Nitrogen assimilation and short term retention in a nutrient-rich tidal freshwater marsh – a whole ecosystem $^{15}$N enrichment study


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Abstract. An intact tidal freshwater marsh system (3477 m$^2$) was labelled by adding $^{15}$N-ammonium as a tracer to the flood water inundating the ecosystem. The appearance and retention of $^{15}$N-label in different marsh components (leaves, roots, sediment, leaf litter and invertebrate fauna) was followed over 15 days. This allowed us to elucidate the direct assimilation and dependence on creek-water nitrogen on a relatively short term and provided an unbiased assessment of the relative importance of the various compartments within the ecosystem. Two separate experiments were conducted, one in spring/early summer (May 2002) when plants were young and building up biomass; the other in late summer (September 2003) when macrophytes were in a flowering or early senescent state.

Nitrogen assimilation rate (per hour inundated) was $>3$ times faster in May compared to September. On both occasions, however, the results clearly revealed that the less conspicuous compartments such as leaf litter and ruderal vegetation are more important in nitrogen uptake and retention than the prominent reed ($Phragmites australis$) meadows. Moreover, short-term nitrogen retention in these nutrient rich marshes occurs mainly via microbial pathways associated with the litter and sediment. Rather than direct uptake by macrophytes, it is the large reactive surface area provided by the tidal freshwater marsh vegetation that is most crucial for nitrogen transformation, assimilation and short term retention in nutrient rich tidal freshwater marshes. Our results clearly revealed the dominant role of microbes in initial nitrogen retention in marsh ecosystems.

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1 Introduction

Tidal freshwater marshes are periodically inundated wetlands fringing rivers. These distinct features of inner estuaries often occur where estuaries are most enriched in particles and nutrients. High nutrient concentrations and regular tidal inundation results in highly productive macrophyte and algal communities, with potential to play an important role in the nitrogen retention. Thus, tidal freshwater marshes potentially attenuate river borne nitrogen load to adjacent coastal waters. The dynamics of nitrogen cycling in tidal freshwater marshes is not well known and most of what is known comes from plant tissue analysis, tidal input/output balance studies, and analogy to more intensively studied salt marshes (Odum, 1988; Bowden, 1986; Bowden et al., 1991; Merrill and Cornwell, 2000; Verhoeven et al., 2001; Hansson et al., 2005).

Net marsh nitrogen retention, i.e. less nitrogen is leaving the marsh system than entering, is governed by the balance of loss processes (gaseous emissions of nitrous oxide and dinitrogen, and tidal export) and processes which import and retain nitrogen within the system (nitrogen fixation, precipitation, tidal imports, plant uptake, recycling, and accretion) (White and Howes, 1994b; Mitch and Gosselink, 2000). While particulate deposition (sedimentation), plant nitrogen uptake and denitrification are generally reported to be the most important sinks for watershed derived nitrogen in (tidal freshwater) wetlands (Bowden, 1986; Hansson et al., 2005; Neubauer et al., 2005), methodological restrictions have limited our understanding of interactions between the various marsh compartments and of the functioning of these ecosystems as a whole.
The Scheldt estuary (Belgium – The Netherlands) is a macrotidal, heterotrophic, low-oxygen, nutrient-rich system (Soetaert et al., 2005). Although many tidal marshes of the Scheldt basin have been reduced to very small size today (mainly by embankment and polder reclamation), this is one of the few European basins where fringing, tidal freshwater marshes are still a prominent feature. Yet the importance of these marshes as a nutrient sink remains largely unassessed. We conducted two temporally separated (May 2002 and September 2003) pulse additions of $^{15}$N-ammonium to the tidal marsh flood water, and traced the (short term) fate of riverine ammonium in a freshwater marsh fringing the Scheldt River. Using this relatively new technique of deliberate additions of trace amounts of heavy nitrogen ($^{15}$N) to aquatic systems allowed us to simultaneously study the dynamics, uptake and transformation of watershed derived ammonium by the marsh biota in an intact marsh ecosystem.

Nutrient transformation and assimilation rates are potentially influenced by seasonally variable factors, such as nutrient loading, developmental stage of macrophytes and associated microbes, and temperature. To maximize contrasts we therefore scheduled our two experiments in spring (May), when plants were young and building up biomass, and late summer (September), when macrophytes were in a flowering or early senescent state, respectively. The detailed results of the water-phase component of these studies have been described previously (Gribsholt et al., 2005, 2006). In both May and September, nitrification accounted for the largest fraction of ammonium transformation, and the large reactive surface area of the marsh played a crucial role in nitrogen transformation. However, a significant part of the added $^{15}$N-NH$_3^+$ was assimilated and stored within the marsh. In these companion papers, however, marsh retention was treated collectively as one unresolved $^{15}$N-sink, and the details of uptake into specific marsh compartments and their relative importance for the nitrogen cycling were ignored.

