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Regeneration of nitrogen (^{15}N) from seagrass litter in tropical Indo-Pacific meadows

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ABSTRACT: The input of nitrogen (N) into litter of the seagrasses *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea rotundata* during decomposition and the uptake of N released from this litter by the surrounding seagrasses were examined simultaneously using ^{15}N-enriched seagrass leaf material ($\delta^{15}N \approx 500$‰) placed in litterbags in seagrass meadows. The biomass (dry weight) decomposition rates (k) for these tropical seagrasses were high (0.023 to 0.070 d^{-1}). Litter N concentration declined during decomposition, indicating an overall release of N during decomposition. Besides release of litter N there was an input of non-litter N, indicated by a decline of $\delta^{15}N$ values. This exogenous N made up an important part of litter N, consisting of ca. 25% of the total standing stock litter N. The increased $\delta^{15}N$ values of the surrounding seagrasses were used to calculate the uptake of N released from the macrophyte litter. The seagrasses efficiently took up the released N. The canopy thickness (aboveground biomass) of the seagrass meadow played a role in the uptake efficiency of N released from litter, increasing the N retention in these tropical meadows. The high litter decomposition rates, substantial input of exogenous N onto litter and efficient uptake of N released from litter suggest that N cycling, outside the living plant but within the meadow via the detrital pathway, is important to retain N in these nutrient-poor tropical meadows, despite the open and highly dynamic shallow coastal environments from which an easy loss of N, either dissolved in the water column or associated with the export of leaf litter, would be expected.

KEY WORDS: Nitrogen cycling · Tropical seagrass · Uptake · Decomposition · ^{15}N

INTRODUCTION

Tropical offshore seagrass meadows are often characterized by clear water and carbonate sediments. These often shallow coastal environments are nutrient poor, but at the same time maintain a high year-round primary production (Erftemeijer & Middelburg 1995, Vermaat et al. 1995). The leaves account for 70% of the total production of tropical seagrass meadows (Vermaat et al. 1995). This combined with the relatively high nutrient content of leaves compared to other plant parts, identifies leaves as the main nutrient sink in these seagrass meadows. The leaf lifespan is short for most tropical seagrass species (median of 50 d) and resorption of nitrogen (N) from the leaves of these species before detachment accounts for only 10% of the leaf N-content (Hemminga et al. 1999). Consequently, a high loss of N is associated with the detachment of leaves and leaf fragments from the living plant, which may easily be exported from the meadow due to the open and highly dynamic conditions of the coastal environment.

However, export of litter from offshore nutrient-poor meadows due to current and tide is estimated to be ca. 10% (Stapel et al. 1996). Direct grazing results in loss of 15 to 50% of the leaf production, but generally does not exceed 25% (Cebrián 1999). Accumulation of decomposing seagrass within the meadow and along the shore or on the outer reef flat does not occur (Lindeboom & Sandee 1989, Nienhuis et al. 1989).

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Erftemeijer et al. (1993), furthermore, showed that autotrophic and heterotrophic processes in these meadows were in balance. The combination of high leaf productivity and low inorganic nutrient concentrations, and high litter production without large accumulations of organic material in the system, limited export and herbivory of organic material and a balance between production and consumption processes, suggest high decomposition rates and a tight coupling between decomposition and (re)assimilation. These observations identify leaf fall as the most important process by which seagrasses lose N, comparable to ca. 50% of the demand for leaf production (Vonk 2008).

The N demand of these seagrass systems therefore seems to be met by an efficient process of trapping of organic material, uptake of dissolved N sources and recycling of seagrass material (Hemminga et al. 1991, Erftemeijer & Middelburg 1995). Uptake efficiency of N released from litter by the seagrass leaves may depend on the density (Koch et al. 2006) and size of the meadow (Stapel et al. 2001). N recycling can be accomplished through rapid *in situ* decomposition of seagrass litter within the seagrass beds (Fenchel et al. 1998) and subsequent uptake of released N by the leaves (Stapel et al. 2001), identifying decomposition rates of seagrass litter as important fluxes in the nutrient cycling of seagrass meadows (Mateo & Romero 1996). During decomposition, input of exogenous N from water column sources or by microbial colonization can result in substantial enrichment in the N content of detritus (White 1994, Tremblay & Benner 2006), which may affect the decomposition rate and N balance.

Stable nitrogen isotope (^{15}N) enrichment of seagrass material allows us to study and quantify *in situ* processes involving N at the scale of the plant or the community (Robinson 2001). At seagrass community level, ^{15}N-tracer experiments have been performed to study the role of benthic vegetation as sinks of N inputs (Lepoint et al. 2004) or the retention efficiency of N in seagrass ecosystems (Stapel et al. 2001, Evrard et al. 2005, Barrón et al. 2006).

We hypothesise that there is a tight coupling between the release and re-assimilation of N from seagrass litter and that this coupling is more efficient in seagrass meadows with higher aboveground biomass. In the present study ^{15}N-enriched leaf litter was used in litterbags in seagrass meadows (1) to measure decomposition rates, (2) to distinguish between the decline of original litter-N and the input of other N sources which were defined as exogenous N and (3) to measure uptake by the seagrasses of N released from the litter. The experiments were executed in 2 adjacent seagrass systems with different aboveground biomass and comparable hydrologic regime and nutrient availability.

MATERIALS AND METHODS

Study site. The study was carried out at Bone Batang, an uninhabited coral island located 15 km offshore in the Spermonde Archipelago, Indonesia (5° 01′ S, 119° 19′ E). The rainy season in this area is from October until March. The island consisted of an inter-tidal sandbank with a surrounding reef flat and was fringed by a barrier reef. An extensive multispecies seagrass meadow covered the reef flat, which consisted of coarse carbonate sand and coral rubble comparable to the nearby island Barang Lompo (93 to 100% $CaCO_3$; Erftemeijer 1994). A mixed stand of *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea rotundata*, interspersed with patches of *Enhalus acoroides,* dominated the meadow. *Halophila ovalis* was found in low densities.

