

Dissolved organic matter uptake in a temperate seagrass ecosystem

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ABSTRACT: We assessed the utilization of inorganic and organic nitrogen compounds of different complexity by primary producers and bacteria in a seagrass ecosystem. Using double-labeled (¹³C and ¹⁵N) substrates, the net transfers from the dissolved nitrogen and carbon pools to phytoplankton, planktonic bacteria, epiphytes, seagrasses (*Zostera noltii* and *Cymodocea nodosa*), and a macroalga (*Caulerpa prolifera*) were quantified in field incubations. Phytoplankton was the largest nitrogen sink, followed by the epiphytic community. In contrast, carbon fixation was dominated by the macrophytes. Although compartment-specific variations existed, NH₄⁺ was generally preferred over NO₃⁻ and urea. Specific uptake rates of individual amino acids were inversely related to their C:N ratio and their structural complexity (glycine > L-leucine > L-phenylalanine). In addition, biomarker-specific measurements (polar lipid-derived fatty acids and D-alanine) indicated increasing bacterial contributions to carbon uptake with increasing amino acid structural complexity. All primary producers acquired nitrogen from a complex pool of algae-derived dissolved organic matter (DOM), but algae-derived dissolved organic carbon (DOC) was almost exclusively used by the planktonic compartment. In contrast, a similar complex pool of bacteria-derived DOM was not taken up in significant quantities by any of the primary producers. Our results illustrate that dissolved organic nitrogen (DON) can provide bioavailable nitrogen to primary producers and bacteria in seagrass ecosystems on very short time-scales, and DON and DOC dynamics are largely uncoupled and must be investigated as 2 separate interacting pools.

KEY WORDS: Dissolved organic matter · Seagrass ecosystems · Uptake · Complexity · Phytoplankton · Epiphytes · *Zostera noltii* · *Cymodocea nodosa* · *Caulerpa prolifera*

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INTRODUCTION

The high productivity of seagrass-dominated ecosystems in a generally low dissolved inorganic nitrogen (DIN) environment implies a high potential for nitrogen (N) limitation (Hemminga 1998) and a strong necessity for efficient dissolved organic nitrogen (DON) recycling. DON is often the largest con-

stituent of total dissolved nitrogen (TDN) in oligotrophic marine systems (Bronk 2002), and therefore a potentially large source of bioavailable N, either through direct uptake or after remineralization (Seitzinger et al. 2002, Bronk et al. 2007). Moreover, because the bioavailability of DON is taxon-specific (Berman & Chava 1999, Harrison et al. 2007), DON may also play an important role in competitive out-

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comes and succession patterns in primary producer communities (Berg et al. 2003, Glibert et al. 2004). Nevertheless, DON is often still considered a black box from which DIN is produced, because it is hard to link the operationally defined fractions within the DON pool with bioavailability (Bronk 2002).

DON utilization turns out to be far more widespread than initially presumed. Besides heterotrophic bacteria and phytoplankton, which are most frequently studied, some macroalgae take up urea and amino acids as well (Tarutani et al. 2004, Tyler et al. 2005). Recently, DON utilization by seagrasses was observed in the laboratory and in the field (Vonk et al. 2008, Van Engeland et al. 2011). The latter is not surprising, given the broad occurrence of organic N uptake in terrestrial macrophytes (Persson et al. 2003) and of N storage as amino acids in some seagrass species (Udy & Dennison 1997, Invers et al. 2002). It is, however, not clear how important DON is in the overall nutrition of seagrasses.

The relative importance of DON as an N source to seagrasses is likely to depend on a variety of factors. For example, the concentrations of certain organic compounds, such as amino acids, are orders of magnitude smaller than those of DIN. In addition, seagrasses and macroalgae have to compete directly with microalgae and bacteria for N in the water column. Competitive outcome may depend on factors such as current velocity, N species (Cornelisen & Thomas 2004, Morris et al. 2008), and the presence of alternative N sources. Furthermore, DON bioavailability may change seasonally, and with eutrophication intensity and DON pool composition (Stepanaukas et al. 1999, Benner 2002, Wiegner & Seitzinger 2004).

Organic matter processing in macrophyte-dominated ecosystems is often characterized by preferential recycling of N over carbon (C) (Ziegler et al. 2004). However, at the level of individual organic substances, N uptake may or may not be coupled to C uptake by primary producers (Veuger & Middelburg 2007), and variability between organisms in different conditions is very high. Mulholland et al. (2004) observed coupled uptake of amino acid N and C by the pelagophyte *Aureococcus anophagefferens* in one bay, but preferential N uptake from urea in another bay. Andersson et al. (2006) reported seasonal variation in the coupling between N and C uptake from urea in a temperate estuary. Tyler et al. (2005) observed coupled uptake of N and the α -carbon from glycine by *Ulva lactuca*, but not from alanine. Price & Harrison (1988) reported coupled C and N uptake from urea in N-starved diatoms, but

not in diatoms with sufficient internal N. These few results illustrate the large variability in the (un-)coupling of dissolved organic carbon (DOC) and DON, with the reasons underlying this variability being poorly understood.

A laboratory experiment has indicated that the dominant macrophytes in the inner Bay of Cadiz (*Zostera noltii*, *Cymodocea nodosa*, *Caulerpa prolifera*) can take up DON substances (Van Engeland et al. 2011), but it is currently not clear to what extent they can profit from this ability when other potential competitors, such as microalgae and heterotrophic bacteria, are abundant. We studied the capacity of co-occurring primary producers (the 3 aforementioned macrophytes, epiphytes, and phytoplankton) and bacteria in this system to acquire DON and DIN compounds from the water column. Using a spectrum of ^{13}C and ^{15}N double-labeled substrates of different complexity and composition, we investigated (1) how much of each substrate flows into each of these ecosystem compartments, (2) whether C and N acquisition was coupled, and (3) how much each sink contributed to total substrate incorporation.

MATERIALS AND METHODS

Study site

The Bay of Cadiz encompasses an area of approximately 12 000 ha. It is subdivided into a deeper outer bay with direct input of continental shelf water and river runoff, and a shallow inner bay which receives water from the outer bay. The system is impacted by aquaculture and wastewater runoff, and dominated by 3 seagrass species: *Zostera marina* L. (not treated in this study), *Cymodocea nodosa* Ucria (Ascherson), *Zostera noltii* Hornem., and a small rhizophyte *Caulerpa prolifera* (Forsskål) J. V. Lamouroux. This experiment was conducted in the inner bay near Santibañez (36° 28' 12.79" N, 6° 15' 7.07" W), where *Z. noltii*, *Cymodocea nodosa*, and *Caulerpa prolifera* co-occur in distinct patches.

Experimental design

The relative contributions of the main players (*Zostera noltii*, *Cymodocea nodosa*, *Caulerpa prolifera*, epiphytes, and suspended matter [phytoplankton + bacterioplankton]; called compartments from here on) with regard to N acquisition from different (in)organic sources in the water column (Table 1) was

Table 1. Substrates (Cambridge Isotope Laboratories, CIL) used for this experiment, their initial concentrations in the incubations, C:N ratio of the organic substances, amino acid abundance according to Cowie & Hedges (1992), and molar mass. DIC: dissolved inorganic carbon, DOC: dissolved organic carbon, DOM: dissolved organic matter, DON: dissolved organic nitrogen, U: universally labeled (all carbon atoms or all nitrogen atoms)

Treatment	Abbrev.	Labeled substrates	Concentrations	C:N ratio	Abundance (mol % N)	Molar mass (g mol ⁻¹)
NH ₄ ⁺ , DIC	NH ₄ ⁺	NH ₄ Cl (¹⁵ N, 99%) + NaHCO ₃ (¹³ C, 99%)	1 μmol N l ⁻¹ , 30 μmol C l ⁻¹			
NO ₃ ⁻ , DIC	NO ₃ ⁻	NaNO ₃ (¹⁵ N, 98%) + NaHCO ₃ (¹³ C, 99%)	1 μmol N l ⁻¹ , 30 μmol C l ⁻¹			
Urea	UR	Urea (¹³ C, 99%; ¹⁵ N ₂ , 98%)	2 μmol N l ⁻¹	0.5		63
Glycine	Gly	Glycine (U- ¹³ C ₂ , 98%; ¹⁵ N, 98%)	0.1 μmol N l ⁻¹	2	7.9–14.5	78
Leucine	Leu	L-leucine (U- ¹³ C ₆ , 98%; ¹⁵ N, 98%)	0.1 μmol N l ⁻¹	6	6–9.3	138
Phenylalanine	Phe	L-phenylalanine (U- ¹³ C ₉ , 98%; ¹⁵ N, 98%)	0.1 μmol N l ⁻¹	9	2.4–4.5	175
Algae-derived DOM	AD	DOC (8.33% ¹³ C) + DON (65.78% ¹⁵ N)	25 μmol C l ⁻¹ , 2.93 μmol N l ⁻¹			
Bacteria-derived DOM	BD	DOC (46.22% ¹³ C) + DON (46.36% ¹⁵ N)	138 μmol C l ⁻¹ , 39 μmol N l ⁻¹			

investigated under circumstances of potential competition in the summer of 2007. Incubations were performed in plastic bags (Fig. 1). The macrophytes were made into a bundle and inserted through a hole in the screw cap with a hydrophobic cotton wool bud wrapped around the base of their aboveground parts, such that the aboveground parts were in the bag and the belowground parts outside the bag. The hydrophobicity effectively prevented water leakage and diffusion. The bags were filled with 5 l of unfiltered bay water containing the natural plankton community. Each incubation therefore involved not only 3 macrophytes and the epiphytes, but also planktonic algae and bacteria (called suspended particulate matter [SPM] from here on). Because the different macrophyte species did not occur in mixed patches, but rather separate patches per species, they had to be removed from the sediment to incubate them together in an enclosure. However, because we were primarily interested in the ability to take up organic N compounds, this artificial aspect is of minor concern for this experiment.

