Regeneration of nitrogen (\(^{15}\text{N}\)) 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 \(^{15}\text{N}\)-enriched seagrass leaf material (\(\delta^{15}\text{N} \approx 500\%\)) 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 \(\delta^{15}\text{N}\) 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 \(\delta^{15}\text{N}\) 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 · \(^{15}\text{N}\)

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 (Ertemeijer & 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).
15N-tracer experiments have been performed to study through rapid (Stapel et al. 2001). N recycling can be accomplished on the density (Koch et al. 2006) and size of the meadow released from litter by the seagrass leaves may depend (Erftemeijer & Middelburg 1995). Uptake efficiency of N cycling of seagrass material (Hemminga et al. 1991, organic material, uptake of dissolved N sources and re-seem to be met by an efficient process of trapping of 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 (15N) 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, 15N-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 15N-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% CaCO3; 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 15N 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 15N-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 15NH4Cl (99% 15N) 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 15N 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 δ\textsubscript{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 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 \textsuperscript{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 were randomly chosen seagrass vegetation quadrats. The other 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 water and oven dried to a constant DW and analysed for δ\textsubscript{15}N.

**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 (Buchbaum et al. 1991, Cebrián et al. 1997, Fourquean & Schrlau 2003). We therefore calculated the decomposition rates using a single first-order exponential decay model (Olson 1963):

\[ W_t = W_0 e^{(-k_{DW} t)} \]  

(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\textsuperscript{-1}). We also calculated the decomposition rate constants for total N (\( k_N \)) and \textsuperscript{15}N (\( k_{15N} \)) using this model, substituting total N and total \textsuperscript{15}N at time 0 and time \( t \) for \( W_0 \) and \( W_t \), respectively. 

**Exogenous N input.** Changes in δ\textsubscript{15}N values during decomposition can be caused by 4 main processes, influencing both natural as well as enriched δ\textsubscript{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 δ15N values of 0 to 5‰ for decomposition and 28 to 33‰ for denitrification (Robinson 2001). The second process could be the assimilation of 15N-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 15N-enriched dissolved N. Uptake of this enriched N source by the microbial community will increase their natural δ15N signature with an unknown ‰. The third process inducing a change in δ15N 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 δ15N values. We labelled the seagrasses 20 d before harvesting to make sure that the added 15N 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 δ15N values of the litter is the input of exogenous N (other than original litter N) with natural δ15N 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 N2-fixation by bacteria may also contribute. This input of exogenous N will result in a decline of the δ15N 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 δ15N, we assume that the decline in δ15N 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 15N 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 15N and N decomposition rate constants, assuming a steady state in the production and decomposition of litter:

$$N_{\text{exogenous}} = \frac{(k_{15N} - k_N)}{k_N} \times 100\%$$  \hspace{1cm} (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 15N 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 15N-enrichment (µg 15N) in the central vegetation quadrats and the decline of 15N 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):

$$\frac{dR}{dt} = ck_{15N}L - aR$$  \hspace{1cm} (3)

where $c$ is the portion of 15N regenerated from the litter that is taken up by the seagrasses, $k_{15N}$ the first-order decomposition rate constant of the cumulative 15N in all 4 litterbags around the central quadrat, L the cumulative amount of 15N in the 4 litterbags, $a$ the first-order rate constant at which 15N declined in the seagrasses from the central quadrat due to leaf turnover and leaching and $R$ the 15N-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 15N-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:

$$c_d = \frac{R_t - R_0}{k_{15N}L_0 (e^{-a} - k_{15N}^\beta - 1)}$$  \hspace{1cm} (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 15N in the litterbags, $R_t$ and $R_0$ are the 15N-enrichments in the seagrass periphery at time $t$ and 0, respectively. All 15N data were expressed as portion of the initial total amount of 15N 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 15N 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 δ^{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.

**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 δ^{15}N values varying from 350 to 950‰, more or less equally distributed over all leaves (Table 2). During the decomposition, the δ^{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 1. Mean ± SD shoot density (× 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Density (× 10^3 m^{-2})</th>
<th>Shoot biomass (g m^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dense canopy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thalassia hemprichii</em></td>
<td>0.85 ± 0.25^A</td>
<td>58 ± 17^A</td>
</tr>
<tr>
<td><em>Halodule uninervis</em></td>
<td>1.66 ± 1.39^B</td>
<td>17 ± 15</td>
</tr>
<tr>
<td><em>Cymodocea rotundata</em></td>
<td>2.27 ± 0.54^A</td>
<td>64 ± 22^A</td>
</tr>
<tr>
<td><em>Halophila ovalis</em></td>
<td>0.18 ± 0.16</td>
<td>9 ± 11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5.03 ± 1.08</td>
<td>149 ± 32^A</td>
</tr>
<tr>
<td><strong>Open canopy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thalassia hemprichii</em></td>
<td>0.45 ± 0.31^B</td>
<td>33 ± 29^B</td>
</tr>
<tr>
<td><em>Halodule uninervis</em></td>
<td>2.92 ± 0.82^A</td>
<td>2 ± 10</td>
</tr>
<tr>
<td><em>Cymodocea rotundata</em></td>
<td>1.67 ± 0.76^B</td>
<td>31 ± 18^B</td>
</tr>
<tr>
<td><em>Halophila ovalis</em></td>
<td>0.37 ± 0.41</td>
<td>10 ± 8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5.23 ± 1.09</td>
<td>98 ± 38^B</td>
</tr>
</tbody>
</table>

### Table 2. Relative biomass fraction of the leaf-age classes and the mean δ^{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 = 5). Leaf 0 is the whitish youngest leaf not yet visible in the field. Leaf class 4 also includes older leaves (na: not applicable).

