biomass in summer, with the reverse coincident with low standing biomass in the beginning of spring. Below, each chemical and isotopic parameter is discussed with reference to growth patterns.

5.1. Seasonal changes in the chemical composition of *Z. noltii* new leaf

The concentration of carbon and nitrogen in new leaf tissue was characterized by a decrease in the transition from spring to summer (Fig. 2), equivalent to 25–27% and 40–60%, respectively, relative to the maximum concentrations measured in early spring (March–April). Consequent on the more pronounced N_{org} than C_{org} decline, the molar C:N ratio of new leaf tissue also varied systematically during the growing season, increasing by a factor of 1.6–2 to a maximum 11–12 in the summer (Fig. 2). Maximum concentration and minimum C:N appear to become established through autumn and winter. The dynamics of nitrogen appears to drive tissue C:N as indicated by the significant correlation between the two parameters, which can be described by the power function, $C{:}N = 25.4\ (\pm 7.7)\ [N_{org}]^{-0.88(\pm 0.22)}$ ($r = -0.9$, $p < 0.001$, $n = 16$; Fig. 6), very similar to the relationships reported for the youngest leaf of *Z. marina* (Papadimitriou et al., 2005) and a large assortment of marine plants (Duarte, 1992). Based on the average measurements taken from all the available plant leaves, Duarte (1990) suggested that N_{org} concentrations less than 1.3 mmol g^{-1} and C:N values greater than 20 could represent conditions where nitrogen demand by seagrasses exceeded the external supply. It is not known whether or not these critical values are appropriate for the assessment of new leaf tissue, but assuming it is, it is noted that the new leaf tissue of *Z. noltii* did not appear to be limited by the supply of external nitrogen at any time of the year (Fig. 6).

The characteristics of the temporal variability of carbon and nitrogen in new leaf of *Z. noltii* are consistent with seasonal fluctuations reported for the same species elsewhere in the temperate coastal zone (Perez-Llorens and Niell, 1993; Plus et al., 2003) and for other seagrass species (*Z. marina*: Stephenson

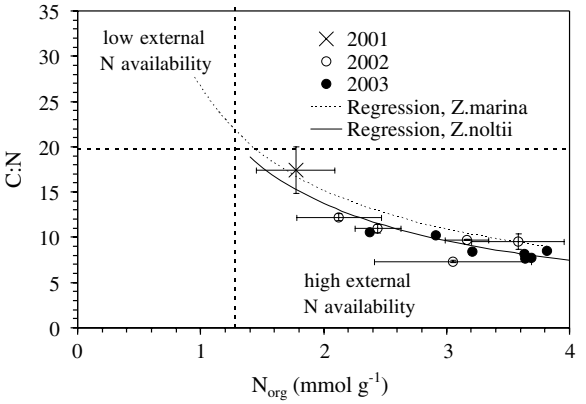

Fig. 6. Molar carbon to nitrogen ratio (C:N) versus nitrogen concentration (N_{org}) in new leaf of *Z. noltii*. Dashed lines indicate the threshold values for N_{org} and the C:N ratio in seagrasses related to the balance between external nitrogen supply and internal demand by the plants (from Duarte, 1990). The regression fitted line for *Z. marina* was taken from Papadimitriou et al. (2005).

et al., 1984; Pedersen and Borum, 1993; Papadimitriou et al., 2005; *Posidonia oceanica*: Alcoverro et al., 1995; *Thalassia testudinum*: Anderson and Fourqurean, 2003). The seasonal trend of the N_{org} mirrored that of the DIN pool in the water column (Fig. 4b) and varied inversely with the standing biomass (Table 1). The standing biomass of temperate *Zostera* species reaches a maximum in the summer, following the seasonal temperature and irradiance increase (Perez-Llorens and Niell, 1993; Plus et al., 2003). Seagrass plants are known to support growth by internal mobilization of compounds stored during the preceding period (winter) of elevated external nutrient availability (Borum et al., 1989; Pedersen and Borum, 1992; Marbá et al., 2002). The potential for relocation of stored nitrogen compounds and for reclamation of assimilated nitrogen from older seagrass tissues to support new growth, along with additional inorganic nitrogen that can be derived from the dissolved ammonium reservoir in the underlying sediments via root uptake throughout the year (Thursby and Harlin, 1982; Short and McRoy, 1984), cannot prevent the distinct seasonality in the N_{org} and C:N of young seagrass tissue.