Eight N- and C-containing substrates were added in quantities amounting to the final realistically low (but widely varying) concentrations as indicated in Table 1. Ammonium and nitrate were used for reference because of the vast body of existing literature on their uptake. In addition, small well-defined organic compounds of contrasting chemical/structural complexity and presumably also reactivity were used. Urea is a simple metabolic product with a rela-

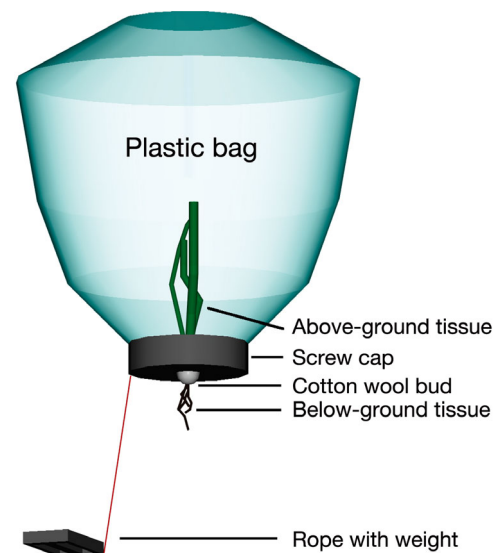


Fig. 1. Incubation setup: a cap with a hole containing 3 macrophyte species in similar biomasses is screwed onto a plastic bag, which contains the incubation medium (water from the bay) with the plankton community

tively high N content. Glycine is an achiral amino acid. Leucine and phenylalanine are chiral amino acids, and phenylalanine has a chemically very stable aromatic ring. $\text{NaH}^{13}\text{CO}_3$ was added to the NH_4^+ and NO_3^- incubations to assess primary production (Table 1). Finally, 2 dissolved organic matter (DOM) substrates of complex composition from different sources (algae versus bacteria) were used to mimic a naturally complex DOM pool.

The complex algae- and bacteria-derived DOM substrates were prepared following Veuger et al. (2004). The algae (axenic culture of *Skeletonema costatum*) were grown on $^{15}\text{NH}_4^+$ and ^{13}C -bicarbonate, and soil bacteria on $^{15}\text{NH}_4^+$ and ^{13}C -glucose. After 1 wk of incubation, algal and bacterial material was harvested through filtration, and suspended in Milli-Q water inside centrifuge tubes. In order to eliminate all the inorganic N remaining, Devarda's alloy was added to convert all inorganic N into ammonium, and MgO was added to convert ammonium (NH_4^+) into ammonia (NH_3). The tubes were vigorously shaken for 48 h to stimulate loss of ammonia gas. The algal and bacterial material was washed and centrifuged 3 times and subsequently extruded with hot water (60°C) and filtered onto 0.2 mm polycarbonate filters (Millipore) to isolate only the dissolved fraction of the organic matter (i.e. DOM). We refer to the paper by Van Engeland et al. (2011) for further details on the DOM processing protocol. Since filtration of substrates added to filtered seawater demonstrated a considerable adherence of the bacteria-derived DOM to the filter (data not shown), the results for bacteria-derived DOM should be interpreted with caution, particularly with respect to uptake by the SPM. Dissolved combined amino acids (DCAA) comprised 84 ± 24 (SD)% of the algae-derived DON and 47 ± 11 % of the bacteria-derived DON, and 34 ± 11 % of the algae-derived DOC and 46 ± 12 % of the bacteria-derived DOC.

After the substrates were added to the incubations, the bags were anchored approximately 0.3 m above the bottom at a depth of 0.5 to 1.5 m (tidal variation). Incubations were started in random order around low water (in the morning) and lasted for approximately 4 h, followed by collection and sampling of the enclosures in random order. This experimental setup was replicated 3 times on different days. This design prevented systematic bias due to changes in environmental conditions due to timing relative to the tides (e.g. light conditions in the water column).

Photosynthetic photon flux (PPF, $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and temperature (°C) were recorded at

0.3 m above the sediment surface during each day of incubations using an ONSET data logger and Apogee photosynthetically active radiation (PAR) sensor. Mean (\pm SD) PPF and temperature were $908 \pm 561 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and $30.1 \pm 2.9^\circ\text{C}$, respectively.

Sampling and sample handling

Dissolved nutrients, amino acids, and DOC were collected after removal of SPM by filtration over pre-combusted GF/F filters (Whatman). Unfiltered water was collected for dissolved inorganic carbon (DIC) measurements in headspace vials. HgCl was added (20 μl per 10 ml of sample) for preservation. SPM samples were collected on preweighed pre-combusted GF/F filters (Whatman) for bulk ^{13}C and ^{15}N analysis, polar lipid-derived fatty acid (PLFA) isotope analysis, and amino acid isotope analysis. All SPM samples were stored on ice in the field and at -20°C in the laboratory until further processing. Epiphytes of *Cymodocea nodosa* and *Zostera noltii* were scraped off with razor blades, and stored on pre-combusted GF/F filters (Whatman) on ice. The macrophytes were dissected, and aboveground and belowground parts stored separately in liquid nitrogen in the field and at -20°C in the laboratory. Reference samples from the environment were taken for all variables mentioned above. In addition, GF/F filters for pigment analysis were collected to assess the phytoplankton community composition (also stored on liquid nitrogen in the field and at -20°C in the laboratory). The macrophytes, SPM, and epiphytes were later freeze-dried, and macrophyte tissues were ground for isotope analysis.

Chemical analyses

DIN components (NH_4^+ , NO_2^- , NO_3^-) and urea were determined colorimetrically. DON was calculated by difference from the TDN, determined as NO_3^- after alkaline persulphate destruction (120°C for 30 min; Grasshoff et al. 1999), and DIN. DOC concentrations were measured with an auto-analyzer (Skalar SK12 organic carbon analyzer). Dissolved free amino acids (DFAA) concentrations were measured by HPLC on a Waters HPLC system with a 996 photodiode array detector (Fitznar et al. 1999). Pigment analysis was performed on a Waters HPLC system with a Waters 474 fluorescence detector (Barranguet et al. 1997).

Concentrations and relative abundances of ^{13}C in DIC (DI^{13}C) were measured with a headspace technique using a Thermo NA2500 elemental analyzer coupled to a Thermo Delta Plus isotope ratio mass spectrometer via a Conflo II interface (Miyajima et al. 1995). C and N content and relative abundances of ^{13}C and ^{15}N in SPM and plant tissue were measured using a Thermo EA 1112 elemental analyzer coupled to a Thermo Delta V Advantage isotope ratio mass spectrometer with a Conflo II interface (EA-IRMS). Concentrations and relative abundances of ^{13}C and ^{15}N ratios in hydrolyzable amino acids in SPM (HAA) were analyzed by gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) using an HP 6890 GC with a Thermo type III combustion interface and Thermo Delta Plus IRMS after extraction and derivatization of the amino acids using the protocol by Veuger et al. (2005). PLFA extractions and derivatization to fatty acid methyl esters (FAME) were performed using a modified Bligh and Dyer protocol (Boschker et al. 1999). FAME concentrations were measured by gas chromatography-flame ionization detection (GC-FID) with separation on a polar column (Scientific Glass Engineering BPX-70; Middelburg et al. 2000). ^{13}C isotope ratios of the individual FAME were measured on an HP 6890 GC gas chromatograph with a Thermo type III combustion interface and Thermo Delta Plus GC-c-IRMS.