In this paper we report in detail the $^{15}$N uptake into the different marsh compartments (sediment, root, leaves, leaf litter and invertebrate macrofauna) and subsequent $^{15}$N retention on a time scale of days. Constrained by the two-directional flow of the tide and the complete drainage of the marsh between tides, tracer could only be added as a short pulse, allowing us to elucidate the direct assimilation of $^{15}$N and dependence on creek-water nitrogen on a relatively short term only (15 days). However, the advantage of the stable isotope approach is that it allows the unbiased assessment of the relative importance of the various compartments within the ecosystem. Our results clearly show that the less conspicuous compartments such as leaf litter with associated microbes and more ruderal vegetation are more important than the prominent reed meadows, and that short term nitrogen retention in these nutrient rich marshes occurs mainly via microbial pathways associated with the litter and sediment.

## 2 Methods

### 2.1 Study area

The study site is located in the northern end of the Tielrode tidal freshwater marsh (51°06′N, 4°10′E) fringing the Scheldt and the Durme rivers, Belgium. This triangular shaped area of 3477 m$^2$ is bordered on two sides by dikes, while the remaining side was closed off by 1 m wooden boards during the experiments (Fig. 1). Boards were dug 10–20 cm into the sediment, allowing water flow only through a 4.5 m wide open span across the tidal creek. A 4.5 m long sampling and labelling bridge was placed across the creek. The study marsh has a patchy vegetation typical for tidal marshes in the region, with the common reed *Phragmites australis* dominating the lower elevations and willows (2–6 m high specimens of *Salix* sp.) and patches of ruderal vegetation (dominated by Policeman’s helmet *Impatiens glandulifera*, Hairy Willow-herb *Epilobium hirsutum* and Stinging Nettle *Urtica dioica*) at the higher elevations (Fig. 1).

Twelve sampling stations were placed within the study site (Fig. 1), three within each vegetation type (reed, willow and ruderal) and three in the unvegetated creek banks (four habi-
The appearance of the study area was very different between the two campaigns. In May the reed (St. 1–3) was approximately 2 m high while the ruderal vegetation (St. 7–9) reached heights of approximately 1 m. There was no (St. 4–5) or limited (St. 6) herbaceous vegetation present under willows (Table 1). Generally the vegetation appeared green. Dense benthic microalgal mats (dominated by filamentous yellow-green algae Vaucheria sp.) were found on the sediment surface especially on creek bank and willow sites, and creek banks were covered by watercress (Rorippa sp.). Sediment Chl a content was high ranging from 263–1022 mg m$^{-2}$. In September, the herbaceous vegetation was considerably taller than in May, reaching up to 3–4 m and both reed and the ruderal key species (Policeman’s helmet, Hairy Willow-herb and Stinging Nettle) were flowering. The reed appeared more light in colour compared to May. Some ruderal vegetation (Policeman’s helmet) reaching up to 2 m was present at the willow sites (Table 1). No vegetation covered the creek banks, and the sediment surface appeared bare. Thick algal mats observed in May were generally absent in September, and sediment Chl a concentrations were considerably lower ranging from 17–169 mg m$^{-2}$.

### Table 1. Habitat (vegetation type), relative area of study site represented by station (Area%), surface elevation (relative to mean sea level), duration of flooding during $T_0$ and standing above-ground biomass at each station in May and September. Locations of sampling stations are shown in Fig. 1.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Station nr.</th>
<th>Relative Area</th>
<th>Surface elevation</th>
<th>Flooding duration ($T_0$)</th>
<th>Standing biomass$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(m)</td>
<td>May (min) Sep (min)</td>
<td>May (kg m$^{-2}$) Sep (kg m$^{-2}$)</td>
</tr>
<tr>
<td>Reed</td>
<td>1</td>
<td>25</td>
<td>3.23</td>
<td>117 70</td>
<td>0.9 2.2±1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>2.97</td>
<td>139 109</td>
<td>0.9 2.3±0.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>3.06</td>
<td>133 100</td>
<td>0.8 1.2±0.4</td>
</tr>
<tr>
<td>Willow</td>
<td>4</td>
<td>12</td>
<td>3.15</td>
<td>124 88</td>
<td>0 0.7±0.2$^b$</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>3.34</td>
<td>105 39</td>
<td>0 0.4±0.3$^b$</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>3.40</td>
<td>92 0</td>
<td>0.2$^b$ 0.6±0.3$^b$</td>
</tr>
<tr>
<td>Ruderal</td>
<td>7</td>
<td>5</td>
<td>3.31</td>
<td>109 48</td>
<td>0.4 1.2±0.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6</td>
<td>3.25</td>
<td>116 68</td>
<td>0.6 0.8±0.7</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5</td>
<td>3.31</td>
<td>109 48</td>
<td>1.1 0.9±0.6</td>
</tr>
<tr>
<td>Creek</td>
<td>10</td>
<td>2</td>
<td>3.11</td>
<td>129 94</td>
<td>0$^c$ 1.3±0.9</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3</td>
<td>2.87</td>
<td>148 118</td>
<td>0$^c$ 0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2</td>
<td>2.67</td>
<td>163 140</td>
<td>0$^c$ 0</td>
</tr>
</tbody>
</table>

$^a$ Above-ground biomass only and willows and benthic microalgae are excluded.