5.2. Seasonal changes in the isotopic composition of *Z. noltii* new leaf

Information on the stable isotope ratio of carbon, nitrogen, and sulphur in *Z. noltii* tissues is rather scarce. Two $\delta^{13}C_{leaf}$ values were cited in the seagrass review of Hemminga and Mateo (1996), averaging $-11.6 \pm 0.5‰$, near the lower end of the current range ($-11.0‰$ to $-6.9‰$), while there are no published $\delta^{15}N_{leaf}$ and $\delta^{34}S_{leaf}$ values. The $\delta^{13}C_{leaf}$ and $\delta^{15}N_{leaf}$ exhibited different periodicity but similar amplitude of 3–4‰ between the highest and lowest values measured during the growing season (Fig. 3). The highest ^{13}C enrichment was measured in spring and was coincident with the lowest ^{15}N enrichment, with the reverse observed in the summer. Intra-annual ranges of 1–9‰ with a clear seasonal pattern have been reported for the $\delta^{13}C$ of *Z. marina* (Stephenson et al., 1984; Papadimitriou et al., 2005) and *T. testudinum* (Anderson and Fourqurean, 2003). Combined with the current observations, it appears that the $\delta^{13}C_{leaf}$ of seagrasses is invariably characterized by considerable cyclical seasonality. The intra-annual ranges of 1–5‰ reported for the $\delta^{15}N$ of the same seagrass species (Anderson and Fourqurean, 2003; Papadimitriou et al., 2005) are similar to the range of the $\delta^{15}N_{leaf}$ of *Z. noltii* but were not characterized by the clear seasonality seen here. The current $\delta^{34}S_{leaf}$ measurements are comparatively restricted but exhibited a systematic decline during the growing season, leading to a pronounced ^{34}S depletion in new leaf in the summer by $-13‰$ relative to the value measured in early April. Our current state of knowledge of the extent of variation of the $\delta^{34}S$ in seagrass leaves (-6.7 to $+17.6‰$) is based on a small data set that often relates to single measurements of $\delta^{34}S$ in different seagrass species at different locations (Fry et al., 1982; Raven and Scrimgeour, 1997; Kharlamenko et al., 2001; Oakes and Connolly, 2004).

The nutrient requirement of aquatic plants is directly related to growth rate, such that the strong seasonality in seagrass growth can impose observable isotopic variation in new biomass (Papadimitriou et al., 2005). Both the $\delta^{13}C_{leaf}$ and $\delta^{15}N_{leaf}$ of *Z. noltii* were significantly correlated with C:N in 2002 and 2003 ($\delta^{13}C_{leaf}$: $r = -0.612$, $p = 0.015$, $n = 15$; $\delta^{15}N_{leaf}$: $r = 0.739$, $p = 0.002$, $n = 15$), with the most ^{13}C depleted and most ^{15}N enriched values measured during the period of highest standing biomass in the summer. Given the distinct growth-related seasonality of C:N, the correlations suggest that growth should influence the carbon and nitrogen isotope ratios in *Z. noltii* new leaf. Below, the observed seasonal isotopic changes will be discussed in relation to the isotopic composition of inorganic solute pools from which seagrasses derive their nutrient requirement, as well as in relation to biological isotopic fractionation due to kinetic constraints of isotope assimilation during plant growth.

5.2.1. Carbon isotopes

The $\delta^{13}C$ of seagrass tissue has been observed to reflect partly the $\delta^{13}C$ of the external dissolved inorganic carbon source (i.e., DIC) and partly the kinetics of assimilation of carbon isotopes (Hemminga and Mateo, 1996; Papadimitriou et al., 2005). Approximately 40% of the intra- and inter-annual variability in the $\delta^{13}C_{leaf}$ of *Z. noltii* can be explained by its modest but significant correlation with the external bulk seawater $\delta^{13}C_{DIC}$ ($r = 0.696$, $p = 0.036$; $n = 9$; Fig. 7). A similar relationship was observed in *Z. marina* meadows in Denmark (Papadimitriou et al., 2005; reproduced in Fig. 7), despite dissimilarities in the two study areas, such as the much wider external $\delta^{13}C_{DIC}$ range (5‰) experienced by the *Z. marina* plants

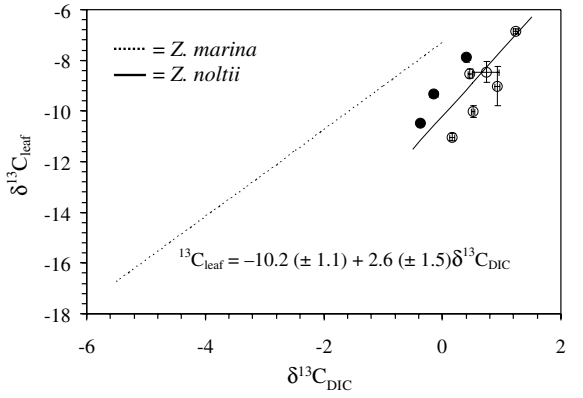

Fig. 7. The $\delta^{13}C_{leaf}$ as a function of the external seawater $\delta^{13}C_{DIC}$. The equation shown is the regression fit to the observations from *Z. noltii*. The regression fit on *Z .marina* new leaf covers the extent of the observations reported in Papadimitriou et al. (2005).