Calculations and conversions

Due to the wide range of labeling intensities, we based all our calculations on atomic fractions (for ^{15}N or ^{13}C) in the incubated samples (F_{sample}) and natural reference samples (F_{nat} ; natural isotope abundance) rather than δ -values. For each compartment, isotope excesses ($E_{\text{sample}} = F_{\text{sample}} - F_{\text{nat}}$; i.e. enrichments) were used to calculate specific uptake rates of heavy isotope (V):

$$V = E_{\text{sample}} \times [X] \times t^{-1} \quad (1)$$

where $[X]$ is the C or N concentration (in $\mu\text{mol g DW}^{-1}$; DW = dry weight) of the relevant compartment, and t represents time in hours. Specific uptake rates of all N (V_{N} ; $^{14}\text{N} + ^{15}\text{N}$) was calculated by dividing the specific uptake rates for the heavy isotope by the fraction of heavy isotope in the substrate, assuming that the background substrate concentration only contained ^{14}N , ^{15}N (and ^{13}C) incorporation was calculated as the product of the isotope excess (E_{sample}) and the amount of biomass in terms of N (or C). Transport rates (ρ) were calculated as the product:

$$\rho_{\text{sample}} = V_{\text{sample}} \times \text{DW} \quad (2)$$

and expressed in $\mu\text{mol } ^{15}\text{N h}^{-1}$. Total incorporation and transport rates were not calculated for the epiphytes because no biomass values were available. Corrections for different initial substrate concentrations were accomplished by dividing V_{sample} by the amount of substrate added (n_{sub} ; $\mu\text{mol } ^{15}\text{N}$ or ^{13}C). This measure will be referred to as the normalized uptake rate (V_{norm}):

$$V_{\text{norm}} = V_{\text{sample}}/n_{\text{sub}} \quad (3)$$

This rate of uptake per amount available gives an indication of the preference for a particular nutrient source and is expressed in $\mu\text{mol } ^{15}\text{N (g DW} \times \text{h} \times \mu\text{mol } ^{15}\text{N}_{\text{added}})^{-1}$. No corrections were made for isotope dilution during the 4 h incubations, and uptake rates should thus be considered minimal estimates.

Three biomarker classes were measured to estimate the bacterial and phytoplankton biomasses and/or their relative contributions to N and C incorporation in SPM. They were (1) PLFA, (2) HAA with bacteria-specific D-alanine, and (3) pigments. The chlorophyll *a* (chl *a*) concentration was converted to phytoplankton biomass in terms of C ($\text{POC}_{\text{phyto}}$, where POC = particulate organic carbon) by assuming a C content of $45 \mu\text{g C per } \mu\text{g of chl } a$ (Cloern et al. 1995). No biomarker-based calculations were performed on the algae- and bacteria-derived DOM additions because biomarkers were introduced by the additions themselves.

Bacterial biomass was determined following Evrard et al. (2008) and references therein. The average bacterial PLFA concentration (PLFA_{bac}) was calculated from the bacteria-specific PLFA concentration ($\text{PLFA}_{\text{bact-spec}}$; aiC15:0, iC15:0, iC14:0, and iC16:0) as:

$$\text{PLFA}_{\text{bac}} = \sum \text{PLFA}_{\text{bact-spec}}/a \quad (4)$$

assuming a ratio of bacteria-specific PLFA to total bacterial PLFA (a) of 0.14 mmol PLFA-C:mmol PLFA-C (Evrard et al. 2008). Next, the bacterial C concentration (POC_{bac}) was determined as:

$$\text{POC}_{\text{bac}} = \text{PLFA}_{\text{bac}}/b \quad (5)$$

where the amount of PLFA C per amount of bacterial C (b) was assumed to be 0.073 mmol PLFA-C:mmol POC_{bac} (Evrard et al. 2008).

The phytoplankton biomass was determined as follows. The concentration of phytoplankton PLFA was calculated as the difference between the total PLFA concentration and the total bacterial PLFA concentration:

$$\text{PLFA}_{\text{phyto}} = \sum \text{PLFA}_{\text{all}} - \text{PLFA}_{\text{bac}} \quad (6)$$

From this, the phytoplankton biomass (C concentration) was calculated as:

$$\text{POC}_{\text{phyto}} = \text{PLFA}_{\text{phyto}}/c \quad (7)$$

assuming a ratio of phytoplankton PLFA C to phytoplankton C (*c*) of $0.077 \pm 0.034 \mu\text{g PLFA-C}:\mu\text{g POC}_{\text{phyto}}$, based on averaged results from Dijkman & Kromkamp (2006) for Chlorophyceae, Trebouxiophyceae, and Bacillariophyceae combined, and on the present phytoplankton community (see first section of 'Results' below).

Bacterial contributions to C and N uptake by the SPM were also estimated by means of the HAA in SPM. Following Veuger et al. (2005), and using a bacterial D-alanine:L-alanine ratio of the isotope excesses in C or N (D:L) of 0.07, bacterial contributions were estimated as (D:L)/0.07, after correction for hydrolysis-induced racemization (Kaiser & Benner 2005, Veuger et al. 2007). The bacterial D:L ratio of 0.07 was chosen because at lower D:L ratios, the phenylalanine addition would result in bacterial contributions to C uptake in HAA of >100%.

Using the area-specific biomasses for *Zostera noltii* ($65.6 \pm 4.4 \text{ g DW m}^{-2}$) and *Cymodocea nodosa* ($123 \pm 10 \text{ g DW m}^{-2}$) reported for the inner Bay of Cadiz by Brun et al. (2006), the area-specific biomass for *Caulerpa prolifera* ($97 \pm 52 \text{ g DM m}^{-2}$) from Morris et al. (2009), epiphyte concentrations kindly provided by Patricia García Marín (pers. comm.; $183.3 \pm 78.2 \text{ mg DW of epiphytes per g DW of } Cymodocea \text{ nodosa leaf}$), and the SPM data from the present study ($21.5 \pm 5.2 \text{ mg DW m}^{-2}$), we extrapolated the N uptake and turnover rates per m^2 of bottom surface. For the SPM and the nutrient stocks, we assumed a water depth of 3.75 m, based on the average water depth and the difference between average low and high tide reported by Morris et al. (2009). Turnover rates were calculated as the quotient of the area-specific uptake rates ($\mu\text{mol m}^{-2} \text{ h}^{-1}$) and the amount of substrate (μmol) per m^2 . These calculations provide a first insight in the substrate turnover rates at the experimental location.

Statistics

Significance of ^{15}N and ^{13}C enrichments (difference in isotope fractions in samples after incubation, relative to reference samples, i.e. natural abundance) was tested by means of *t*-tests (3 replicates per substrate). Thus, significant aboveground enrichments indicated significant substrate uptake, whereas sig-

nificant belowground enrichments indicated significant translocation. Logarithmic transformations of response variables were performed when needed to obtain normality of the residuals. Differences in variance per factor level were corrected by introducing a variance function (Kutner et al. 2005).

Dependence of ^{15}N -normalized uptake rates on the biotic compartment and substrates was tested using a 2-way ANOVA. Individual differences between substrates and biotic compartment were tested by post hoc Tukey HSD tests. For the amino acids, the relationship between substrate C:N ratio and ^{15}N -normalized uptake was investigated by means of a linear regression analysis.

Dependence of total ^{15}N incorporation on substrate type and differences in the contribution of different biotic compartments to total incorporation of ^{15}N were tested through variance analyses. Differences between biotic compartments and substrate treatments were investigated by means of post hoc Tukey HSD tests.

Bacterial contributions to ^{13}C and ^{15}N incorporation in SPM were not statistically analyzed, because the HAA were not measured in replicate treatments due to logistic constraints. Standard errors are provided for the PLFA-based measurements.

RESULTS

Environmental conditions and microbial community composition

Water column DIN concentrations were low ($<1 \mu\text{mol N l}^{-1}$), while DON was almost 20 times higher (Table 2). Urea comprised 13.2% of the DON. The leucine concentration was much smaller than the glycine concentration and exhibited considerably more variation between sampling days (standard deviations in Table 2).

Based on chl *a* and PLFA data, phytoplankton biomass was estimated to be 10 to 12 $\mu\text{mol C l}^{-1}$, and represented approximately $25 \pm 12(\text{SD})\%$ of the C pool of SPM. Based on both PLFA and accessory pigment data, diatoms were a major contributor to the phytoplankton community, whereas cryptophytes were a smaller constituent, and cyanobacterial numbers were negligible (data not shown). Abundant chl *b* indicated a chlorophyte contribution, which may partially be due to *Caulerpa prolifera* rather than microalgae. Based on PLFA data, bacterial biomass was $4 \pm 1 \mu\text{mol C l}^{-1}$, which corresponds to $8 \pm 3\%$ of the POC.