$^b$ Biomass of understory ruderals (e.g., Policeman’s helmet (L. glandulifera)).

$^c$ Biomass of watercress (Rorippa sp.) not included.

#### 2.2 Isotopic labelling

The marsh was labelled with $^{15}$NH$_4^+$ on two separate occasions, 25 May 2002 and 11 September 2003, by adding the label to the flood water in the tidal creek as it entered the study area. The $^{15}$N addition was deliberately scheduled in early (May) and late (September) summer, respectively, to represent seasonal variation in macrophyte growth and associated variation in microbial activity.

In May 1.97 mol $^{15}$N-NH$_4^+$ was added while 1.41 mol $^{15}$N-NH$_4^+$ was added in September. This increased the $^{15}$N content of the ammonium pool from 0.37% to 1.3% and 4.5% and increased the average total NH$_4^+$ concentration by 14 and 73% in May and September, respectively. The higher degree of labelling in September compared to May was due to a combination of significantly lower ammonium concentrations in the flood water (Fig. 2a) and lower tidal height (see Discussion; Gribsholt et al., 2006). Thus only half the volume of water flooded the marsh during September labelling compared to May (Table 2). The label solution consisted of 1 kg 10% $^{15}$N labelled ($^{15}$NH$_4$)$_2$SO$_4$ and 50 kg NaBr (conservative tracer) dissolved in 250 L of water. In May nearly all label solution was added, while only 180 L was added in September. $^{15}$N release was initiated when the first flood water arrived at the labelling platform and ended at the turn of the tide, and the label solution was released proportional to the volume of water entering the marsh as described in detail in Gribsholt et al. (2005). This ensured an even distribution of $^{15}$NH$_4^+$ over the entire study area, as was confirmed by evaluation of the conservative tracer (Br$^-$) distribution.
Table 2. Summary of water inundation parameters for the two sampling occasions. Duration of main tide (flood and ebb), relative area inundated, maximum water height above creek bed (2.47 m above mean sea level) below measuring platform and flood water volume (calculated water budget). All data are for tides prior to marsh station sampling, except for September T5, where stations were sampled at T4.

<table>
<thead>
<tr>
<th>Tide</th>
<th>Flood (min)</th>
<th>Ebb (min)</th>
<th>Area inundated (%)</th>
<th>Water height (cm)</th>
<th>Flood (m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maya</td>
<td>Sepb</td>
<td>May</td>
<td>Sep</td>
<td>May</td>
</tr>
<tr>
<td>T0</td>
<td>80</td>
<td>77</td>
<td>131</td>
<td>107</td>
<td>100</td>
</tr>
<tr>
<td>T1</td>
<td>77</td>
<td>71</td>
<td>119</td>
<td>103</td>
<td>100</td>
</tr>
<tr>
<td>T5</td>
<td>78</td>
<td>58</td>
<td>139</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>T31</td>
<td>76</td>
<td>ndc</td>
<td>106</td>
<td>nd</td>
<td>100</td>
</tr>
</tbody>
</table>

a Compiled from Gribsholt et al. (2005).
b Compiled from Gribsholt et al. (2006).
c Not determined.

Fig. 2. (a) dissolved ammonium (b), dissolved nitrate + nitrite, (c) suspended particulate nitrogen (SPN), and (d) O₂ saturation during T0 (tracer addition) in May and September. Only data from T0 are shown, as the temporal patterns of all parameters were quite similar among tides on both occasions. (For details see Gribsholt et al., 2005, 2006).

2.3 Marsh sampling and analysis

2.3.1 ¹⁵N and total nitrogen

Marsh stations were sampled during low tide when the marsh surface was air-exposed prior to tracer addition (T-2) to establish natural abundance levels of ¹⁵N, and just after labelling (T0). In May samples were also collected after two subsequent tides (T5 and T31), while T1, T2, T4 and T29 were sampled in September. T denotes the tide inundating the marsh and the subscript denotes the tide number relative to tracer addition. Samples were collected more frequently in September, because May results revealed a rapid formation of the ¹⁵N immediately following tracer addition. The entire study area is flooded only by the highest tides, and each experiment was initiated just before a spring tide when a maximum of high tides followed, and sampling continued until the following spring tide cycle, when the study area was flooded again. As there were two tides per day, this means that label retention was followed for about 15 days. At all stations samples of sediment, above ground vegetation (live macrophyte stems and leaves, onwards collectively referred to as leaves), below ground vegetation (roots), dead macrophyte material on the sediment surface (litter), invertebrate macrofauna (benthic infauna and epifauna) and suspended matter settling on the sediment surface (sedimentation traps) were collected for analysis of total nitrogen and ¹⁵N content.