than by the *Z. noltii* plants (external $\delta^{13}C_{DIC}$ range: 1.6‰). The dissimilarity between the two *Zostera* species extends in that the *Z. noltii* plants are intertidal and experience periods of emersion in the course of a tidal cycle, with concomitant variation in their photosynthetic carbon assimilation rate (Peralta et al., 2002; Silva et al., 2005, and references therein). Further, the isotopic composition of the external carbon source during the emersion period can be different from that of the DIC in the bulk water column as a result of inorganic carbon exchange with the atmosphere and the benthic environment of the small water volume retained around the plants. It appears, however, that the isotopic composition of the bulk seawater DIC can be reflected in the $\delta^{13}C$ of new *Z. noltii* leaf in this location.

Fractionation due to faster biological assimilation of the light carbon isotope (^{12}C) results in isotopically depleted biomass relative to the external dissolved inorganic source of the assimilated substrate. Overall this will result in departure of tissue $\delta^{13}C$ from that of the external source of carbon and is driven by an imbalance in the rates of supply of the external substrate into the cell and of demand for it by the plant due to a combination of physiological and environmental factors (Fogel and Cifuentes, 1993). The occurrence and magnitude of the growth-related biological carbon isotope effect can be explored by calculating the apparent isotope enrichment factor of new *Z. noltii* leaf relative to external DIC, $\varepsilon_{seagrass\text{-}DIC} = \delta^{13}C_{leaf} - \delta^{13}C_{DIC}$. The available $\delta^{13}C_{leaf}$ measurements integrate inorganic carbon uptake over several diurnal tidal cycles, but information on the $\delta^{13}C$ of the external inorganic carbon source is currently limited to the bulk water column DIC measurements. The observed correlation between the two parameters (Fig. 7) suggests that the bulk seawater $\delta^{13}C_{DIC}$ can be considered representative of the isotopic composition of the external inorganic carbon source in this location with the restricted tidal regime. However, the expected variation in the $\delta^{13}C$ of the external carbon source over a tidal cycle that involves emersion (i.e., during neap tides) renders the calculated $\varepsilon_{seagrass\text{-}DIC}$ only an approximation of the true value.

The $\varepsilon_{seagrass\text{-}DIC}$ can be shown to be a function of the DIC species utilized, i.e., $CO_2(aq)$ and HCO_3^-, as well as of their relative fluxes in and out of the cell driven by the balance between substrate availability and demand (Papadimitriou et al., 2005, and references therein). The maximum $\varepsilon_{seagrass\text{-}DIC}$ will approach the temperature-dependent equilibrium isotopic difference between $CO_2(aq)$ and HCO_3^-, $\varepsilon_{CO_2(aq)\text{–}HCO_3^-}$, when external $CO_2(aq)$ is utilized ($\varepsilon_{CO_2(aq)\text{–}HCO_3^-} = -12‰$ to $-9‰$ in the temperature range of 0 to 25 °C, respectively; Zhang et al., 1995). It will be higher (i.e., less negative) than $\varepsilon_{CO_2(aq)\text{–}HCO_3^-}$ ($\varepsilon_{CO_2(aq)\text{–}HCO_3^-} < \varepsilon_{seagrass-DIC} \leqslant 0$) when active transport of external HCO_3^- into the cell provides the assimilated carbon (Papadimitriou et al., 2005). Maximum $\varepsilon_{seagrass\text{-}DIC}$ and, therefore, maximum ^{13}C enrichment in new leaf should be expected during the period of fast growth rate, when high carbon demand relative to its supply can hinder the expression of the strongly fractionating enzymatic step of assimilation (enzymatic carbon isotope enrichment factor (ε_R) range: -30 to $-19‰$; Guy et al., 1993).

The calculated $\varepsilon_{seagrass\text{-}DIC}$ had a distinct seasonality from a maximum of $-8.1‰$ in May to a minimum of $-11.2‰$ in August (Fig. 8a). The calculated $\varepsilon_{seagrass\text{-}DIC}$ was mostly higher (i.e., less negative) than $\varepsilon_{CO_2(aq)\text{–}HCO_3^-}$, especially during spring, by a maximum of approximately $+2‰$ (Fig. 8a), which suggests utilization of external HCO_3^-. This pathway of inorganic carbon acquisition has been documented experimentally in *Z. noltii* (Mercado et al., 2003). A similar behaviour was noted for *Z. marina* on account of the seasonal pattern of the carbon isotope enrichment factor during the period of fast growth rate, in phase with the growth-related C:N increase (Papadimitriou et al., 2005). However, the current data indicate a negative rather than a positive relationship of $\varepsilon_{seagrass\text{-}DIC}$