The present study was performed using the seagrass species *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea rotundata*. We also tried to measure the decomposition rates of the co-occurring macroalgae *Sargassum* sp. and *Padina* sp., but the macroalgae fragments used continued to grow inside the litterbags. The results were not used to estimate the decomposition rate of these macroalgae, but the release of ^{15}N was used to study uptake of regenerated N by seagrass plants. We performed the experiment in May through June and in November through December 2004. The influence of seagrass leaf biomass on the capacity to take up released N by the seagrasses was analysed by executing the first experiment in a meadow with a dense canopy structure (~150 g DW [dry weight] leaf m^{-2}) and the second in a meadow with an open canopy structure (~100 g DW leaf m^{-2}).

Labelling macrophytes. In the field we created ^{15}N-enriched macrophyte material to be used in the experiments. Macrophytes were incubated *in situ* by placing boxes of 1 × 1 × 0.5 m (length × width × height) closed with transparent plastic foil over the vegetation. We added 0.25 g $^{15}NH_4Cl$ (99% ^{15}N) to the water column in the boxes. A water pump was used to enhance mixing of the water and homogenous uptake of the label by the plants. The macrophytes were incubated for 3 h after which the boxes were removed. All aboveground macrophyte material of the incubated plots was harvested 3 wk later (half leaf age for the used seagrass species; Hemminga et al. 1999) to ensure incorporation of the ^{15}N in the leaf chemical compounds and equal distribution of the label in all plant parts (Stapel et al. 2001). This fresh macrophyte material was collected 2 d before the start of the experiment by cutting off all aboveground material from the enriched plots and transported to the laboratory in cool boxes. The material was kept submerged during the period of transport and pro-

cessing in the laboratory to prevent desiccation. The day before the start of the experiment we sorted the macrophyte material by species. We took 3 random shoot samples from each seagrass species (10 shoots for *Thalassia hemprichii*, 40 for *Halodule uninervis* and 20 for *Cymodocea Rotundata,* respectively) to determine the labelling distribution over the different leaf ages. The leaves were divided by age, oven dried for 48 h at 70°C, weighed and analysed for $\delta^{15}N$.

Litterbags. The remaining macrophyte material was cut into pieces of max. 5 cm length and placed on tissue at room temperature for 1 h to remove excess water. Ten samples of ca. 5 g FW (fresh weight) per species were weighed to estimate the FW:DW ratio of the material. This ratio was used to estimate the starting DW biomass in each of the litterbags. For each of the macrophytes, ca. 5 g FW (~ 0.7 g DW) litter (weighed to ±0.01 g accuracy) and a plastic identification label were put in 30 nylon litterbags (0.10 × 0.10 m, mesh size 1 mm).

Field setup. The day after filling the litterbags (the second day after the ^{15}N-labelled material was harvested from the field) we transported the litterbags submerged in seawater in a cool box to the field. Four litterbags with different macrophyte species (the 3 seagrass species and 1 of the macroalgae) were clustered and placed on top of the sediment in a subtidal seagrass meadow around a central vegetation quadrat (0.25 × 0.25 m during May to June and 0.10 × 0.10 m during the November to December decomposition experiment; Fig. 1). In total, 27 of these clusters of 4 litterbags were randomly deployed.

Sampling. At each sampling occasion, 3 clusters of 4 litterbags and the central vegetation quadrat, which was harvested up to 0.15 m deep, were taken from the field. The central vegetation quadrats and the litterbags containing the same macrophyte species were treated as replicates. The first sampling on day t = 0 consisted of 3 litterbags for each species and 3 randomly chosen seagrass vegetation quadrats. The other samplings were done every 5 d until t = 40 d. At each harvest we removed excessive sediment from the remaining litterbags if necessary to prevent burial causing unequal environmental conditions among litterbags. In the laboratory, the litterbag material was washed out and all fauna and sediment material was removed. The samples were briefly rinsed with freshwater and oven dried to a constant DW and analysed for carbon (C) and N concentrations and $\delta^{15}N$. The seagrass material from the central quadrat was washed over a 1 mm sieve, sorted by species, divided into the different plant parts (leaf, sheath and rhizome; except for *Halophila ovalis*), briefly rinsed with freshwater to remove salt and oven dried. The DW was measured

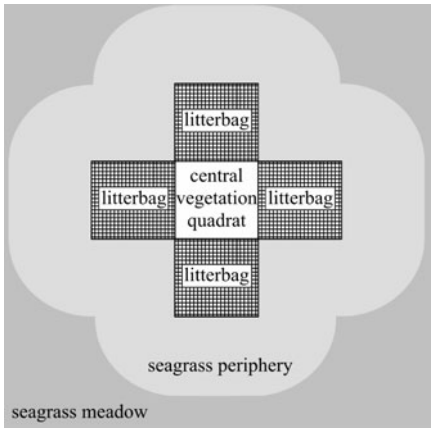

Fig. 1. Schematic overview of the clustered litterbags (0.10 × 0.10 m) and central vegetation quadrant harvested during the experiment

and all samples were analysed for $\delta^{15}N$ and N concentration to calculate uptake of released ^{15}N by the seagrasses.

Decomposition rate. The loss of organic matter DW from the seagrass litterbags was used as a measure for decomposition. The first stages of decomposition are characterized by leaching of soluble components from the litter, which usually occurs within the first hours to days (Godshalk & Wetzel 1978, Anesio et al. 2003). We assumed that most of the leaching had already occurred before the start of the experiment since we harvested the living macrophyte material for the litterbags 2 d before the decomposition experiment started and measured a decline in N concentration of ca. 0.25% of DW over this period. The final stages of decomposition are characterized by slow decay of refractory material. We expected that during the relatively short duration (40 d) of the experiment the decomposition was not yet dominated by refractory material decomposition (Buchsbaum et al. 1991, Cebrián et al. 1997, Fourqurean & Schrlau 2003). We therefore calculated the decomposition rates using a single first-order exponential decay model (Olson 1963):

$$W_t = W_0 e^{(-k_{DW}t)} \quad (1)$$

where W_t is the weight (g DW) of litter left after time t (d) from the initial weight W_0 with a biomass decomposition rate constant k_{DW} (d^{-1}). We also calculated the decomposition rate constants for total N (k_N) and ^{15}N (k_{15N}) using this model, substituting total N and total ^{15}N at time 0 and time t for W_0 and W_t, respectively.