At each station the surface layer (0–0.5 cm, including benthic algae) of three sediment cores (internal diameter 6 cm) were pooled while one additional core was sectioned into 0.5–2.5, 2.5–5 and 5–10 cm depth intervals to trace any downward transfer of ¹⁵N. Triplicates were pooled (surface sediment as well other compartments, see below) in order to obtain a more representative sample and to limit financial cost of isotope analysis. In May, a sub-sample (~4 g) of the surface sediment was immediately transferred to 10 ml 2 M KCl solution and total extractable inorganic nitrogen (includes both free inorganic nitrogen and nitrogen loosely sorbed on sediment surfaces; Bremner, 1965; Bowden et al., 1991) was extracted the next day (shaken 1 h). Following centrifugation the supernatant was removed and the sediment was rinsed in milliQ water and subsequently centrifuged three times before the remaining sediment pellet was frozen. Sediment samples (untreated and KCl extracted) were frozen and then freeze dried. Sorbed nitrogen (Nsob) was inferred from Ntot=Nsob+Norg, where Norg equals organic nitrogen remaining after KCl extraction and Ntot is total nitrogen determined in untreated samples. Immobilization of ¹⁵N in the Norg pool is attributed to microbial incorporation. Although ¹⁵N incorporation into microbial biomass can now be quan-
tified more directly by analysing the incorporation into hydrolysable amino acids (HAAs), including the specific bacterial biomarker D-alanine (D-Ala) (Veuger et al., 2005), we rely on the less laborious standard soil science extraction approach (Bremner, 1965). In a follow-up study we have compared the two approaches and found comparable results, supporting the validity of the method applied here (Gribsholt, unpubl.).

Suspended particulate matter settling on the sediment surface was trapped on 60 mm diameter GF/A filters placed on the sediment surface. Each filter was placed on top of a 100 mm diameter filter paper (to keep undersides clean) and held down by three wire clips. New filter traps were placed at each station before each tide and collected immediately at low tide. Filters were dried at 60°C for 24 h.

Leaves and roots were sampled by gently pulling individual plants out of the sediment. Three plants of each sampled species (per station) were pooled after separating leaves and roots. In St. 1–3 only the very dominant reed was collected, and reed shoot tops were collected separately (May only). From ruderal stations (St. 7–9), where the vegetation is more heterogenous, the two most dominant species were sampled separately (3 plants each). In addition to randomly handpicking willow leaves, the dominant scrub (Policeman’s helmet) covering the sediment floor below willows was collected (St. 4–6) when applicable. In May, samples of the small macrophyte watercress (Rorippa) which covered the otherwise unvegetated creek banks were also collected. Watercress was not present in September. All samples were dried to constant weight (70°C) before further handling.

Litter was collected randomly from the sediment surface of all stations except creek banks St. 11 and 12 in May. The litter composition reflected local vegetation consisting of reed leaves and stems (St. 1–3), willow leaves (St. 4–6), and herbs (St. 7–10), and no distinction was made between old and new litter. In May additional sub-samples of all litter fragments incubated in nylon litterbags (mesh size 300 µm, filled with local litter at T<sub>−2</sub>) were collected at stations 2, 4 and 7 at T<sub>−2</sub>, T<sub>0</sub> and T<sub>5</sub>. Litterbags were deployed in order to follow the temporal evolution of isotopic signatures of homogenized litter materials. This minimizes the interference between temporal and spatial variability in isotopic signatures that may occur when time series are based on randomly collected litter samples.

For macro-invertebrate infauna, 3 sediment cores (0–5 cm depth, internal diameter 6 cm) were collected from 4 representative stations (St. 2, 5, 8 and 11) in September only. Triplicates were pooled and immediately preserved in formalin (4%) with rose Bengal. After sieving (1 mm mesh) invertebrates were identified under a dissecting microscope to the taxonomic class or order and quantified. Samples were rinsed in water and freeze dried for subsequent isotopic signature analysis of pooled material from each group. Similarly, invertebrates handpicked from the sediment surface and vegetation covering an area of several m<sup>2</sup> at each station were identified to taxonomic class or order and analyzed separately for N. Abundances were not quantified.

All samples described above were analyzed for isotopic composition and total nitrogen. Dried leaf and root samples were initially shredded by a Retsch cutting mill. After mixing, a sub-sample (∼20 g) was grinded to a fine, homogene powder in a Ball Mill. The freeze-dried sediment samples were homogenized similarly (Ball Mill), before total nitrogen analysis on a Carlo Erba Elemental Analyzer EA following Nieuwenhuize et al. (1994), and N analysis on a Fisons elemental analyzer (EA-1500) coupled on line, via a Finnigan CONFOLO II interface, with a Finnigan Delta S isotope ratio mass-spectrometer (EA-IRMS). Sub-samples of invertebrate infauna were analyzed without further treatment. Ground litter and handpicked invertebrate samples were analysed on a Thermo Finnigan Delta<sup>PLUS</sup>XL mass spectrometer connected on line to an elemental analyzer (EA, Flash series 1112) via a continuous flow interface (Finnigan Confo III).