Table 2. Concentrations of nutrients ($\mu\text{mol N l}^{-1}$) and chl *a* ($\mu\text{g l}^{-1}$) in the environment. For the nitrogen compounds, contribution to total dissolved nitrogen (TDN) are given as well. Amino acid concentrations are given for the free (DFAA; dissolved free amino acids) and combined form (DCAA; dissolved combined amino acids). All values are mean \pm SD. DON: dissolved organic nitrogen

Nutrient	Concentration	% of TDN
NH_4^+	0.29 ± 0.11	1.7 ± 0.7
NO_3^-	0.72 ± 0.65	3.5 ± 3.3
DON	16.41 ± 1.34	94 ± 2.5
Urea	2.17 ± 0.41	12.5 ± 2.3
TDN	17.42 ± 1.82	
Glycine		
DFAA	0.018 ± 0.006	
DCAA	0.444 ± 0.116	
Leucine		
DFAA	0.00021 ± 0.00041	
DCAA	0.042 ± 0.039	
Phenylalanine		
DFAA	0.003 ± 0.001	
DCAA	0.012 ± 0.016	
Chl <i>a</i>	2.7 ± 2.6	

Isotope enrichments, specific uptake rates and substrate preferences

^{15}N enrichments in the SPM, epiphytes, and above-ground macrophyte parts were significant for all substrates (*t*-tests, all $p < 0.05$), except phenylalanine in the seagrasses. ^{15}N enrichments in *Caulerpa prolifera* incubations with urea and NO_3^- were not significant because of a strong outlier and resulting high variance.

In the belowground macrophyte parts, some significant ^{15}N excesses were detected, e.g. *Zostera noltii* after NH_4^+ addition (*t*-test: $t = 2.6$, $p = 0.03$), indicating translocation. However, belowground incorporations were in these cases below 3% of the aboveground incorporation. Therefore, it is justified to ignore translocation in the remainder of this study.

^{15}N -normalized uptake rates (V_{norm}) varied significantly with substrate type (ANOVA: $F_{7,78} = 146$, $p < 0.0001$), but the power of the Tukey HSD procedures was only sufficient to distinguish the extremely low values in the phenylalanine-N and bacterial DON treatments from the remainder of the substrates in the macrophytes and epiphytes (Fig. 2). For SPM, normalized uptake rates of algae-derived DON were higher than those of urea-N, phenylalanine-N, and bacteria-derived DON (Tukey HSD, all $p < 0.05$). ^{15}N -normalized uptake rates also varied among compartments (ANOVA: $F_{4,78} = 729$, $p < 0.0001$), with higher

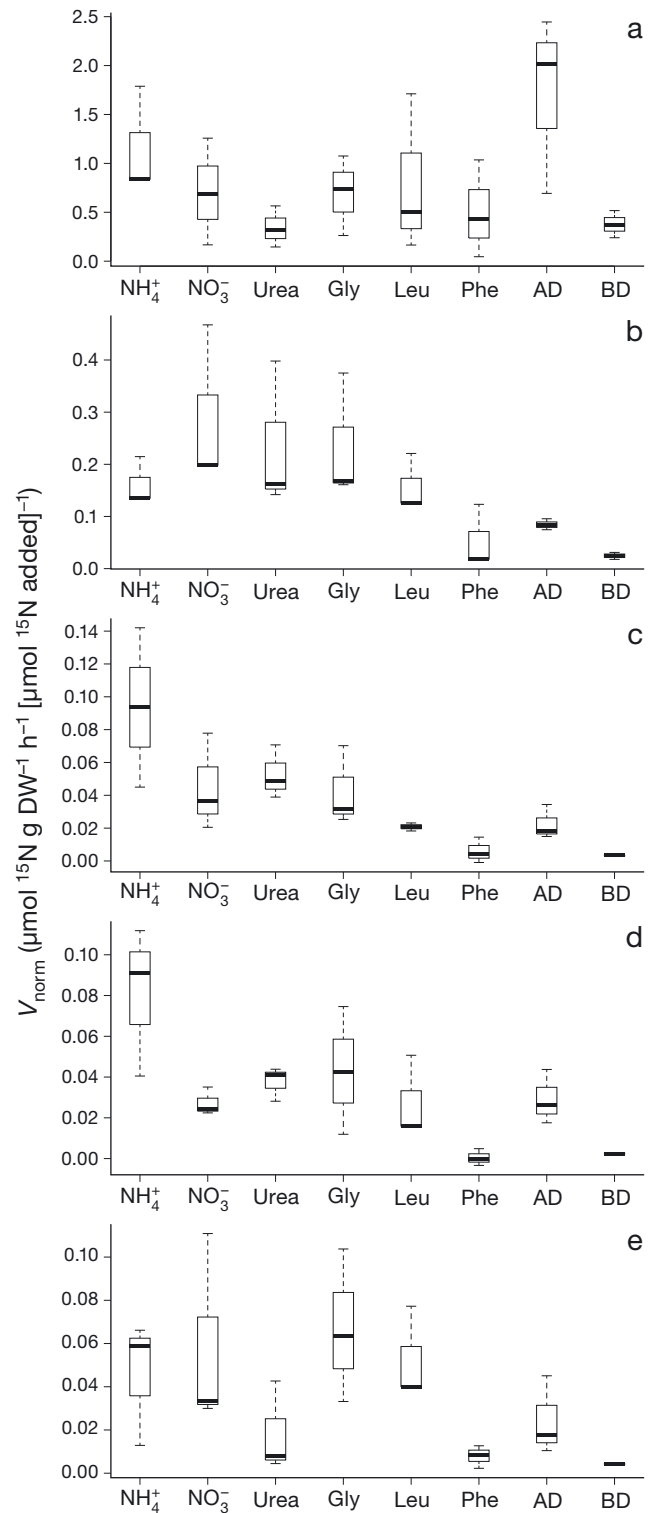


Fig. 2. Normalized nitrogen uptake rates (V_{norm}) for (a) suspended particulate matter (SPM; containing detritus, phytoplankton, and bacterioplankton), (b) epiphytes, and 3 macrophyte species: (c) *Zostera noltii*, (d) *Cymodocea nodosa*, and (e) *Caulerpa prolifera*. DW: dry weight. See Table 1 for treatment abbreviations. Boxplots: whiskers are extremes, boxes are inter-quartile range, and thick lines are median

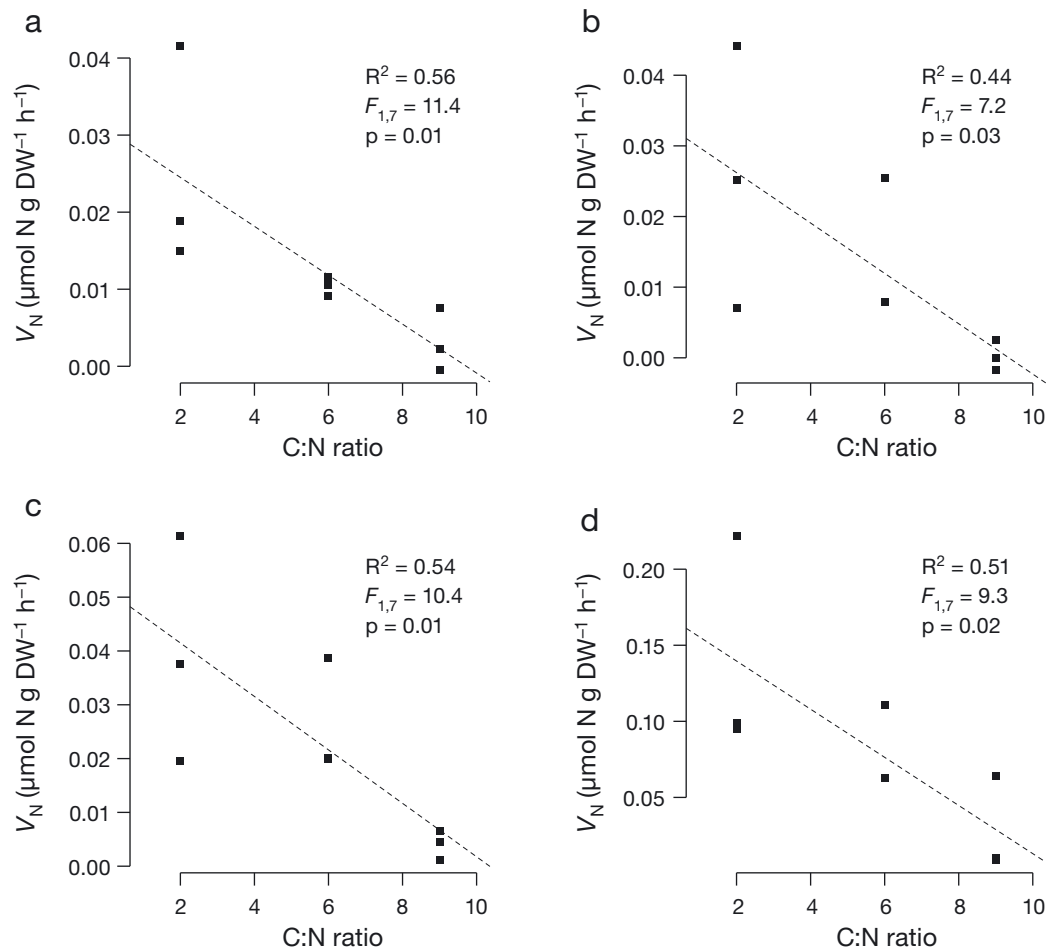


Fig. 3. Linear regression analyses of specific nitrogen uptake rate (V_N) versus C:N ratio of the added amino acids for (a) *Zostera noltii*, (b) *Cymodocea nodosa*, (c) *Caulerpa prolifera*, and (d) epiphytes. Coefficient of determination and F -test results are indicated in each graph. DW: dry weight

values in SPM than the other compartments (Tukey HSD, $p < 0.05$).