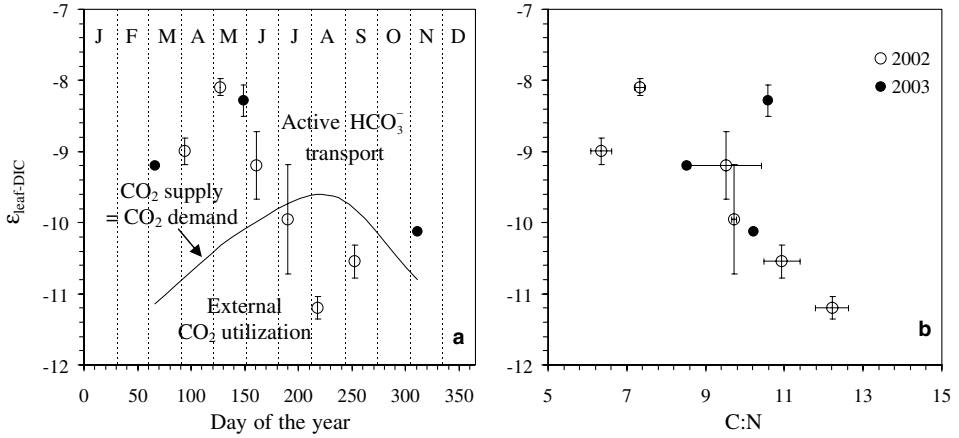

Fig. 8. (a) Seasonality in the apparent carbon isotopic enrichment factor in *Z. noltii* new leaf relative to the external DIC ($\varepsilon_{seagrass\text{-}DIC}$), and (b) as a function of new leaf C:N. The curve in (a) indicates the equilibrium isotope enrichment factor between CO_2(aq) and HCO_3^- calculated from the temperature function of Zhang et al. (1995).

with C:N (Fig. 8b), suggesting that the imbalance in carbon assimilation in *Z. noltii* is out of phase with that of nitrogen assimilation. In other words, excess carbon demand appears to occur early in the growing season (spring), while excess nitrogen demand occurred later during the summer, both relative to their external supply rates. This hypothesis requires validation, for example, in physiological factors, such as the potential effect of amino acid synthesis or overall growth stimulation on carbon demand in *Z. noltii* early in the growing season (e.g., Touchette and Burkholder, 2000), when external nitrogen supply is augmented by the availability of inorganic nitrogen in the water column.

5.2.2. Nitrogen isotopes

A similar variation with season may be expected in the $\delta^{15}N_{leaf}$ on account of the temporally opposing patterns of biomass (Table 1), and, hence, DIN demand, and DIN availability, especially in the form of NO_3^-, in the water column (Fig. 4). In contrast to seagrass $\delta^{13}C$, however, seagrass $\delta^{15}N$ has often been deemed a reliable reflection of the $\delta^{15}N$ of the external DIN assimilated by the plants with minimal biological isotope effect due to the limited amount of external DIN available crucially during the period of maximum growth (McClelland and Valiela, 1998; Papadimitriou et al., 2005). In addition, root uptake of the NH_4^+ released in the pore waters of the underlying sediments via benthic heterotrophic activity is well documented on seagrasses (Thursby and Harlin, 1982; Short and McRoy, 1984; Hemminga et al., 1994). As such, the pore water NH_4^+ pool will contribute to the $\delta^{15}N$ of the biomass, conferring a potentially differing isotopic signal on it from that of the water column DIN. Comparison of the $\delta^{15}N_{leaf}$ with the available pore water $\delta^{15}N_{NH_4^+}$, which was largely invariant (Fig. 5b), and the seawater $\delta^{15}N_{NO_3^-}$ at the seasonal nitrate maximum in early spring (March) showed that the $\delta^{15}N_{leaf}$ deviated from both by up to −2.5‰ and −4.1‰, respectively, at the standing biomass minimum in spring (March–April; Fig. 3b). The ^{15}N depletion in *Z. noltii* very early in the growing season would be expected when the standing biomass and, hence, demand by the seagrass plants is still low relative to the supply of external inorganic nitrogen. Isotopically depleted above-ground biomass relative to the external source of inorganic nitrogen was observed in other seagrass species under natural (*Z. marina*: −2‰ to −6‰; McClelland and Valiela, 1998) or experimentally fertilized conditions (various species including *Z. capricorni*: −4‰ to −11‰; Udy and Dennison, 1997) but was not related to seasonality.