Exogenous N input. Changes in $\delta^{15}N$ values during decomposition can be caused by 4 main processes, influencing both natural as well as enriched $\delta^{15}N$ values. The first process is isotope fractionation, which is the extent to which the nitrogen isotopes are separated be-

tween the litter and released matter during microbial processes (Robinson 2001). Heavier isotopes form bonds of greater energy than their isotopically lighter counterparts and thus are less likely to undergo chemical reactions (Adams & Sterner 2000). In natural litter this results in an increase of $\delta^{15}N$ values of 0 to 5‰ for decomposition and 28 to 33‰ for denitrification (Robinson 2001). The second process could be the assimilation of ^{15}N-enriched components of the litter by the microbial community. Changes in nitrogen isotopic composition are thought to arise from assimilation of dissolved (in)organic N by the microbial community during decomposition (Caraco et al. 1998). The enriched litter used in the present experiment also released ^{15}N-enriched dissolved N. Uptake of this enriched N source by the microbial community will increase their natural $\delta^{15}N$ signature with an unknown ‰. The third process inducing a change in $\delta^{15}N$ value could be due to unequal labelling of the leaves or components. Not all leaves and components decompose at the same rate (high rate for proteins and low for fibres; Harrison 1989), and different labelling of these materials results in changes in $\delta^{15}N$ values. We labelled the seagrasses 20 d before harvesting to make sure that the added ^{15}N was incorporated in all chemical components of the litter and was distributed through all leaves to prevent influence of this process. The forth process changing the $\delta^{15}N$ values of the litter is the input of exogenous N (other than original litter N) with natural $\delta^{15}N$ values (close to 0; White 1994). The largest contribution to exogenous N in litter is assumed to result from microbial colonization (Anesio et al. 2003, Tremblay & Benner 2006), but N_2-fixation by bacteria may also contribute. This input of exogenous N will result in a decline of the $\delta^{15}N$ values that depends on the amount of input, as measured in enriched cord grass (White 1994). Since the first 3 processes result in small changes and/or increases of the $\delta^{15}N$, we assume that the decline in $\delta^{15}N$ of the litter originated from the attachment of exogenous N to the original litter material.

We calculated the amount of exogenous N input into the litterbags during the course of the experiment as a portion of the total litter-N at $t = 0$ by subtracting the relative amount of ^{15}N left in the litter from the relative amount of total litter-N left during the experiment. The total percentage of exogenous N in standing stock seagrass litter in the field was calculated using the observed difference in the ^{15}N and N decomposition rate constants, assuming a steady state in the production and decomposition of litter:

$$N_{\text{exogenous}} = \frac{(k_{15N} - k_N)}{k_N} 100\% \quad (2)$$

where $N_{\text{exogenous}}$ is the percentage of exogenous N of the total litter-N, k_N and k_{15N} the decomposition rate constants for total N and ^{15}N respectively. Since the litter was washed to remove sediment, only strongly attached exogenous N is taken into account. Loosely attached exogenous N is considered to be easily washed away from litter by hydrodynamic forces in the field.

Uptake of released N. From the measured ^{15}N-enrichment (µg ^{15}N) in the central vegetation quadrats and the decline of ^{15}N in the litterbags due to decomposition during the 40 d of the present experiment, we estimated the uptake by the seagrasses of N released from the litterbags using the following model (modified from Stapel et al. 2001):

$$\frac{dR}{dt} = ck_{15N}L - aR \quad (3)$$

where c is the portion of ^{15}N regenerated from the litter that is taken up by the seagrasses, k_{15N} the first-order decomposition rate constant of the cumulative ^{15}N in all 4 litterbags around the central quadrat, L the cumulative amount of ^{15}N in the 4 litterbags, a the first-order rate constant at which ^{15}N declined in the seagrasses from the central quadrat due to leaf turnover and leaching and R the ^{15}N-enrichment in the living seagrass periphery around the litterbags. The periphery (Fig. 1) was defined as the total area of seagrass meadow around the litterbags with radius equal to the length of the vegetation quadrat (0.25 m in the dense canopy and 0.10 m in the open canopy meadow). The ^{15}N-enrichment measured in the central quadrats was extrapolated to this periphery area according to the size of the central vegetation quadrats and the total periphery areas. The extrapolation factors were 4.74 for the dense canopy (0.25 m periphery) and 7.14 for the open canopy (0.10 m periphery) vegetation. Solving Eq. (3) becomes:

$$c_d = \frac{\left(\frac{R_t}{e^{(-at)}} - R_0\right)(a - k_{15N})}{k_{15N}L_0(e^{(a-k_{15N})t} - 1)} \quad (4)$$

where c_d is the portion of N released from the litter that is taken up by the seagrass periphery, L_0 is the initial amount of ^{15}N in the litterbags, R_t and R_0 are the ^{15}N-enrichments in the seagrass periphery at time t and 0, respectively. All ^{15}N data were expressed as portion of the initial total amount of ^{15}N in the litterbags (100%). The value for a (0.037 d^{-1}) was taken from Stapel et al. (2001).

Analyses. Carbon and nitrogen concentrations were measured with a Carlo Erba NA 1500 elemental analyzer, and for nitrogen stable isotope composition, this was coupled online via a Finnigan Conflo III interface with a ThermoFinnigan DeltaPlus mass spectrometer.