The macrophytes and epiphytes exhibited significantly decreasing ^{15}N -specific uptake rates (V) with increasing amino acid C:N ratios (Fig. 3). No relationship between the amino acid C:N ratio and ^{15}N uptake rates was found for SPM (data not shown).

DIC, urea, and the complex DOM substrates caused significant ^{13}C enrichments for all biotic compartments (t -tests, all $p < 0.01$). ^{13}C enrichments in the individual amino acid additions were only significant in the SPM and epiphytes (t -tests, all $p < 0.05$), but not in the macrophytes.

Substrate incorporation in the enclosures

SPM dominated total ^{15}N incorporation per enclosure in all substrate treatments (data not shown). For

instance, if SPM was included, the percentage of added NH_4^+ that was incorporated in biomass increased from 12 to 59%, while for algal DON this increased from 4 to 81% (data not shown). Bacteria-derived DON incorporation increased from 1 to 22% when the SPM was included. However, the latter may be a severe overestimation, given the adherence of this substrate to the filters. Because the possibility exists that specifically the macrophytes and epiphytes were limited in their ^{15}N supply by concentration gradients in the enclosure (insufficient mixing, see 'Discussion'), and because no biomass data were available for our epiphyte samples, only the macrophytes are compared in Fig. 4.

Total ^{15}N incorporation in the macrophytes, as a percentage of the added amount (Fig. 4a, percentages above the barplots), varied significantly with substrate type (ANOVA: $F_{7,16} = 4.7$, $p = 0.006$; numbers above the bars in Fig. 4a). Percentage substrate

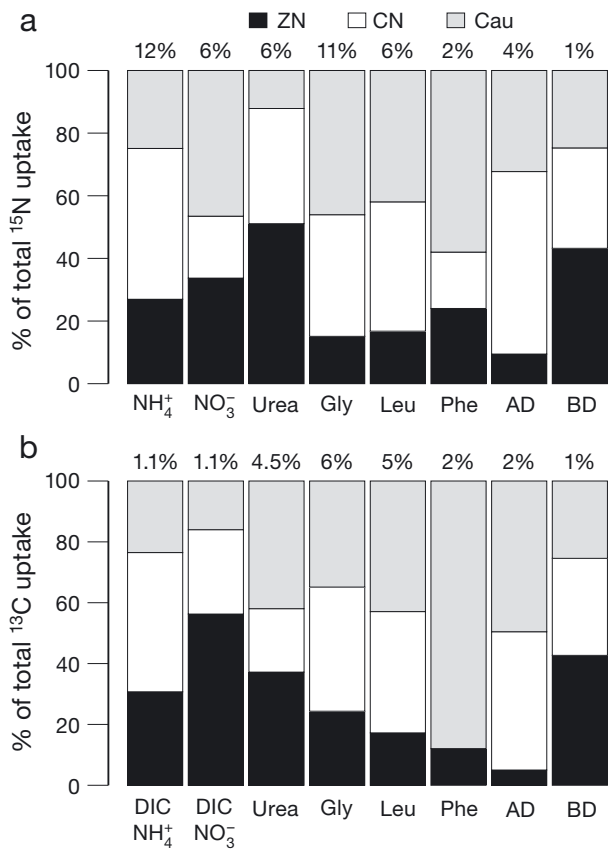


Fig. 4. Percent contribution of *Zostera noltii* (ZN), *Cymodocea nodosa* (CN), and *Caulerpa prolifera* (Cau) to the total uptake of (a) ¹⁵N and (b) ¹³C per enclosure for each of the treatments. Numbers above the bars are the percentages of available substrate taken up. ¹³C-DIC was added to the inorganic ¹⁵N treatments (2 columns on the left in panel b). See Table 1 for treatment abbreviations

incorporation decreased with increasing amino acid C:N ratios (and chemical complexity; see 'Materials and methods; Experimental design'; Fig. 4a), a reflection of the negative relationships between specific ¹⁵N uptake rates of macrophytes and epiphytes, and amino acid C:N ratios (Fig. 3). Contributions of the individual macrophyte species to the total ¹⁵N incorporation by all macrophytes (subdivision of bars in Fig. 4) varied significantly among substrates (ANOVA: $F_{7,62} = 5.4$, $p = 0.0001$). A significant interaction effect of substrate with macrophyte species was also found (ANOVA: $F_{14,62} = 2.7$, $p = 0.006$).

In contrast to the ¹⁵N incorporation, which was dominated by the SPM, macrophytes dominated DI¹³C and urea-¹³C incorporation with a total contribution of ~85% (data not shown). The macrophytes were responsible for only 2% of the algal DOC incorporation (Fig. 4b).

Contribution of bacteria and algae to planktonic N and C uptake

Bacterial contributions to total ¹⁵N incorporation into HAA increased with substrate complexity, and seemed decoupled from the fate of the substrate ¹³C (Fig. 5). Bacterial contributions to ¹³C incorporation into HAA appeared considerably higher for the leucine and phenylalanine substrates (Fig. 5). Approximately 26% of the DI¹³C incorporation into the HAA pool was in the bacterial compartment. The increased bacterial contribution for leucine and phenylalanine was also supported by the higher and significant contributions of bacteria in total ¹³C incorporation into PLFAs (t -test, all $p < 0.05$) (Fig. 5).

The ratios of the incorporation of substrate ¹³C over substrate ¹⁵N into HAA ranged from 0.05 for the NH₄⁺ (+ DIC) treatment to 0.5 for the glycine addition. This is well below the ¹³C:¹⁵N ratio in the added substrates, indicating that the added N was incorporated into amino acids more readily than the C. The ¹³C:¹⁵N ratio in the urea treatments was of the same order of magnitude as the NH₄⁺ and NO₃⁻ treatments (which also received DI¹³C). The HAA ¹³C:¹⁵N ratios in the amino acid additions were higher (0.5, 0.24, and 0.46 for the glycine, leucine, and phenylalanine additions, respectively), indicating relatively higher C incorporation from amino acids than from urea or DIC.

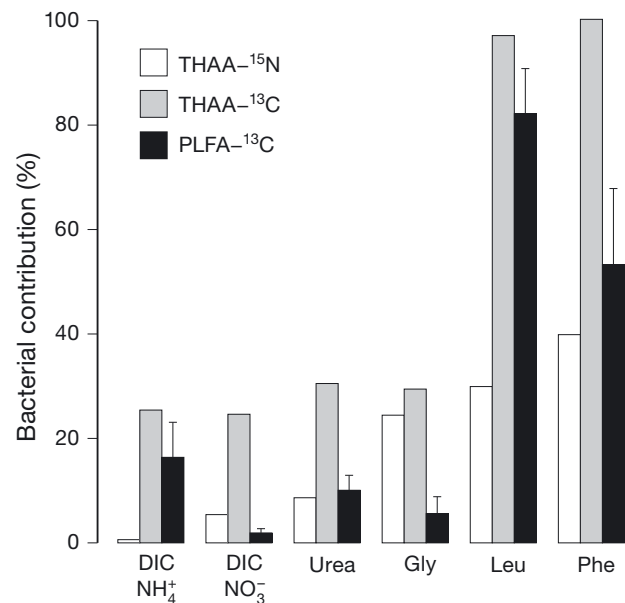


Fig. 5. Bacterial contribution to ¹⁵N (THAA-¹⁵N) and ¹³C (THAA-¹³C) incorporation by the suspended particulate matter into total hydrolyzable amino acids and polar lipid-derived fatty acids (PLFA-¹³C) per substrate treatment. See Table 1 for treatment abbreviations

Table 3. Area-based carbon fixation ($\mu\text{mol C m}^{-2} \text{h}^{-1}$ for DIC) and nitrogen transport rates ($\mu\text{mol N m}^{-2} \text{h}^{-1}$ for the other treatments) for the various biotic compartments from this study, turnover times for all compartments, and total amount of substrate available to the biota per unit surface area. Means \pm SD are shown. All calculations were based on the assumption of a water column depth of 3.75 m, and using biomass values from Brun et al. (2006), Morris et al. (2009), Patricia García Marín (pers. comm.), and the present study. SPM: suspended particulate matter. See Table 1 for other abbreviations