2.3.2 Biomass estimates and sediment characteristics

Standing biomass was determined by harvesting all plant material in three 30×30 cm plots at each station. In September leaf material was separated into live and dead, counted and dried separately, while no distinction was made in May. Although a striking feature of the marsh, willow tree biomass was neglected due to methodological restrictions. Litter biomass was determined by collecting all material lying on the sediment surface in triplicate 30×30 cm plots. No distinction was made between old and new litter. Dry weight was determined by drying at 70°C till constant weight. Root biomass was not quantified in this study; instead values from similar habitat types just outside the study area determined in May and September 2002 were used for budget calculations (Gribsholt, unpublished). Sediment density was obtained from wet weight of a known sediment volume. Porosity was calculated from water loss of a known sediment volume after freeze drying. Molar C:N ratio was determined according to Nieuwenhuize et al. (1994). Separate surface sediment (0–0.5 cm depth) samples were collected for pigment analysis. Samples were freeze dried and stored at −80°C before analysis. In May total Chl a was extracted and determined spectrophotometrically following Jeffrey and Humphrey (1975), while pigments were extracted and analyzed by high performance liquid chromatography (Rijstenbil, 2003) in September.

2.4 Discharge characteristics and creek water sampling

Adveective water fluxes in and out of the study area were determined for all the tides except September T<sub>29</sub> (Gribsholt et al., 2005, 2006). Creek water nitrogen concentrations and N<sub>15</sub>N in dissolved (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub>−, NO<sub>2</sub>−, N<sub>2</sub>O and N<sub>2</sub>) and suspended (SPN) inorganic nitrogen pools, as well
as Br\(^-\) (conservative tracer) were determined 12 times over each main tidal cycle and three times during seepage (Gribsholt et al., 2005, 2006), and water column stock size for all components subsequently calculated from concentration and discharge measurements (mass balance budget). Dissolved oxygen, specific conductivity, temperature, pH and turbidity were recorded continuously (2 min intervals) using a Hydrolab Datasonde 3. Detailed descriptions of the water phase sampling, analysis and results can be found in Gribsholt et al. (2005 [May]; 2006 [September]).

2.5 Calculations

For nitrogen standing stock calculations, measurements of total nitrogen content (%N) of the various compartments as well as bulk sediment density were grouped by station (n=4–6) within each year. Repeated-measures analysis of variance (ANOVA) was used to determine any effects of tide, sampling station and season.

Nitrogen isotopic ratios were measured as delta values (\(\delta^{15}N\), \%), relative to atmospheric nitrogen and given as \(\Delta\delta^{15}N\) (isotopic enrichment), which were corrected for natural abundance levels of \(^{15}N\) by subtracting the \(\delta^{15}N\) value of similar samples collected at \(T_{i-2}\). For stations where more macrophyte species were sampled (St. 7–9) a weighted mean enrichment (according to the relative species abundance) was used for calculations. Furthermore, the label content (excess \(^{15}N\)) in each pool was determined from the isotopic enrichment and nitrogen stock size and a total \(^{15}N\) inventory was constructed for each tide. Surface sediment nitrogen and excess \(^{15}N\) concentrations, respectively, were converted to pool size (g m\(^{-2}\); 0–0.5 cm depth) using corresponding bulk sediment density. Plant tissue (leaves, roots, litter) concentrations were converted to pool size (g m\(^{-2}\)) using corresponding biomass estimates. Each compartment nitrogen standing stock and \(^{15}N\) content at the different tides was weighted by factors proportional to the area represented by each station and habitat class using a GIS based digital terrain model (Gribsholt et al., 2005) to derive the average marsh value for each component (Table 1).

Retention and export of \(^{15}N\) were calculated by mass balance of \(^{15}N\) added. In addition, for each sampling station compartment-specific (leaves, roots, litter, and sediment), \(^{15}N\)-ammonium uptake rates (\(\mu \text{mol} \ ^{15}\text{N} \text{m}^{-2} \text{h}^{-1}\)) during \(T_0\) flooding were calculated by dividing the amount of \(^{15}N\) recovered (\(\mu \text{mol} \ ^{15}\text{N} \text{m}^{-2}\)) by the inundation duration (\(h_i\)) at each station (determined from GIS based digital terrain model). Habitat specific (reed, willow, ruderal, and creek bank) and whole ecosystem \(^{15}N\) uptake rates into each component were calculated as weighted mean according to the area represented by each sampling station (Table 1, determined as described above). Finally, to allow appropriate comparison of both spatial and temporal uptake rates (\(T_0\)) between the May and September experiments, habitat and whole ecosystem total nitrogen uptake rates (\(\mu \text{mol} \ ^{15}\text{N} \text{m}^{-2} \text{h}^{-1}\)) were determined by dividing the \(^{15}N\) uptake rate by the average percentage of \(^{15}N\) labelling of the floodwater ammonium pool.