The biomass, total N and ^{15}N decomposition data per species, were expressed as a relative remaining frac-

tion and ln-transformed. The differences between decomposition rates were tested using linear regression (Sokal & Rohlf 1995). The C and N concentrations, C:N ratio and $\delta^{15}N$ during the decomposition experiment were analyzed per species using linear regression and Pearson correlation. The differences between the dense canopy and open canopy meadow were analysed using independent *t*-tests. Probability (p) < 0.05 was considered significant.

RESULTS

Seagrass meadows

The main difference between the meadows was the significantly higher leaf biomass in the dense canopy meadow (149 g DW m^{-2}) compared to the open canopy meadow (98 g DW m^{-2}), due to the higher abundance of the large species *Thalassia hemprichii* and *Cymodocea rotundata* in the dense canopy meadow (Table 1). However, the total shoot density was comparable for both meadows, due to the high number of tiny *Halodule uninervis* shoots in the open canopy meadow.

Table 1. Mean ± SD shoot density ($\times 10^3$ m^{-2}) and biomass (g m^{-2}) of the dense canopy and open canopy meadow measured in the central quadrats (0.25 × 0.25 m and 0.10 × 0.10 m, respectively). Significant differences (independent *t*-test) of the individual species or totals between the 2 meadows are marked where ^{A} denotes the higher value (n = 27); for *Halophila ovalis*, whole plant biomass is shown

Species	Density ($\times 10^3$ m^{-2})	Shoot biomass (g m^{-2}) Leaf	Sheath	Rhizome
Dense canopy				
Thalassia hemprichii	0.85 ± 0.25^{A}	58 ± 17^{A}	35 ± 12	263 ± 91^{A}
Halodule uninervis	1.66 ± 1.39^{B}	17 ± 15	2 ± 3	51 ± 55^{B}
Cymodocea rotundata	2.27 ± 0.54^{A}	64 ± 22^{A}	29 ± 11	119 ± 28^{A}
Halophila ovalis	0.18 ± 0.16	9 ± 11		
Total	5.03 ± 1.08	149 ± 32^{A}	65 ± 21	433 ± 98^{A}
Open canopy				
Thalassia hemprichii	0.45 ± 0.31^{B}	33 ± 29^{B}	34 ± 30	145 ± 69^{B}
Halodule uninervis	2.92 ± 0.82^{A}	2 ± 10	6 ± 8	92 ± 45^{A}
Cymodocea rotundata	1.67 ± 0.76^{B}	31 ± 18^{B}	44 ± 51	62 ± 31^{B}
Halophila ovalis	0.37 ± 0.41	10 ± 8		
Total	5.23 ± 1.09	98 ± 38^{B}	83 ± 55	299 ± 91^{B}

Seagrass litter

We used fresh leaf material for the experiment, but the relative contribution of the different leaf-age classes to the litter biomass showed that the litter material used consisted largely of older leaves (Table 2). These leaves are considered to make the large bulk of the natural leaf litter in the meadow. The ^{15}N-enriched seagrass leaf material used in the decomposition experiment had $\delta^{15}N$ values varying from 350 to 950‰, more or less equally distributed over all leaves (Table 2). During the decomposition, the $\delta^{15}N$ values of the litter material declined significantly for all seagrasses, ranging from 0.80 to 1.16‰ d^{-1} for the different species (Fig. 2A,B; see Table 4), indicating that the different components of the seagrass litter were comparably labelled with ^{15}N.

Decomposition

We measured no difference between both experiments in the litter decomposition rate (k_{DW}) for the same seagrass species (p > 0.05, linear regression for biomass decomposition rate per species). We pooled the litter data of both experiments per species

Table 2. Relative biomass fraction of the leaf-age classes and the mean $\delta^{15}N$ values (‰) measured in the different leaf-age classes (0 to 4) for the seagrass species used during the first (1) (May to June) and second (2) (November to December) decomposition experiment (n = 3). Leaf 0 is the whitish youngest leaf not yet visible in the field. Leaf class 4 also includes older leaves (na: not applicable)

Species	Expt	% litter DW 0	1	2	3	4	$\delta^{15}N$ 0	1	2	3	4
Thalassia hemprichii	1	1.3	12.2	29.6	34.2	22.7	662	544	505	793	756
Thalassia hemprichii	2	2.7	10.9	27.9	38.1	20.4	329	365	411	460	521
Halodule uninervis	1	0.8	15.1	37.4	38.5	8.2[a]	480	454	756	863	536[a]
Halodule uninervis	2	1.7	17.0	35.8	35.5	10.0[a]	523	537	617	914	669[a]
Cymodocea rotundata	1	1.9	20.7	42.4	35.0	na	425	389	420	433	na
Cymodocea rotundata	2	2.4	15.7	38.6	43.3	na	432	483	592	948	na