As the growing season progressed towards the standing biomass maximum in mid-summer (July), the $\delta^{15}N_{leaf}$ became increasingly ^{15}N-enriched and surpassed the seawater $\delta^{15}N_{NO_3^-}$ measured in early spring towards values equivalent to the pore water $\delta^{15}N_{NH_4^+}$ (Fig. 3b). On this evidence, growth exclusively on external seawater nitrate as a source of inorganic nitrogen must be precluded; this would require that the limit of $\delta^{15}N_{leaf}$ should be $\delta^{15}N_{NO_3^-}$, because of the rapid exhaustion of nitrate early in the growing season (March–April; Fig. 4)

and of conservation of the isotopic mass balance between the major nitrogen source in the water column (NO_3^-) and its sink (seagrass biomass). Contribution to the seagrass nitrogen demand from the pore water ammonium pool can explain the measured $\delta^{15}N_{leaf}$, at least during the growing season, when NO_3^- in the external seawater remains low and is at its prolonged seasonal minimum to sub-μM levels. Shifts in the external inorganic nitrogen source from the seasonally limited seawater NO_3^- pool to the seasonally sustained pore water NH_4^+ pool are known to occur in seagrasses (Short and McRoy, 1984; Pedersen and Borum, 1993; Lepoint et al., 2002). The mean pore water $\delta^{15}N_{NH_4^+}$, furthermore, appears to be an upper limit for the $\delta^{15}N_{leaf}$ (Fig. 3b). On the axiom of minimal isotopic fractionation between source and sink when biomass demand severely exceeds inorganic source supply (e.g., Fogel and Cifuentes, 1993), this implies some degree of limitation in the pore water ammonium supply to the seagrass plants relative to their demand at the biomass maximum despite pore water ammonium concentrations mostly exceeding the proposed threshold of 100 μM (Dennison et al., 1987).

The current results from the anoxic incubation experiments indicated fast benthic nitrogen remineralization rates, in the order of 8–14 mmol N m^{-2} d^{-1}, which expectedly exhibited a temperature-dependent magnitude (Table 2). The incubation-derived values of the net NH_4^+ remineralization rate compare well with similarly derived values from *Z. marina* colonized sediments (5.7–91.0 mmol N m^{-2} d^{-1}; Dennison et al., 1987, and references therein) but are minimum estimates of the total benthic nitrogen remineralization rate. An additional contribution is expected from the dissolved organic nitrogen pool, which is unknown here but can be substantial in similar environments (McGlathery et al., 2001; Eyre and Ferguson, 2002). Furthermore, the standing stock of pore water NH_4^+ on which the current estimates are based is the net product of addition via heterotrophic organic nitrogen oxidation modified by removal via NH_4^+ adsorption onto sediment particles and biological uptake. While seagrass uptake or release of NH_4^+ was not a factor during the anoxic incubations, the extent of bacterial NH_4^+ uptake can be a considerable fraction of the total nitrogen remineralization rate in sediments (Hoch et al., 1992, and references therein), which is also unknown in this setting. Accounting for the effect of NH_4^+ adsorption raised the NH_4^+ remineralization slightly, but it cannot be discerned from the net rate owing to the large error (Table 2) propagated from that of the NH_4^+ adsorption coefficient (see Section 4.4). The observed benthic remineralization rates of nitrogen are higher than the respective approximate acquisition rate by *Z. noltii* (1.5–4.0 mmol N m^{-2} d^{-1}; Section 4.1) by up to a factor of 2–3. This suggests a non-limiting supply of NH_4^+ available to the seagrass plants from the sedimentary pore waters, which is consistent with the N_{org} and C:N measurements (Fig. 6). If indeed there are conditions of sedimentary NH_4^+ limitation during the period of maximum standing seagrass biomass in the summer as the $\delta^{15}N_{leaf}$ suggests (Fig. 3b), it must not be related to its availability; a limiting step in the acquisition pathway in the rhizosphere, such as solute diffusion within the sedimentary matrix (Lepoint et al., 2002), is potentially a more likely factor.

5.2.3. Sulphur isotopes

The $\delta^{34}S$ of submerged marine plants reflects that of the main source of sulphur, i.e., seawater SO_4^{2-}, owing to minimal isotopic fractionation during the biological uptake of sulphur (Trust and Fry, 1992). It is evident from the available measurements that the $\delta^{34}S_{leaf}$ deviated from the $\delta^{34}S_{SO_4^{2-}}$ (approximately +20‰ in oceanic water; Rees et al., 1978) by −10‰ (April) to −23‰ (July). Isotopic depletion of the $\delta^{34}S_{leaf}$ by up to −26.7‰ relative to the oceanic water value has been measured in *Z. marina* (Kharlamenko et al., 2001). Isotopically depleted $\delta^{34}S$ values in rooted aquatic macrophytes are associated with the microbial redox cycle of inorganic sulphur compounds in the benthic environment, including sulphide production during the heterotrophic oxidation of organic matter by SO_4^{2-} reduction. The $\delta^{34}S$ of sedimentary sulphide phases is much more negative than that of their parent compound due to large isotopic fractionations associated with the heterotrophic processing of inorganic sulphur compounds in anoxic sediments (Trust and Fry, 1992; Habicht and Canfield, 1996). Isotopically depleted $\delta^{34}S$ values in rooted aquatic macrophytes can develop due to uptake of isotopically depleted SO_4^{2-} generated from sulphide oxidation in the immediate vicinity of the roots or when ingress of sedimentary dissolved sulphide occurs. The former process has not yet been documented and remains a possibility, but sulphide invasion has been measured directly in