Source	Rate ($\mu\text{mol m}^{-2} \text{h}^{-1}$)					Turnover time (h)	Nutrient stocks ($\mu\text{mol m}^{-2}$)
	SPM	Epiphytes	<i>Zostera noltii</i>	<i>Cymodocea nodosa</i>	<i>Caulerpa prolifera</i>		
DIC	747 \pm 207	323 \pm 135	1133 \pm 280	1982 \pm 553	724 \pm 659	1.9	6280 \pm 739
NH ₄ ⁺	600 \pm 280	18 \pm 5	40 \pm 17	64 \pm 24	28 \pm 22	1.5	1100 \pm 401
NO ₃ ⁻	467 \pm 324	32 \pm 15	24 \pm 13	28 \pm 6	45 \pm 41	4.1	2438 \pm 2444
Urea	573 \pm 327	53 \pm 27	72 \pm 19	96 \pm 19	36 \pm 43	9.8	8125 \pm 1539
Gly	33 \pm 18	2.6 \pm 1.1	1.7 \pm 0.8	3.1 \pm 1.9	3.7 \pm 2.7	1.5	68 \pm 23
Leu	32 \pm 29	1.8 \pm 0.5	0.7 \pm 0.1	1.7 \pm 1	2.5 \pm 1.6	0.06	2.3 \pm 2.1
Phe	21 \pm 18	0.6 \pm 0.6	0.2 \pm 0.2	0.03 \pm 0.2	0.4 \pm 0.3	0.6	13 \pm 5
AD	12658 \pm 6421	18 \pm 2	135 \pm 52	328 \pm 126	211 \pm 184	45.6	61525 \pm 5016

Area-specific N uptake and C fixation in the inner bay

Total C fixation per m² was mainly accomplished by the seagrasses, in particular *Cymodocea nodosa* (Table 3). *Caulerpa prolifera* and the SPM had an equal share, which was highly variable. In contrast to the DIC consumption, SPM was by far the largest sink for dissolved N regardless of the source (Table 3), but this may have been due to local depletion and strong concentration gradients limiting N uptake by the macrophytes. The area-based DIN turnover time amounted to 0.11 d in our simplified system. The DIN turnover time, if *Zostera noltii* was the only consumer, would be 2.3 d, and for *Cymodocea nodosa* it would be 1.6 d. In the absence of fluxes into the substrate pools, the total uptake of NH₄⁺ and glycine by the investigated consumers would drive these pools close to depletion after 1.5 h (Table 3). This was less pronounced for NO₃⁻ and urea (Table 3). The area-based uptake rates of leucine-N and phenylalanine-N were large compared to the standing stock, indicating a rapid turnover of these N compounds as well. The calculations for the alga-derived DON were based on the assumption that the entire DON pool is derived from algae, which is obviously a crude approximation.

DISCUSSION

It has been established that many marine macrophytes are able to directly take up organic N (Bird et al. 1998, Tarutani et al. 2004, Tyler et al. 2005, Vonk et al. 2008). In a companion study, Van Engeland et al. (2011) showed that the macrophytes in the present

study have the capacity to use DON for their nutrition under circumstances of a strongly reduced microbial community. Their results suggest that either biomass-specific bacterial breakdown had to be very fast (exo-enzyme activity cannot be reduced by filtration over a glass-fiber filter), or these macrophytes took up organic N without interference from a bacterial community. The exact uptake mechanisms in seagrasses are not known, but could resemble those in terrestrial vascular plants, which may acquire as much as 20% of their total N demand from organic forms (cf. Touchette & Burkholder 2000, and references therein). The present study shows that the same macrophytes also take up N from DOM in the water column in the presence of a well-equipped and potentially competing microbial community, which can take up N from a complex DON pool in quantities similar to or higher than those from inorganic N pools (Veuger et al. 2004, the present study).

However, our results also support the inferior position of slow-growing and C-rich macrophytes as short-term N sinks in the water column relative to a fast-growing N-rich microbial community (Duarte 1990, 1995, Malta et al. 2005), and suggest an even weaker direct influence on water-column organic N concentrations. Although insufficient mixing in our enclosures may partially compromise comparison of the phytoplankton and macrophyte uptake rates in terms of competition, we believe the inferiority of macrophytes as a short-term sink for water-column N relative to microbes is real, considering that the epiphytes were subject to the same concentration gradients caused by insufficient mixing, and still showed a higher specific uptake rate than the macrophytes. Insufficient mixing essentially strengthens the effect of differences in proximity to nutrients between the

phytoplankton and the benthic community, a potentially major factor in the competitive outcome between macrophytes and microbes in acquiring sufficient nutrients from the water column. Because of their association with the bottom, macrophytes have less access to water column nutrients than phytoplankton, and epiphytes may act as nutrient filters to macrophytes (Cornelisen & Thomas 2004; i.e. a competitive inferiority). Note that an effect of the exclusion of root-mediated uptake as an alternative N source for macrophytes on their leaf-mediated uptake cannot be ruled out. However, if an effect existed on the uptake rates, it would probably be a positive effect, since macrophytes were in our study solely dependent on leaf-mediated N uptake. The difference in competitive abilities for DON and DIN uptake implies that changes in the DON:DIN ratio, for instance due to targeting dissolved inorganic nutrients in nutrient-reduction measures (Pehlivanoglu-Mantas & Sedlak 2006), may not only cause phytoplankton community shifts (Glibert et al. 2007), but also affect seagrasses that may be overgrown by epiphytes, shaded by phytoplankton (McGlathery 2001), or pushed towards increased dependence on nutrient uptake from the sediment (including organic N; Vonk et al. 2008, Van Engeland et al. 2011). Effects of organic-to-inorganic nutrient ratio could be particularly relevant for the Bay of Cadiz, considering the aquaculture activities in this system (Hernández et al. 2002). Note that a similar competitive interaction arises in the benthic environment, with a competitive advantage for the macrophytes over microalgae, since the former may alter the fluxes to the water column and therefore the availability of N to the latter (Tyler et al. 2001).

The present study illustrates a difference in bioavailability among the individual organic compounds. This was most clear for the macrophytes and epiphytes, for which a negative relationship existed between the specific ^{15}N uptake rates (V) and the amino acid C:N ratio. Preferences for simple N-rich amino acids over complex C-rich amino acids have also been observed for terrestrial plants (Harrison et al. 2007). The relationship with the substrate C:N ratio may in our case be attributable to molecular complexity/stability (cf. Table 1). Enantiomery (glycine is achiral) and the presence of chemically stable aromatic rings (phenylalanine) can be important determinants of chemical and biological reactivity. The higher bacterial contributions to ^{15}N incorporation for more complex molecules support this view (Fig. 5), since heterotrophic bacteria are better equipped to process the complex amino acid. How-

ever, considering that urea did not fit in this regression analysis, substrate complexity does not provide the entire explanation. The increasing gradient in the amino acid C:N ratio also corresponded with decreasing amino acid abundance in the organisms (Cowie & Hedges 1992), our studied system (Table 2, DFAA + DCAA), and some seagrass sediments (Hansen et al. 2000). From an evolutionary perspective, high relative amino acid abundance in organisms and their environment and high amino acid bioavailability may well be opposite sides of the same coin. For abundant amino acids (e.g. glycine) that are needed and available in larger amounts, it is probably easier to invest in uptake (more transporter and assimilation proteins) than in new production. These considerations illustrate that more research is needed to understand the relationship between DOM composition and bioavailability.

The differences in preference for individual organic compounds were also reflected in strong differences in the utilization of complex DON pools from either algal (more amino acids) or bacterial (less amino acids) origins. Besides the lower amino acid content, the refractory nature of peptidoglycans may be a part of the explanation for lower bacterial DON bioavailability (Veuger et al. 2006). Our findings are in line with the reported dependence of DON bioavailability on pool composition, which in turn is related to variability in flow conditions of rivers (Stepanaukas et al. 2000), land use in river tributaries (Wiegner & Seitzinger 2004), coastal versus inland waters (Stepanaukas et al. 1999), and algal versus bacterial origin (McCallister et al. 2006, the present study), and other factors. In the investigated system, aquaculture and sewage treatment are 2 DOM sources that could have compositions and bioavailability different from *in situ* DOM production by primary producers. For such shallow seagrass ecosystems with potentially strong benthic-pelagic couplings, this dependence of DOM bioavailability on the source and composition implies a potentially strong variability in the intensity of nutrient cycling, since the importance of fluxes from different sources (e.g. sediment versus water column) may vary throughout the growth season (Ziegler & Benner 1999a).