3 Results

3.1 Hydrodynamics and label distribution (waterphase components)

Details of the similarities and differences in the water-phase component of the system between the two campaigns have been discussed previously (Gribsholt et al., 2006). Here, only the main differences and similarities between the two campaigns will be highlighted as they add to the understanding of the labelling experiments and the functioning of the system. On both occasions the timing of the label addition was carefully selected based on the predicted tidal heights, and while there was very little difference in predicted and observed heights in May, the September tides were much lower than predicted. Thus the maximum water height in the creek and the total volume entering the study site were generally much reduced in September compared to May (Table 2). Consequently the inundation durations were shorter, and a significantly smaller reactive litter and plant surface area was inundated in September, potentially limiting periphyton mediated N processing compared to May (Gribsholt et al., 2006). Especially critical is the relatively low September-T\(_0\) tide (label addition) where only half as much water flooded the study area as in May-T\(_0\). While no part of the 3477 m\(^2\) study marsh surface escaped labelling in May, the most elevated marsh area (St. 6) was not exposed to labelled flood water in September.

The ambient nitrate (Fig. 2b) and especially ammonium (Fig. 2a) concentrations were much higher in May than in September. The combination of lower tidal volume and low ammonium concentrations resulted in a higher degree of labelling (4.5%) of the ammonium pool in September compared to May (1.5%). Similarly, the label addition increased the total T\(_0\) NH\(_4\)+ pool 73% in September, compared to 14% in May. The flood water was hypoxic (<50 \(\mu\text{mol} \ L^{-1}\)) during most of the main tide (Fig. 2d). \(O_2\) saturation was inversely correlated with [NH\(_4\)+], which showed a bell shaped distribution pattern over the tidal cycle and matched the main river only at maximum tidal height (Gribsholt et al., 2005, 2006).

3.2 Marsh N standing stock

Surface sediment (0–0.5 cm) and especially macrophyte biomass were the major nitrogen pools in the marsh, with the above and belowground plant biomass contributing about equally (Table 3). Surface sediment (0–0.5 cm) nitrogen content ranged from 0.32 to 1.06 wt%, with highest values observed at St. 4 and St. 10 in May. There was no significant difference in average sediment nitrogen content between
Fig. 3. Isotopic enrichment (Δδ) (above natural abundance levels) in the main marsh compartments (a, e) leaves, (b, f) roots, (c, g) litter and (d, h) sediment in the four habitats (reed, willow, ruderal and creek bank) during May and September (Mean±SE, n=3; n.d.: not determined). T₁ and T₂ were only sampled in September. In May T₃ and T₃₁ were sampled, while T₄ and T₂₉ were sampled in September. Note the different scales on the y-axis.

May (0.64±0.16%) and September (0.61±0.14%), and surface sediment pool size (0–0.5 cm) was similar among habitats as well as between seasons (Table 3). Average sediment nitrogen content decreased with depth (0–10 cm) to 0.48±0.08 wt% (May and September). Molar sediment C:N ratio was 12–14, with no significant difference among habitats or between seasons (data not shown). Root nitrogen content ranged from 0.72±0.22 wt% in reed to 1.50±0.44 wt% in the ruderal vegetation. No significant difference was observed in reed root nitrogen content between May and September, while ruderal roots had a significantly (P<0.01) higher nitrogen content in May. Root nitrogen pool was
Fig. 4. Amount of $^{15}$N (excess $^{15}$N) per unit surface area ($\mu$mol m$^{-2}$) recovered in the four main compartments (a, e) leaves, (b, f) roots, (c, g) litter and (d, h) sediment in the four marsh habitats (reed, willow, ruderal and creek bank) during May and September (Mean±SE, n=3; n.d.: not determined). $T_1$ and $T_2$ was only sampled in September. In May $T_5$ and $T_{31}$ was sampled, while $T_4$ and $T_{29}$ was sampled in September. Note the different scale on the y-axis in panes d and h.