seagrasses under specific environmental conditions, such as reduced oxygen concentration in the water column (Pedersen et al., 2004; Borum et al., 2005). The current $\delta^{34}S_{leaf}$ values, therefore, suggest that toxic dissolved sulphide penetrated the *Z. noltii* plants, while their seasonality implies some temporal fluctuation in this process. The temperature-dependent increase in benthic microbial activity indicated by the current anoxic incubations will lead to enhancement of benthic sulphide production in the rhizosphere in the summer and, hence, of the potential for occurrence of sulphide ingress in the seagrass plants.

6. Summary

The current data indicated considerable and systematic seasonal periodicity in the nitrogen concentration and molar C:N, as well as in the stable isotope ratio of carbon and nitrogen in *Z. noltii* new leaf tissue. The $\delta^{13}C_{leaf}$ reflected the $\delta^{13}C$ of the external bulk seawater DIC but was further modified by isotope fractionation during carbon assimilation. Assuming the bulk seawater DIC is the major source of inorganic carbon, variation in biological fractionation due to the growth-driven imbalance in external inorganic carbon supply and demand can explain the periodicity in the carbon isotope enrichment and depletion pattern in new seagrass leaf, while the extent of isotopic enrichment was consistent with utilization of external bicarbonate ions as a source of inorganic carbon. These indications require validation because of the unaccounted effect of emersion periods in the stable carbon isotope dynamics in this intertidal seagrass species. Two of the major external DIN sources to seagrass plants, seawater nitrate, as measured at the early spring concentration maximum, and pore water NH_4^+ had distinctly different $\delta^{15}N$. By comparison with these values, the $\delta^{15}N_{leaf}$ was consistent with biological isotope fractionation briefly during the period of slow growth rate and appeared to record the $\delta^{15}N$ of pore water NH_4^+ later in the growing season at or near the standing biomass maximum. The current data do not constrain well the utilization of seawater NO_3^- as an external source of inorganic nitrogen, but the reflection of the $\delta^{15}N$ of pore water NH_4^+ in the $\delta^{15}N_{leaf}$ as the growing season progressed indicated contribution to the demand of *Z. noltii* from the benthic inorganic nitrogen pool. The $\delta^{34}S_{leaf}$ appears to be similarly influenced by the benthic environment; its deviation from the stable isotope ratio of oceanic sulphate ions suggests sulphide ingress into the plants of seasonal intensity. These findings call attention to a substantial natural annual range of the stable isotope ratio of carbon, nitrogen, and sulphur in seagrass tissues that should be expected within meadows.

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References

Al-Azri, A.R.N., 2002. Seasonal variation of dissolved organic carbon and nitrogen in relation to primary production and yellow substances (g_{440}) in the Menai Strait, North Wales, UK. Ph.D. Thesis, University of Wales, Bangor, UK.

Alcoverro, T., Duarte, C.M., Romero, J., 1995. Annual growth dynamics of *Posidonia oceanica*: contribution of large-scale versus local factors to seasonality. Marine Ecology Progress Series 120, 203–210.

Anderson, W.T., Fourqurean, J.W., 2003. Intra- and inter-annual variability in seagrass carbon and nitrogen stable isotopes from south Florida, a preliminary study. Organic Geochemistry 34, 185–194.

Auby, I., Labourg, P.J., 1996. Seasonal dynamics of *Zostera noltii* Hornem. in the Bay of Arcachin (France). Journal of Sea Research 35, 269–277.

Beer, S., Bjork, M., Hellbom, F., Axelsson, L., 2002. Inorganic carbon utilization in marine angiosperms (seagrasses). Functional Plant Biology 29, 349–354.

Borum, J., Pedersen, O., Greve, T.M., Frankovich, T.A., Zieman, J.C., Fourqurean, J.W., Madden, C.J., 2005. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass *Thalassia testudinum*. Journal of Ecology 93, 148–158.

Borum, J., Murray, L., Kemp, W.M., 1989. Aspects of nitrogen acquisition and conservation in eelgrass plants. Aquatic Botany 35, 289–300.