The C and N component of the DOM pool are generally considered decoupled. Strong preferential recycling of DON relative to DOC is observed when DIN concentrations are very low (Thomas et al. 1999, Osterroht & Thomas 2000, Ziegler et al. 2004), but not necessarily under N-replete conditions (Aminot & Kérouel 2004, Bradley et al. 2010). Preferential uptake of N was also found in the present study. In fact, no

significant ^{13}C enrichment was observed, suggesting that the amino acid and urea N entered the macrophytes after extracellular processing (deamination). The fates of amino acid and urea C and N were partially decoupled when taken up by the pelagic community. The $^{13}\text{C}:$ ^{15}N ratios of uptake by the pelagic community were in favor of N uptake, but ^{13}C uptake did occur. Since DI^{13}C resulting from extracellular remineralization would have been lost in a large background pool, the most likely explanation for the ^{13}C enrichment in the primary producers would be uptake of entire molecules, rather than DI^{13}C uptake. Similar patterns were found for intertidal microphyto-benthic communities (Veuger & Middelburg 2007). Both uptake of intact compounds and of parts after breakdown have been documented for microalgae (Palenik & Morel 1991, Legrand & Carlsson 1998).

The degree of coupling depended on the substrate and biotic compartment. Urea, for instance, was largely remineralized prior to uptake, since the $^{13}\text{C}:$ ^{15}N ratio of the influx in SPM was much lower than the 0.5 expected from uptake of intact molecules. Increasing C content coincided with increasing bacterial contributions to N incorporation, suggesting increasing support of bacterial growth and/or energy storage. Smaller N-rich compounds, however, were more channeled into the primary producer compartments, and were probably remineralized rather than incorporated by the bacteria. It is possible that bacterial activity and growth are supported or stimulated by a certain fraction of DOM, which contributes to the bioavailability of another (perhaps partially overlapping) DOM fraction to primary producers. Ziegler & Benner (1999b), for instance, reported a stimulation of ammonium regeneration and bacterial growth efficiencies (BGE) by spring and summer DOC fluxes, most likely originating from seagrass carbohydrate exudation. This increased BGE resulted in a bioavailable fraction of DOM with lower C:N ratio (Ziegler et al. 2004). Overall, a heterotrophic community, stimulated by a DOC flux from autotrophs, produced a bioavailable DOM fraction enriched in N that was available for both autotrophs and heterotrophs. It is therefore not surprising that changes in DOC–DON coupling throughout the growth season have been observed (Andersson et al. 2006), which may be related to varying interactions between autotrophs and heterotrophs. The decoupling of DON and DOC processing implies that these pools should be treated as covarying but separate pools, and that an observed coupling of DOC and DON dynamics is largely determined by the spatio-temporal scale, ecosystem compartments, and the

level of detail at which the composition of the DOM is examined.

The water column of the inner Bay of Cadiz is characterized by a very fast turnover of dissolved N. Our back-of-the-envelope calculations suggest that some of the substrates could be depleted within half a tidal cycle in the absence of fluxes replenishing the substrate pools (Table 3). These fluxes may come from the sediment, as well as through DOM exudation by the macrophytes directly into the water column. The occurrence of very fast remineralization is also supported by the measured bacterial DI^{13}C incorporation in the absence of cyanobacteria (Fig. 5), which can only occur through DOC uptake after DOM loss from primary producers (Findlay et al. 1986, Van den Meersche et al. 2004). Nutrient recycling intensity is in general positively related to the potential for nutrient limitation (Thomas et al. 1999, Osterroht & Thomas 2000, Ziegler et al. 2004). When external inputs are low, concentrations of bioavailable nutrients are low and remineralization fluxes have to support biological activity/growth. Dead organic matter leaks DOM into the water column, of which the useful bioavailable part is quickly stripped, and fixed again in living organic matter (partially after conversion to inorganic nutrients). Therefore, fast turnover may improve nutrient retention in the system, depending on its nutrient state (Vonk & Stapel 2008).

The present work contributes to a growing body of evidence that DON is an underestimated ecosystem component in general, and in seagrass-dominated systems in particular. Very often, DON concentrations exceed DIN concentrations, implying a strong potential for DON as source of fixed N (Bronk et al. 2007) that is bioavailable through direct uptake or after fast remineralization. In the Bay of Cadiz, DON is at least partially available to macrophytes within 1 tidal cycle, either through direct uptake or after microbial breakdown. In addition, the high bioavailability of DON to phytoplankton combined with particle trapping by seagrasses (Marbà et al. 2006) suggests an indirect and prolonged DON availability to seagrasses through phyto-detritus dissolution and burial as well (Evrard et al. 2005, Barrón et al. 2006, Vonk & Stapel 2008). Our discussion also illustrates that a close but complex relationship exists among the cycles of the major elements in DOM (C, N, phosphorus). The interactions between these elemental cycles are governed by DOM bioavailability, composition, and sources, and autotroph–heterotroph interactions, but are currently not fully understood. Therefore, DOM cycling in all its complexity deserves further investigation.

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LITERATURE CITED

- Aminot A, K erouel R (2004) Dissolved organic carbon, nitrogen and phosphorus in the N-E Atlantic and the N-W Mediterranean with particular reference to non-refractory fractions and degradation. *Deep-Sea Res I* 51:1975–1999
- Andersson MGI, van Rijswijk P, Middelburg JJ (2006) Uptake of dissolved inorganic nitrogen, urea and amino acids in the Scheldt estuary: comparison of organic carbon and nitrogen uptake. *Aquat Microb Ecol* 44:303–315
- Barranguet C, Herman PMJ, Sinke JJ (1997) Microphytobenthos biomass and community composition studied by pigment biomarkers: importance and fate in the carbon cycle of a tidal flat. *J Sea Res* 38:59–70
- Barr n C, Middelburg JJ, Duarte CM (2006) Phytoplankton trapped within sea-grass (*Posidonia oceanica*) sediments are a nitrogen source: an in situ isotope labeling experiment. *Limnol Oceanogr* 51:1648–1653
- Benner R (2002) Chemical composition and reactivity. In: Hansell DA, Carlson CA (eds) *Biogeochemistry of marine dissolved organic matter*. Academic Press, San Diego, CA, p 59–90
- Berg GM, Balode M, Purina I, Bekere S, B chemin C, Maestrini SY (2003) Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen. *Aquat Microb Ecol* 30:263–274
- Berman T, Chava S (1999) Algal growth on organic compounds as nitrogen sources. *J Plankton Res* 21:1423–1437
- Bird KT, Johnson JR, Jewett-Smith J (1998) In vitro culture of the seagrass *Halophila decipiens*. *Aquat Bot* 60:377–387
- Boschker HTS, de Brouwer JFC, Cappenberg TE (1999) The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: stable carbon isotope analysis of microbial biomarkers. *Limnol Oceanogr* 44:309–319
- Bradley PB, Lomas MW, Bronk DA (2010) Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay, measured using a flow cytometric sorting approach. *Estuar Coast* 33:971–984
- Bronk DA (2002) Dynamics of DON. In: Hansell DA, Carlson CA (eds) *Biogeochemistry of marine dissolved organic matter*. Academic Press, San Diego, CA
- Bronk DA, See JH, Bradley P, Killberg L (2007) DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences* 4:283–296
- Brun FG, Vergara JJ, Peralta G, Garc a-S nchez MP, Hern ndez I, P rez-Llor ns JL (2006) Clonal building, simple growth rules and phylloclimate as key steps to develop functional–structural seagrass models. *Mar Ecol Prog Ser* 323:133–148
- Cloern JE, Grenz C, Vidergar-Lucas L (1995) An empirical model of the phytoplankton chlorophyll:carbon ratio—the conversion factor between productivity and growth rate. *Limnol Oceanogr* 40:1313–1321
- Cornelisen CD, Thomas FIM (2004) Ammonium and nitrate uptake by leaves of the seagrass *Thalassia testudinum*: impact of hydrodynamic regime and epiphyte cover on uptake rates. *J Mar Syst* 49:177–194
- Cowie GL, Hedges JI (1992) Sources and reactivities of amino acids in a coastal marine environment. *Limnol Oceanogr* 37:703–724
- Dijkman NA, Kromkamp JC (2006) Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: application for inferring phytoplankton composition. *Mar Ecol Prog Ser* 324:113–125
- Duarte CM (1990) Seagrass nutrient content. *Mar Ecol Prog Ser* 67:201–207
- Duarte CM (1995) Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41:87–112
- Evrard V, Kiswara W, Bouma TJ, Middelburg JJ (2005) Nutrient dynamics of seagrass ecosystems: ¹⁵N evidence for the importance of particulate organic matter and root systems. *Mar Ecol Prog Ser* 295:49–55
- Evrard V, Cook PLM, Veuger B, Huettel M, Middelburg JJ (2008) Tracing carbon and nitrogen incorporation and pathways in the microbial community of a photic subtidal sand. *Aquat Microb Ecol* 53:257–269
- Findlay S, Carlough L, Crocker MT, Gill HK, Meyer JL, Smith PJ (1986) Bacterial-growth on macrophyte leachate and fate of bacterial production. *Limnol Oceanogr* 31:1335–1341
- Fitznar HP, Lobbes JM, Kattner G (1999) Determination of enantiomeric amino acids with high-performance liquid chromatography and pre-column derivatisation with *o*-phthaldialdehyde and *N*-isobutyrylcysteine in seawater and fossil samples (mollusks). *J Chromatogr A* 832:123–132
- Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S (2004) Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. *Mar Ecol Prog Ser* 280:73–83
- Glibert PM, Wazniak CE, Hall MR, Sturgis B (2007) Seasonal and interannual trends in nitrogen and brown tide in Maryland's coastal bays. *Ecol Appl* 17(Suppl):S79–S87
- Grasshoff K, Kremling K, Ehrhardt M (1999) *Methods of seawater analysis*. Wiley-VCH, Weinheim
- Hansen JW, Udy JW, Perry CJ, Dennison WC, Lomstein BA (2000) Effect of the seagrass *Zostera capricorni* on sediment microbial processes. *Mar Ecol Prog Ser* 199:83–96
- Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* 88:989–999
- Hemminga MA (1998) The root/rhizome system of seagrasses: an asset and a burden. *J Sea Res* 39:183–196
- Hern ndez I, Mart nez-Arag n JF, Tovar A, P rez-Llor ns JL, Vergara JJ (2002) Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. *Ammonium*. *J Appl Phycol* 14:375–384
- Invers O, P rez M, Romero J (2002) Seasonal nitrogen speciation in temperate seagrass *Posidonia oceanica* (L.) Delile. *J Exp Mar Biol Ecol* 273:219–240
- Kaiser K, Benner R (2005) Hydrolysis-induced racemization of amino acids. *Limnol Oceanogr Methods* 3:318–325
- Kutner MH, Nachtsheim CJ, Neter J, Li W (2005) *Applied linear statistical models*. McGraw-Hill, Chicago, IL
- Legrand C, Carlsson P (1998) Uptake of high molecular weight dextran by the dinoflagellate *Alexandrium catenella*. *Aquat Microb Ecol* 16:81–86
- Malta EJ, Ferreira DG, Vergara JJ, P rez-Llor ns JL (2005) Nitrogen load and irradiance affect morphology, photosynthesis and growth of *Caulerpa prolifera* (Bryopsi-