[a]Sheath

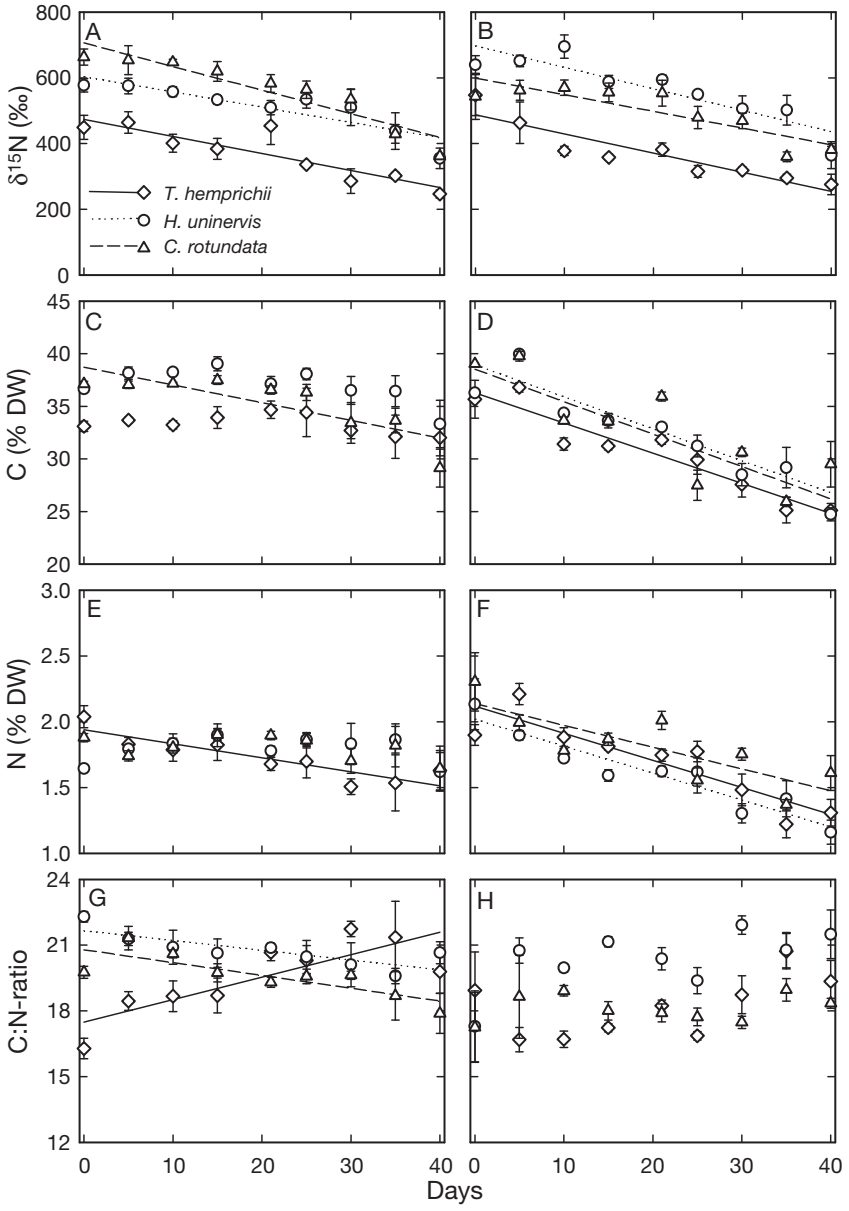

Fig. 2. (A,B) $\delta^{15}N$ values, (C,D) %C of dry weight (DW), (E,F) %N of DW, and (G,H) C:N ratio of litter material during decomposition experiments (Expt 1: A,C,G,E; Expt 2: B,D,F,H) for *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea rotundata* (mean ± SE, n = 3). Significant regression lines are shown (see Table 4 for statistics and coefficient of correlation)

to calculate the decomposition rates. The first order biomass decomposition rates of *Thalassia hemprichii* and *Halodule uninervis* were significantly lower than that of *Cymodocea rotundata* (Table 3). In most cases, the litter C and N concentration declined significantly during decomposition (Fig. 2C–F; Table 4) but at different rates. This resulted in some cases in an increase of the C:N ratio of the litter material; in other cases the ratio remained the same or decreased (Fig. 2G,H).

Table 3. Litter biomass decomposition rates (k_{DW}) for the different macrophyte species. Significantly different biomass decomposition rates are denoted with superscripts

Species	Mean k_{DW} (d^{-1})	95% confidence interval
Thalassia hemprichii	0.042^{A}	0.034–0.050
Halodule uninervis	0.031^{A}	0.023–0.039
Cymodocea rotundata	0.062^{B}	0.055–0.070

Exogenous N input

For all macrophytes in both experiments k_{15N} values were larger compared to k_N (Table 5), indicating an input of exogenous N into the litter during decomposition. The amount of exogenous N input into the litter was expressed graphically as a percentage of the total litter N at $t = 0$ by subtracting the course of ^{15}N (representing litter-N) from total N (representing litter- plus exogenous N) during the decomposition experiment (Fig. 3). The litter showed a fast increase in exogenous N followed by a decrease, but relative to leaf litter-N the exogenous N continued to increase. The calculated amount of exogenous N in the seagrass litter standing stock using Eq. (2) was on average 25% (Table 5). Due to the large range of exogenous N input percentages, no differences between species were identified.

Uptake of released N

The increase in ^{15}N of the seagrass growing around the litterbags was comparable for all species, indicating equal use of the released N by the seagrasses. About half of the ^{15}N-enrichment in the central vegetation quadrants was present in the leaves, the remainder in underground parts. Using the measured ^{15}N-enrichment in the central vegetation quadrants, the course of ^{15}N in the periphery was described by Eq. (4) (Fig. 4). For k_{15N} we substituted a decomposition rate that was obtained using Eq. (1) after adding up the remaining ^{15}N contents in all 4 litterbags (3 seagrass species and

Table 4. *F*-statistics for the linear regression analysis of the chemical composition (%C, %N, C:N ratio and $\delta^{15}N$) of litter material during decomposition (see Fig. 2). *F*-values were significant (F-critical$_{1,26}$ = 5.59), unless noted (ns = not significant). Significant R^2 and coefficient of correlations (r; Pearson correlation, all $p < 0.01$) are given

Species	Expt	$\delta^{15}N$			%C			%N			C:N ratio		
		F	R^2	r	*F*	R^2	r	*F*	R^2	r	*F*	R^2	r
Thalassia hemprichii	1	25.77	0.58	−0.76	1.81	ns		25.68	0.79	−0.61	14.53	0.45	0.67
	2	37.56	0.63	−0.79	70.10	0.90	−0.89	29.74	0.70	−0.84	3.34	ns	
Halodule uninervis	1	25.43	0.54	−0.73	4.49	ns		0.01	ns		13.18	0.27	−0.52
	2	30.49	0.67	−0.82	50.53	0.87	−0.89	60.14	0.72	−0.85	4.59	ns	
Cymodocea rotundata	1	50.60	0.76	−0.87	15.60	0.69	−0.73	1.93	ns		13.23	0.38	−0.62
	2	21.44	0.53	−0.73	19.46	0.73	−0.81	14.27	0.51	−0.72	0.19	ns	