Burkhardt, S., Riebesell, U., Zondervan, I., 1999. Effects of growth rate, CO_2 limitation and cell size on the stable carbon isotope fractionation in marine phytoplankton. Geochimica et Cosmochimica Acta 63, 3729–3741.

Chambers, R.M., Fourqurean, J.W., Macko, S.A., Hoppenot, R., 2001. Biogeochemical effects of iron availability on primary producers in a shallow marine carbonate environment. Limnology and Oceanography 46, 1278–1286.

Dennison, W.C., Aller, R.C., Alberte, R.S., 1987. Sediment ammonium availability and eelgrass (*Zostera marina*) growth. Marine Biology 94, 469–477.

Duarte, C.M., 1989. Temporal biomass variability and production/biomass relationships of seagrass communities. Marine Ecology Progress Series 51, 269–276.

Duarte, C.M., 1990. Seagrass nutrient content. Marine Ecology Progress Series 67, 201–207.

Duarte, C.M., 1992. Nutrient concentration of aquatic plants: patterns across species. Limnology and Oceanography 37, 882–889.

Duarte, C.M., 2002. The future of seagrass meadows. Environmental Conservation 29, 192–206.

Duarte, C.M., Chiscano, C.L., 1999. Seagrass biomass and production: a reassessment. Aquatic Botany 65, 159–174.

Eyre, B.D., Ferguson, A.J.P., 2002. Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperate Australian lagoons. Marine Ecology Progress Series 229, 43–59.

Fogel, M.L., Cifuentes, L.A., 1993. Isotope fractionation during primary production. In: Engel, M.H., Macko, S.A. (Eds.), Organic Geochemistry: Principles and Applications. Plenum Press, New York, pp. 73–98.

Fourqurean, J.W., Moore, T.O., Fry, B., Hollibaugh, J.T., 1997. Spatial and temporal variation in C:N:P ratios, $\delta^{15}N$, and $\delta^{13}C$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology Progress Series 157, 147–157.

Fry, B., Silva, S.R., Kendall, C., Anderson, R.K., 2002. Oxygen isotope corrections for online $\delta^{34}S$ analysis. Rapid Communications in Mass Spectrometry 16, 854–858.

Fry, B., Scalan, R.S., Winters, J.K., Parker, P.L., 1982. Sulphur uptake by salt grasses, mangroves and seagrasses in anaerobic sediments. Geochimica et Cosmochimica Acta 46, 1121–1124.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of Seawater Analysis. Verlag Chemie, Weinheim, Germany.

Guy, R.D., Fogel, M.L., Berry, J.A., 1993. Photosynthetic fractionation of the stable isotopes of oxygen and carbon. Plant Physiology 101, 37–47.

Habicht, K.S., Canfield, D.E., 1996. Sulphur isotope fractionation in modern microbial mats and the evolution of the sulphur cycle. Nature 382, 342–343.

Hemminga, M.A., Mateo, M.A., 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. Marine Ecology Progress Series 140, 285–298.

Hemminga, M.A., Koustaal, B.P., van Soelen, J., Merks, A.G.A., 1994. The nitrogen supply to intertidal eelgrass (*Zostera marina*). Marine Biology 118, 223–227.

Hill, A.E., 1994. Fortnightly tides in a lagoon with variable choking. Estuarine Coastal Shelf Science 38, 423–434.

Hoch, M.P., Fogel, M.L., Kirchman, D.L., 1992. Isotope fractionation associated with ammonium uptake by a marine bacterium. Limnology and Oceanography 37, 1447–1459.

Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B., Peterson, B.J., 1998. Measuring $^{15}N\text{-}NH_4^+$ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Marine Chemistry 60, 235–243.

Holmes, R.M., Aminot, A., Kerouel, R., Hocker, B.A., Peterson, B.J., 1999. A simple and precise method for measuring ammonium in marine and fresh water. Canadian Journal of Fisheries and Aquatic Sciences 56, 1801–1808.

Jones, D.C., 1978. Ecology of two coastal lagoons. M.Sc. Thesis, University of Wales, Bangor, UK..

Kennedy, H., Gacia, E., Kennedy, D.P., Papadimitriou, S., Duarte, C.M., 2004. Organic carbon sources to SE Asian coastal sediments. Estuarine Coastal Shelf Science 60, 59–68.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., Vyshkvartezev, D.I., 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur isotope ratio and fatty acid analyses. Marine Ecology Progress Series 220, 103–117.

Lepoint, G., Millet, S., Dauby, P., Gobert, S., Bouquegneau, J.M., 2002. Annual nitrogen budget of the seagrass *Posidonia oceanica* as determined by in situ uptake experiments. Marine Ecology Progress Series 237, 87–96.

Mackin, J.E., Aller, R.C., 1984. Ammonium adsorption in marine sediments. Limnology and Oceanography 29, 250–257.