- dales: Chlorophyta). *Mar Ecol Prog Ser* 298:101–114
- Marbà N, Holmer M, Gacia E, Barrón C (2006) Seagrass beds and coastal biogeochemistry In: Larkum AWD, Orth RJ, Duarte CM (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht
- McCallister SL, Bauer JE, Canuel EA (2006) Bioreactivity of estuarine dissolved organic matter: a combined geochemical and microbiological approach. *Limnol Oceanogr* 51: 94–100
- McGlathery KJ (2001) Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *J Phycol* 37:453–456
- Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon: an *in situ* ¹³C-labeling study. *Limnol Oceanogr* 45:1224–1234
- Miyajima T, Yamada Y, Hanba YT, Yoshii K, Koitabashi T, Wada E (1995) Determining the stable isotope ratio of total dissolved inorganic carbon in lake water by GC/C/IRMS. *Limnol Oceanogr* 40:994–1000
- Morris EP, Peralta G, Brun FG, van Duren L, Bouma TJ, Pérez-Lloréns JL (2008) Interaction between hydrodynamics and seagrass canopy structure: spatially explicit effects on ammonium uptake rates. *Limnol Oceanogr* 53: 1531–1539
- Morris EP, Peralta G, Benavente J, Freitas R and others (2009) *Caulerpa prolifera* stable isotope ratios reveal anthropogenic nutrients within a tidal lagoon. *Mar Ecol Prog Ser* 390:117–128
- Mulholland MR, Boneillo G, Minor EC (2004) A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. *Harmful Algae* 3:361–376
- Osterroht C, Thomas H (2000) New production enhanced by nutrient supply from non-redfield remineralisation of freshly produced organic material. *J Mar Syst* 25:33–46
- Palenik B, Morel FMM (1991) Amino oxidases of marine phytoplankton. *Appl Environ Microbiol* 57:2440–2443
- Pehlivanoglu-Mantas E, Sedlak DL (2006) Wastewater-derived dissolved organic nitrogen: analytical methods, characterization, and effects—a review. *Crit Rev Environ Sci Technol* 36:261–285
- Persson J, Högberg P, Ekblad A, Högberg MN, Nordgren A, Näsholm T (2003) Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. *Oecologia* 137:252–257
- Price NM, Harrison PJ (1988) Uptake of urea-C and urea-N by the coastal marine diatom *Thalassiosira pseudonana*. *Limnol Oceanogr* 33:528–537
- Seitzinger SP, Sanders RW, Styles R (2002) Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. *Limnol Oceanogr* 47:353–366
- Stepanuskas R, Edling H, Tranvik LJ (1999) Differential dissolved organic nitrogen availability and bacterial aminopeptidase activity in limnic and marine waters. *Microb Ecol* 38:264–272
- Stepanuskas R, Laudon H, Jorgensen NOG (2000) High DON bioavailability in boreal streams during a spring flood. *Limnol Oceanogr* 45:1298–1307
- Tarutani K, Niimura Y, Uchida T (2004) Short-term uptake of dissolved organic nitrogen by an axenic strain of *Ulva pertusa* (Chlorophyceae) using ¹⁵N isotope measurements. *Bot Mar* 47:248–250
- Thomas H, Ittekkot V, Osterroht C, Schneider B (1999) Preferential recycling of nutrients—the ocean's way to increase new production and to pass nutrient limitation? *Limnol Oceanogr* 44:1999–2004
- Touchette BW, Burkholder JM (2000) Review of nitrogen and phosphorus metabolism in seagrasses. *J Exp Mar Biol Ecol* 250:133–167
- Tyler AC, McGlathery KJ, Anderson IC (2001) Macroalgae mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon. *Estuar Coast Shelf Sci* 53:155–168
- Tyler AC, McGlathery KJ, Macko SA (2005) Uptake of urea and amino acids by the macroalgae *Ulva lactuca* (Chlorophyta) and *Gracilaria vermiculophylla* (Rhodophyta). *Mar Ecol Prog Ser* 294:161–172
- Udy JW, Dennison WC (1997) Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. *J Exp Mar Biol Ecol* 217:253–277
- Van den Meersche K, Middelburg JJ, Soetaert K, van Rijswijk P, Boschker HTS, Heip CHR (2004) Carbon-nitrogen coupling and algal-bacterial interactions during an experimental bloom: modeling tracer experiment. *Limnol Oceanogr* 49:862–878
- Van Engeland T, Bouma TJ, Morris EP, Brun FG and others (2011) Potential uptake of dissolved organic matter by seagrasses and macroalgae. *Mar Ecol Prog Ser* 427:71–81
- Veuger B, Middelburg JJ (2007) Incorporation of nitrogen from amino acids and urea by benthic microbes: role of bacteria versus algae and coupled incorporation of carbon. *Aquat Microb Ecol* 48:35–46
- Veuger B, Middelburg JJ, Boschker HTS, Nieuwenhuize J, van Rijswijk P, Rochelle-Newall EJ, Navarro N (2004) Microbial uptake of dissolved organic and inorganic nitrogen in Randers Fjord. *Estuar Coast Shelf Sci* 61: 507–515
- Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2005) Analysis of ¹⁵N incorporation into D-alanine: a new method for tracing nitrogen uptake by bacteria. *Limnol Oceanogr Methods* 3:230–240
- Veuger B, van Oevelen D, Boschker HTS, Middelburg JJ (2006) Fate of peptidoglycan in an intertidal sediment: an *in situ* ¹³C-labeling study. *Limnol Oceanogr* 51:1572–1580
- Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2007) Update of 'Analysis of N incorporation into D-alanine: a new method for tracing nitrogen uptake by bacteria' (Veuger et al. 2005, *Limnol Oceanogr Methods* 3:230–240). *Limnol Oceanogr Methods* 5:192–194
- Vonk JA, Stapel J (2008) Regeneration of nitrogen (¹⁵N) from seagrass litter in tropical Indo-Pacific meadows. *Mar Ecol Prog Ser* 368:165–175
- Vonk JA, Middelburg JJ, Stapel J, Bouma TJ (2008) Dissolved organic nitrogen uptake by seagrasses. *Limnol Oceanogr* 53:542–548
- Wiegner TN, Seitzinger SP (2004) Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands. *Limnol Oceanogr* 49: 1703–1712
- Ziegler S, Benner R (1999a) Nutrient cycling in the water column of a subtropical seagrass meadow. *Mar Ecol Prog Ser* 188:51–62
- Ziegler S, Benner R (1999b) Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. *Mar Ecol Prog Ser* 180:149–160
- Ziegler S, Kaiser E, Benner R (2004) Dynamics of dissolved organic carbon, nitrogen and phosphorus in a seagrass meadow of Laguna Madre, Texas. *Bull Mar Sci* 75: 391–407