Table 5. Litter decomposition rates for total N (k_N) and ^{15}N (k_{15N}) for the different seagrass species per experiment and the exogenous N as percentage of the total standing stock litter-N, calculated using Eq. (2)

Species	Expt	k_N (d^{-1})	k_{15N} (d^{-1})	Exogenous N (% total litter-N)
Thalassia hemprichii	1	0.049	0.064	30
	2	0.058	0.073	26
Halodule uninervis	1	0.030	0.040	34
	2	0.044	0.054	23
Cymodocea rotundata	1	0.054	0.068	26
	2	0.081	0.091	13

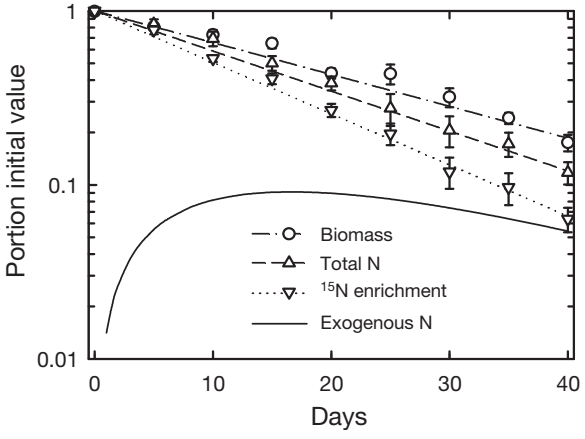

Fig. 3. *Thalassia hemprichii*. Calculated decline of biomass, total N, ^{15}N and the input of exogenous N into the litter during the decomposition (mean ± SE). Biomass, total N and ^{15}N are given as a portion of their initial values, while the exogenous N input is given as the difference between total N and ^{15}N

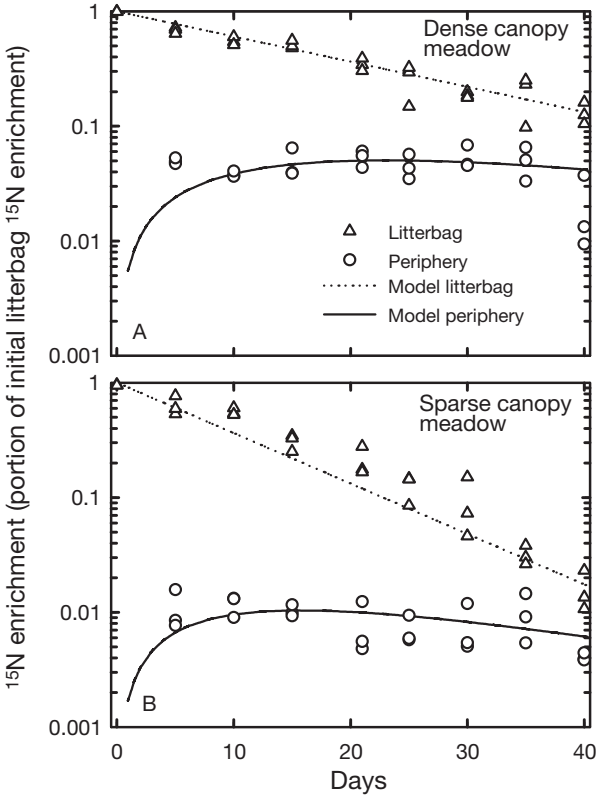

Fig. 4. Decline of total ^{15}N from the litterbags, measured ^{15}N-enrichment in the seagrass periphery (0.25 m for dense canopy, 0.10 m for open canopy meadow) around the litterbags and modelled ^{15}N in the periphery as a portion of the initial amount of ^{15}N in the litterbags in the (A) dense canopy and (B) open canopy meadow

1 macroalgae) at each sampling time. The resulting k_{15N} was 0.051 d^{-1} in the dense canopy and 0.101 d^{-1} in the open canopy meadow. Of the N released from the litterbags 11.8% was taken up by the seagrasses in a 0.25 m periphery in the dense canopy meadow and 1.9% in a 0.10 m periphery in the open canopy meadow.

DISCUSSION

The decomposition rates for seagrasses found in the present study (0.023 to 0.070 d^{-1}) were amongst the highest values found in the literature (0.005 to 0.1 d^{-1}; Harrison 1989, Mateo & Romero 1996, Cebrián et al. 1997, Machás et al. 2006). The fast decomposition rates can be explained by stimulated microbial activity due

to the high temperatures in the tropics (Godshalk & Wetzel 1978, Harrison 1989). For temperate seagrasses a positive correlation between temperature and decomposition rates are found for the most-studied seagrass species, *Zostera marina* and *Posidonia oceanica* (Buchsbaum et al. 1991, Mateo & Romero 1996). No trend between decomposition rate and seagrass species size or initial N concentration was observed. *Thalassia hemprichii* and *Halodule uninervis* had comparable decomposition rates, while *Cymodocea rotundata* had higher rates. The use of fresh leaf material with N concentrations of ca. 2% DW may have enhanced the decomposition rates (ca. 20% of the litter material consisted of the youngest leaves), since the litter in the field consisted mainly of the oldest leaves with N concentrations ranging from 1 to 1.5% DW (Vonk et al. 2008). However, foraging by fauna species in seagrass meadows also results in the detachment of relatively young leaf material, comparable to the material used in this experiment. The initial N concentrations of the seagrass litter were relatively high, but within the range for tropical seagrasses N reported by others (Hemminga et al. 1999, Stapel et al. 2001). The C and N concentration of the litter declined for most of the seagrass species during the decomposition, as found in most decomposition studies of fresh seagrass material in the field (Rublee & Roman 1982, Bourgues et al. 1996, Machás et al. 2006).