<table>
<thead>
<tr>
<th>Species</th>
<th>Expt</th>
<th>% litter DW</th>
<th>δ^{15}N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Thalassia hemprichii</em></td>
<td>1</td>
<td>1.3</td>
<td>12.2</td>
</tr>
<tr>
<td><em>Thalassia hemprichii</em></td>
<td>2</td>
<td>2.7</td>
<td>10.9</td>
</tr>
<tr>
<td><em>Halodule uninervis</em></td>
<td>1</td>
<td>0.8</td>
<td>15.1</td>
</tr>
<tr>
<td><em>Halodule uninervis</em></td>
<td>2</td>
<td>1.7</td>
<td>17.0</td>
</tr>
<tr>
<td><em>Cymodocea rotundata</em></td>
<td>1</td>
<td>1.9</td>
<td>20.7</td>
</tr>
<tr>
<td><em>Cymodocea rotundata</em></td>
<td>2</td>
<td>2.4</td>
<td>15.7</td>
</tr>
</tbody>
</table>
| ^aSheath
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).

### Exogenous N input

For all macrophytes in both experiments *k*<sub>15N</sub> values were larger compared to *k*<sub>N</sub> (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 *15N* (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 *15N* 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 *15N*-enrichment in the central vegetation quadrants was present in the leaves, the remainder in underground parts. Using the measured *15N*-enrichment in the central vegetation quadrants, the course of *15N* in the periphery was described by Eq. (4) (Fig. 4). For *k*<sub>15N</sub> we substituted a decomposition rate that was obtained using Eq. (1) after adding up the remaining *15N* contents in all 4 litterbags (3 seagrass species and

### Table 3. Litter biomass decomposition rates (*k*<sub>DW</sub>) for the different macrophyte species. Significantly different biomass decomposition rates are denoted with superscripts

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean <em>k</em>&lt;sub&gt;DW&lt;/sub&gt; (d&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thalassia hemprichii</em></td>
<td>0.042&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.034–0.050</td>
</tr>
<tr>
<td><em>Halodule uninervis</em></td>
<td>0.031&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.023–0.039</td>
</tr>
<tr>
<td><em>Cymodocea rotundata</em></td>
<td>0.062&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.055–0.070</td>
</tr>
</tbody>
</table>
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.

### 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 ($F$-critical1, 26 = 5.59), unless noted (ns = not significant). Significant $R^2$ and coefficient of correlations (r; Pearson correlation, all p < 0.01) are given.

<table>
<thead>
<tr>
<th>Species</th>
<th>Expt</th>
<th>$\delta^{15}$N</th>
<th>%C</th>
<th>%N</th>
<th>C:N ratio</th>
<th>Exogenous N (%) total litter-N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$R^2$</td>
<td>r</td>
<td>$F$</td>
<td>$R^2$</td>
<td>r</td>
</tr>
<tr>
<td>Thalassia hemprichii</td>
<td>1</td>
<td>25.77</td>
<td>0.58</td>
<td>1.81</td>
<td>ns</td>
<td>25.68</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>37.56</td>
<td>0.63</td>
<td>-0.79</td>
<td>70.10</td>
<td>0.90</td>
</tr>
<tr>
<td>Halodule uninervis</td>
<td>1</td>
<td>25.43</td>
<td>0.54</td>
<td>-0.73</td>
<td>4.49</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.49</td>
<td>0.67</td>
<td>-0.82</td>
<td>50.53</td>
<td>0.87</td>
</tr>
<tr>
<td>Cymodocea rotundata</td>
<td>1</td>
<td>50.60</td>
<td>0.76</td>
<td>-0.87</td>
<td>15.60</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>21.44</td>
<td>0.53</td>
<td>-0.73</td>
<td>19.46</td>
<td>0.73</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Expt</th>
<th>$k_N$ (d–1)</th>
<th>$k_{15N}$ (d–1)</th>
<th>Exogenous N (%) total litter-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassia hemprichii</td>
<td>1</td>
<td>0.049</td>
<td>0.064</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.058</td>
<td>0.073</td>
<td>26</td>
</tr>
<tr>
<td>Halodule uninervis</td>
<td>1</td>
<td>0.030</td>
<td>0.040</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.044</td>
<td>0.054</td>
<td>23</td>
</tr>
<tr>
<td>Cymodocea rotundata</td>
<td>1</td>
<td>0.054</td>
<td>0.068</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.081</td>
<td>0.091</td>
<td>13</td>
</tr>
</tbody>
</table>

**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 (Buchseries 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 understimation 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 major-
We made the following assumptions: (1) N released of the N released to the water column were measured, meadows. Since no hydrodynamics and concentration declined with ca. 0.25% of DW (about 1/8 of the deployment of the litterbags in the field, the N concentration and expressed the uptake of 15N and assumed uptake of released 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. 15N-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 15N 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:

$$c_r = 1 - [N]_{\text{remaining}}$$

(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:

$$[N]_{\text{remaining}} = e^{-c_r \frac{r^2}{2}}$$

(6)

where $c_r$ 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:

$$c_c = c_d [N]_{\text{remaining}}$$

(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:

$$\frac{\ln[N]_{\text{remaining}}}{[N]_{\text{remaining}}} = -\frac{rc_d}{d}$$

(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 $r$,
the resulting $\left[N\right]_{\text{remaining}}$ 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 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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