6 (May) and 2 (September) times higher in reed compared to ruderal (Table 3), but total marsh root nitrogen pool was relatively similar in May (8.5 g m$^{-2}$) and September (10.3 g m$^{-2}$). The nitrogen pool size in leaves varied greatly among habitat types, ranging from none in the creek bank to 26.2 g m$^{-2}$ in the reed habitat. The weighted average nitrogen pool in leaves was 38% higher in September (15.6 g m$^{-2}$) than in May (11.3 g m$^{-2}$), while the spatial distribution was similar. Note, however, that willow biomass was not included in our estimates (see methods), thus total marsh nitrogen standing stocks are underestimated. Average marsh litter nitrogen stock was similar (4.6 g m$^{-2}$) in May
Table 3. Nitrogen pool size in the four main compartments (sediment (0–0.5 cm), roots, leaves and litter) in the different habitats in May and September. Numbers in parenthesis are percentage of total N in the four compartments.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sediment (g m⁻²)</th>
<th>Root (g m⁻²)</th>
<th>Leaves (g m⁻²)</th>
<th>Litter (g m⁻²)</th>
<th>Total (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsh</td>
<td>12.9 (35)</td>
<td>8.5 (23)</td>
<td>11.3 (30)</td>
<td>4.6 (12)</td>
<td>37</td>
</tr>
<tr>
<td>Reed</td>
<td>12.5±0.9</td>
<td>17.1±1.7</td>
<td>17.7±3.4</td>
<td>7.7</td>
<td>55</td>
</tr>
<tr>
<td>Willow</td>
<td>14.0±4.3</td>
<td>1.1±0.3</td>
<td>2.6±3.8</td>
<td>0.8</td>
<td>18</td>
</tr>
<tr>
<td>Ruderal</td>
<td>11.6±0.7</td>
<td>2.7±1.8</td>
<td>17.5±10.4</td>
<td>1.9</td>
<td>34</td>
</tr>
<tr>
<td>Creek</td>
<td>13.6±4.4</td>
<td>0</td>
<td>0.3±0.06</td>
<td>0.8</td>
<td>15</td>
</tr>
<tr>
<td>September</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsh</td>
<td>13.0 (30)</td>
<td>10.3 (24)</td>
<td>15.6 (36)</td>
<td>4.6 (11)</td>
<td>44</td>
</tr>
<tr>
<td>Reed</td>
<td>12.5±1.1</td>
<td>20.2±1.7</td>
<td>26.2±8.2</td>
<td>5.9±0.4</td>
<td>65</td>
</tr>
<tr>
<td>Willow</td>
<td>13.5±3.7</td>
<td>n.d</td>
<td>n.d</td>
<td>3.4±1.2</td>
<td>17</td>
</tr>
<tr>
<td>Ruderal</td>
<td>12.5±0.3</td>
<td>9.1±3.5</td>
<td>9.3±3.1</td>
<td>4.7±1.6</td>
<td>36</td>
</tr>
<tr>
<td>Creek</td>
<td>12.6±4.0</td>
<td>0</td>
<td>0</td>
<td>2.6±2.0</td>
<td>15</td>
</tr>
</tbody>
</table>

a Area-weighted average.

b Policeman’s helmet (I. glandulifera) only.
c Watercress (Rorippa sp.).

and September, but while the reed litter nitrogen pool was highest in May the opposite was observed in the other habitats (Table 3). Mean litter nitrogen content ranged from 0.9 to 1.2 wt% with no significant difference among habitats.

3.3 Marsh ¹⁵N labelling

Isotopic enrichment (Δδ¹⁵N, Fig. 3), as well as the absolute amount of ¹⁵N assimilated (excess ¹⁵N) per unit surface area (Fig. 4), varied greatly among stations, habitats and marsh compartments in both May and September. In May, the ruderal vegetation (leaves and roots) assimilated added ¹⁵N and the isotopic enrichment increased with time (up to 8‰), while very little enrichment occurred in reed (Fig. 3a).

No significant difference in isotopic signature was observed between shoot tops and the remaining leaves, thus the reed data have been pooled. Highest enrichment (up to 32‰) was observed in the small watercress (Rorippa sp.) covering the otherwise un-vegetated creek banks in May. However, since the watercress biomass was very low, the impact for total ecosystem ¹⁵N-content was negligible (Fig. 4a). A similar enrichment pattern was observed in the leaf compartment in September (Fig. 3e), except watercress was absent at creek banks, and 50% less enrichment occurred in the ruderal vegetation. In spite of lower biomass, leaves ¹⁵N content per unit area was one order of magnitude higher in ruderal (24.3±11.0 μmol m⁻²) compared to reed habitats (2.2±2.2 μmol m⁻²) in May (Fig. 4a). Patterns were similar but less clear in September, due to large heterogeneity in the reed ¹⁵N content among tides. Enrichment to the root compartment was observed in the ruderal habitat with highest enrichment (up to 11‰) in May (Fig. 3b, f). While excess ¹⁵N content was much higher in ruderal compared to reed in May (Fig. 4b), no clear difference was observed in September (Fig. 4f).

Except for watercress on creek banks (see above), litter (Fig. 3c, g) was generally the most enriched compartment at all stations in both May and September. Generally the isotopic enrichment decreased with time. There was no significant difference between ¹⁵N in in situ litter and litter incubated in litterbags (data not shown). Litter ¹⁵N content was generally the most important pool in reed and the litter compartment in reed was higher than in other habitats (Fig. 4c, g).

The sediment compartment (0–0.5 cm) was more enriched in May (Fig. 3d) than in September (Fig. 3h), with little temporal variation among tides in both May and September. No ¹⁵N enrichment was detected in deeper sediment layers and only data for the top layer are reported. While the sediment ¹⁵N enrichment and total ¹⁵N was similar (and low) in all habitats in September (Fig. 3h and 4h), the enrichment of the sediment was much higher in the willow and creek bank compared to the other habitats in May. This was, however, largely due to a very high enrichment at willow St. 4 (up to 30‰) and creek bank St. 10 (up to 33‰), which were both covered by a dense algae mat (Chl a>500 mg m⁻²).