During the decomposition we measured a decline of $\delta^{15}N$ values in the litter. Combining the 4 processes mentioned earlier which influence the measured decline of $\delta^{15}N$ during decomposition (fractionation, uptake of ^{15}N-enriched material by the microbial community, unequal labelling and microbial colonization), we conclude that the measured decline in $\delta^{15}N$ of the litter was caused by the input of exogenous N. This exogenous N is assumed to consist of both microbial colonization and N_2-fixation by bacteria, as shown for cord grass litter (White 1994). The decline of $\delta^{15}N$ was significantly linear, indicating that during the whole decomposition experiment there was a steady N input and that the initial material was equally labelled. Fractionation and uptake of ^{15}N-enriched material by the microbial community may have led to an underestimation of the original litter-N decline. Therefore our modelled input of exogenous N during decomposition and amount of exogenous N in litter represents a minimum value and only consists of strongly attached material.

Exogenous N is an important part of standing stock seagrass litter-N, comparable for all seagrasses studied and calculated to be 25% on average. This estimation indicates a minimum, since the percentage of exogenous N will even increase when the litter enters the phase of refractory decomposition (Tremblay & Benner 2006). For mangroves and cord grass, White (1994) and Tremblay & Benner (2006) found that the contribution of exogenous N to the total litter-N pool, including the refractory material, was 50 to 75% of standing stock litter-N. The amount of refractory material in seagrass litter, however, is low compared to mangroves and cord grass (White 1994, Fourqurean & Schrlau 2003), suggesting that exogenous N in seagrass litter will most likely not contribute more than 50% of the total standing stock. Since direct grazing seldom exceeds 30% of the leaf production, it is thought that the majority of the energy flow from seagrasses to higher trophic levels occurs via the detrital pathway (Cebrián 1999, Fourqurean & Schrlau 2003). During the decomposition we measured a decline in the N concentration of the litter, due to release of N from the litter (cf. Fourqurean & Schrlau 2003, Machás et al. 2006), which may be partly caused by denitrification. Under anaerobic conditions in the sediment denitrification could significantly contribute to N release, but in the case of litter decomposition under mostly aerobic conditions on top of the sediment, as in the present study, this process is considered to result in a smaller loss of N. Nitrification of the released N in the water column is not expected to influence the uptake, since seagrasses can also take up nitrate with their leaves (Vonk et al. 2008b). At the same time of the release of N we also measured an input of N in the litter. We conclude that this exogenous N input counterbalances to some extent the loss of N from the litter and that detritus functions as an important component in the N budget of tropical seagrass ecosystems.

The macrophyte N concentrations are well above the level that indicates N limitation reported by Duarte (1990) for seagrass leaves. Nonetheless, the dissolved N concentrations were very low in the meadows (Vonk et al. 2008b). The loss of N from seagrasses through the detachment of N-rich leaf material in nutrient-poor, but highly productive, environments emphasizes the necessity of reusing N regenerated from decomposing materials. Seagrasses surrounding the litterbags directly took up part of the N released from the decomposing litter on top of the sediment, either as dissolved inorganic (NH_4^+ and NO_3^-) or organic (amino acids and urea) N sources (Vonk et al. 2008b). All 3 seagrass species present in the meadow were equally able to use the released N, as indicated by the comparable ^{15}N-enrichment values of the seagrasses harvested from the central vegetation quadrat.

The overall N-reuptake efficiency of the meadow community is probably higher than the values we presented in the present study for uptake by seagrasses of only microbial decomposition products. Our experiment did not include the first phase of rapid leaching of N-rich (organic) compounds from the macrophyte litter. During the 2 d between harvesting of the macro-

phyte material that was used in the litterbags and the deployment of the litterbags in the field, the N concentration declined with ca. 0.25 % of DW (about ⅛ of the total N content; cf. Anesio et al. 2003). Forms of N leaching from organic matter may form an easily accessible source for uptake, assumably better available for the plants compared to N products from microbial decomposition. Other components of the seagrass community that were not examined in our uptake study, e.g. epiphytes, most likely also take up part of the regenerated N and will enhance the N-reuptake efficiency of the seagrass community.

Efficient uptake of N released from decomposing material by seagrasses has been shown before in the sediment. ^{15}N-labelled detritus consisting of phytoplankton injected into or trapped in the sediment was taken up by the roots and translocated to the leaves (Evrard et al. 2005, Barrón et al. 2006). Together with the uptake of decomposition products via the leaves, shown in the present study, this explains the high retention of N found in these tropical seagrass meadows (cf. Stapel et al. 2001). The ability of seagrasses to take up small organic N sources like amino acids, besides inorganic N with leaves and roots (Vonk et al. 2008b), may contribute to the efficient reuse of N regenerated from organic matter. In tropical meadows growing in nutrient-poor clear water, without influences of upwelling or river discharges, the importance of phytoplankton as a source of regenerated N is expected to be rather limited. Seagrass litter, however, is considered an important source of N in these meadows as shown by the large allocation of N to leaf production (Stapel et al. 2001), the short leaf age and low resorption of N from senescing leaves (Hemminga et al. 1999). This litter material is efficiently retained within the meadows, due to the particle trapping capacity of the seagrasses (Koch et al. 2006) and the collection of litter by burrowing fauna species (Vonk et al. 2008a), as shown also by the low export of litter from these meadows (Stapel et al. 1996). Combined with the low amount of detritus and the high decomposition rates of litter in the meadows, this indicates that N associated with detached leaf material is efficiently regenerated and forms an important source of N for uptake by seagrasses.