Marbá, N., Hemminga, M.A., Mateo, M.A., Duarte, C.M., Mass, Y.E.M., Terrados, J., Gacia, E., 2002. Carbon and nitrogen translocation between seagrass ramets. Marine Ecology Progress Series 226, 287–300.

McClelland, J.W., Valiela, I., 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography 43, 577–585.

McGlathery, K.J., Berg, P., Marino, R., 2001. Using porewater profiles to assess nutrient availability in seagrass-vegetated carbonate sediments. Biogeochemistry 56, 239–263.

Mercado, J.M., Niell, F.X., Silva, J., Santos, R., 2003. Use of light and inorganic carbon acquisition by two morphotypes of *Zostera noltii* Hornem. Journal of Experimental Marine Biology and Ecology 297, 71–84.

Oakes, J.M., Connolly, R.M., 2004. Causes of sulphur isotope variability in the seagrass *Zostera capricorni*. Journal of Experimental Marine Biology and Ecology 302, 153–164.

Papadimitriou, S., Kennedy, H., Kennedy, D.P., Borum, J., 2005. Seasonal and spatial variation in the organic carbon and nitrogen concentration and their stable isotopic composition in *Zostera marina* (Denmark). Limnology and Oceanography 50, 1084–1095.

Pedersen, M.F., Borum, J., 1993. An annual nitrogen budget for a seagrass *Zostera marina* population. Marine Ecology Progress Series 101, 169–177.

Pedersen, M.F., Borum, J., 1992. Nitrogen dynamics of eelgrass *Zostera marina* during a late summer period of high growth and low nutrient availability. Marine Ecology Progress Series 80, 65–73.

Pedersen, O., Binzer, T., Borum, J., 2004. Sulphide intrusion in eelgrass (*Zostera marina* L.). Plant Cell and Environment 27, 595–602.

Peralta, G., Perez-Llorens, J.L., Hernandez, I., Vergara, J.J., 2002. Effects of light availability on growth and nutrient content of the seagrass *Zostera noltii* Hornem. Journal of Experimental Marine Biology and Ecology 269, 9–26.

Perez-Llorens, J.L., Niell, F.X., 1993. Seasonal dynamics of biomass and nutrient content in the intertidal seagrass *Zostera noltii* Hornem. from Palmones River estuary, Spain. Aquatic Botany 46, 49–66.

Plus, M., Chapelle, A., Menesquen, A., Deslous-Paoli, J.M., Auby, I., 2003. Modelling seasonal dynamics of biomasses

and nitrogen contents in a seagrass meadow (*Zostera noltii* Hornem.) application to the Thau lagoon (French Mediterranean coast). Ecological Modelling 161, 213–238.

Raven, J.A., Scrimgeour, C.M., 1997. The influence of anoxia on plants of saline habitats with special reference to the sulphur cycle. Annals of Botany London Supplement A 79, 79–86.

Rees, C.E., Jenkins, W.J., Monster, J., 1978. Sulphur isotopic composition of ocean water sulphate. Geochimica et Cosmochimica Acta 42, 377–381.

Short, F.T., Wyllie-Echeverria, S., 1996. Natural and human induced disturbance of seagrasses. Environmental Conservation 23, 17–27.

Short, F.T., McRoy, C.P., 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. Botanica Marina 27, 547–555.

Sigman, D.M., Altabet, M.A., Michener, R., McCorkle, D.C., Fry, B., Holmes, R.M., 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry 57, 227–242.

Silva, J., Santos, R., Calleja, M.L., Duarte, C.M., 2005. Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. Journal of Experimental Marine Biology and Ecology 317, 87–95.

Stephenson, R.L., Tan, F.C., Mann, K.H., 1984. Stable carbon isotope variability in marine macrophytes and its implication for food web studies. Marine Biology 81, 223–230.

Thursby, G.B., Harlin, M.M., 1982. Leaf–root interaction in the uptake of ammonium by *Zostera marina*. Marine Biology 72, 109–112.

Touchette, B.W., Burkholder, J.M., 2000. Review of nitrogen and phosphorus metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology 250, 133–167.

Trust, B.A., Fry, B., 1992. Stable sulphur isotopes in plants – a review. Plant Cell and Environment 15, 1105–1110.

Udy, J.W., Dennison, W.C., 1997. Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. Journal of Experimental Marine Biology and Ecology 217, 253–277.

Vermaat, J.E., Verhagen, F.C.A., 1996. Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: coupling demographic and physiological patterns. Aquatic Botany 52, 259–281.

Zhang, J., Quay, P.D., Wilbur, D.O., 1995. Carbon isotope fractionation during gas–water exchange and dissolution of CO_2. Geochimica et Cosmochimica Acta 59, 107–114.