In an attempt to illustrate the difference between uptake efficiency of dense and open canopy meadows and to understand the scale of the calculated portion of N regenerated from the litter that is taken up by the seagrasses, we mathematically extrapolated the measured uptake of ^{15}N and expressed the uptake of released N over surface area of the different seagrass meadows. Since no hydrodynamics and concentration of the N released to the water column were measured, we made the following assumptions: (1) N released from a point source, (2) low concentrations of released N (R), (3) a fixed water depth (h), (4) full vertical mixture of the released N in the water column, (5) an equal distribution of the released N in all directions, (6) constant seagrass biomass area index (B) and (7) a decline of released N over distance from the release point only due to uptake by seagrasses. Since the concentration of released N is low, uptake is only concentration-limited and not controlled by the physiological uptake limitations of leaves. Therefore there is a linear correlation between the uptake and the concentration and a linear correlation between uptake and seagrass biomass. The uptake of released N was measured in the present study over a distance of 0.10 or 0.25 m. Since equal distribution was assumed, water depth was assumed fixed and the area of seagrass around the litter is expressed as function of the radius (r) around a point source, the product of seagrass biomass ($B \times \pi \times r^2$) times concentration of released N [$R\ (\pi \times r^2 \times h)^{-1}$] will result in a constant value [$(B \times R)\ h^{-1}$] for any value of r. Since uptake by other primary producers (e.g. epiphytes) was not taken into account, the modelled values represent a minimum efficiency of regenerated N uptake capacities by the meadows.

Our assumptions resulted in the following equations to model the cumulative uptake of released N over area in the seagrass meadows:

$$c_r = 1 - [N]_{\text{remaining}} \quad (5)$$

where c_r is the cumulative uptake of released N by the seagrasses in the meadow over area with radius r and $[N]_{\text{remaining}}$ the portion of released N remaining in the water column. The portion of released N from the source that remained in the water column declined following the equation:

$$[N]_{\text{remaining}} = e^{-c_c \frac{r}{d}} \quad (6)$$

where c_c is the amount-dependent uptake of released N, r the radius of the meadow around the litter and d the radius of the periphery for which the N uptake was actually measured (0.25 m for dense canopy and 0.10 m for open canopy meadow). The concentration-dependent uptake of released N is computed using:

$$c_c = c_d [N]_{\text{remaining}} \quad (7)$$

where c_d is the measured uptake of released N in the present study (cf. Eq. 4). Substitution of Eq. (7) in Eq. (6), the expression becomes:

$$\frac{\ln [N]_{\text{remaining}}}{[N]_{\text{remaining}}} = \frac{-r c_d}{d} \quad (8)$$

This expression was solved iteratively for different values of r, knowing that $[N]_{\text{remaining}}$ in the water column was between 0 and 1. For different values of r,

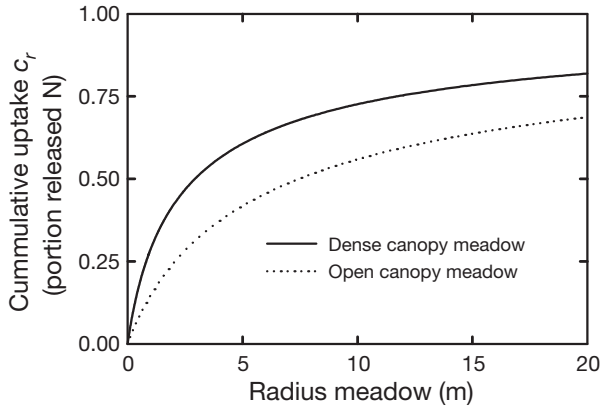

Fig. 5. Cumulative uptake of released N (c_r) as a portion of the released N from litter in the dense canopy and open canopy meadow. Uptake is given as function of the meadow radius around the point source of decomposing material. Cumulative uptake was mathematically extrapolated using the assumptions detailed in 'Discussion' prior to Eqs. (5) to (8)

the resulting $[N]_{remaining}$ was substituted in Eq. (5) to yield an estimation of c_r and expressed graphically (Fig. 5).

Using Eq. (8), we estimated that in the dense canopy meadow half of the released N from litter is taken up by the seagrass canopy within a 2.9 m radius or 27 m^2 area around the source, while in the open canopy meadow this is within a 7.5 m radius or 175 m^2 area. Combining these estimations with the leaf biomass, 149 g DW m^{-2} in the dense canopy and 98 g DW m^{-2} in the open canopy meadow, resulted in an estimated 4 kg DW leaf material in the dense canopy meadow responsible for taking up half of the released N compared to 17 kg DW in the open canopy meadow. It should be stressed that this mathematical exercise merely illustrates the difference between open and dense seagrass meadows and that the calculated figures should be cautiously interpreted. Nevertheless, although largely simplified, this mathematically expressed cumulative uptake of released N from litter indicates that the dense canopy meadow is not only more efficient due to higher biomass, but also that the uptake efficiency of released N g^{-1} biomass is higher in this meadow. This difference may be caused by the reduced water exchange in dense meadows (Koch et al. 2006). The dense canopy seagrass meadow benefits from the longer residence time of water with the released N in the canopy compared to the open canopy meadow. Increasing seagrass canopy density, in addition to increasing patch size as hypothesised by Stapel et al. (2001), coincides with increasing N-retention efficiency, increasing the chances of survival of seagrasses especially in nutrient-poor environments.

We conclude that N cycling outside the living plant but within the meadow via the detrital pathway is important for tropical seagrass meadows. The decomposition rates indicate a fast release of N from tropical seagrass litter, supporting a fast N recycling in the meadow. Decomposing litter material is not only a source of N, but also acts as a sink for N as shown by the exogenous N input from the water column, pore water or by microbial colonization. In spite of the dynamic and open environment in which most of these meadows flourish, these tropical seagrasses efficiently take up N released from litter, contributing significantly to the preservation of N in the meadows. Our mathematical extrapolation of the ^{15}N uptake over distance from its release point indicates that the biomass-specific effectiveness of re-absorption of regenerated N (and maybe the absorption of nutrients in general) increases with meadow canopy density.

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