The spatial-temporal pattern in enrichment (Fig. 3d) was directly reflected in the ¹⁵N content of the surface sediment (Fig. 4d) as sediment nitrogen stocks were similar among stations. The creek bank and willow sediments were the largest pools in May (on a surface area basis).

After T₀ the ¹⁵N enrichment to the sediment was largely due to sorption while almost all of the ¹⁵N was found in the organic N pool (nitrogen remaining after KCl treatment, see methods) after T₅ (Fig. 5). Following Tₛ, most enrichment was again found in the KCl extractable pool, especially in the
most enriched stations. We speculate that $^{15}$N was initially sorbed to the surface sediment ($T_0$), then assimilated by living algae and bacteria ($T_5$), and eventually transferred to a different pool ($T_{31}$) which probably consists of extractable organics (such as dead microbes). More studies using alternative approaches (e.g. Veuger et al., 2005) are needed to elucidate the dynamics of sediment nitrogen pools.

The particulate matter settling on the sediment surface (filter traps) was highly enriched in $^{15}$N after $T_0$ (Fig. 6a, b). Contrary to the sediment compartment, the enrichment in the settling particles was higher (up to 8 times) in September (Fig. 6b) than in May, especially on the creek bank. This suggests that relatively more sediment $^{15}$N was acquired directly from the dissolved $^{15}$NH$_4^+$ pool in May. Generally the settling PN was only enriched after the first tide, consistent with observations in the suspended particulate nitrogen pool (SPN) (Gribsholt et al., 2005, 2006). Enrichment ($\Delta^{15}$N) of settled particles was similar to that observed in SPN (up to 80 and 100% in May and September, respectively; Fig. 2c), except for the much higher September creek bank values. The total amount of $^{15}$N settling on the sediment surface was relatively similar among habitats in May (Fig. 6c), and 3–9 times higher than in September, except for the creek bank where 2.4±2.2 $\mu$mol $^{15}$N settled per m$^2$ after $T_0$–September (Fig. 6d).

The invertebrates collected from leaves, litter and sediment surfaces in September were identified into twelve groups classified according to their taxonomic class or order (Fig. 7a). Not all groups were represented on all stations and/or tides. Gastropods (Gastropoda-prosobranchia) and arachnids (Arachnida) were the only groups found at all stations and on most occasions. Significantly enriched $^{15}$N-values were only observed after $T_4$ in the sap-sucking aphids (Aphidae) at the willow St. 6 (37.0‰) and in one caterpillar (Lepidoptera) (31.1‰) from willow St. 4. Even in the biofilm-grazing gastropods clear enrichments were only observed on few occasions (Fig. 7b). Likewise, no significant $^{15}$N enrichment was observed in the macro-invertebrate infauna (Fig. 7c), which was numerically dominated by Tubificidae (5072±2692 m$^{-2}$) and Nematodae (1555±2837 m$^{-2}$). Specimens of Hirudines, Trichoptera, Lumbricidae and Talitridae were also present. Considerable heterogeneity occurred in natural abundance $^{15}$N values for all macro-invertebrates, and from our (limited) dataset no clear relationship between neither natural abundance $^{15}$N or subsequent $\Delta^{15}$N and habitat type or topographic level (inundation duration) could be determined for any macro-invertebrate group.

### 3.4 $^{15}$N mass balance and uptake rates

Overall, 79–135 and 53–126 mmol $^{15}$N was recovered in the marsh compartments in May and September, respectively (Table 4). On both occasions a similar small fraction (4%) of the added label was assimilated at $T_0$. Total marsh $^{15}$N pools, however, varied by more than a factor 2 among tides, and within compartments the $^{15}$N pool size varied by up to a factor 5 (September roots). In May the highest $^{15}$N content was observed after $T_5$. This increase was largely (87%) of the sediment uptake in May was due to sorption, but after $T_5$ microbial assimilation accounted for 81% (57 mmol) of the $^{15}$N uptake to the sediment compartment. After $T_{31}$ the organic pool decreased to 28%. The root and leaf compartments were
also more enriched after T₅ than after T₀ in May. In September, the highest total marsh enrichment was observed after T₁. This was mainly due to a high enrichment in the litter, accounting for 54% of the increase compared to T₀.

In May the sediment and the litter were the most important sinks for ¹⁵N accounting for 40–52% and 20–40% of the assimilated ¹⁵N, respectively. In September the litter was the most important pool accounting for 29–50% of marsh ¹⁵N assimilation, while only 5–16% was assimilated by the sediment and associated microbes. In total 1.2 mmol ¹⁵N settled on the marsh surface (September T₀ filter traps), corresponding to 0.1% of the added label or 13% of the T₀ sediment ¹⁵N content. Eight times more ¹⁵N was exported as suspended particulate matter (9.5 mmol) during T₀-September, than settled on the marsh surface (data not shown).

Average marsh ¹⁵N uptake rate (weighted by factors proportional to the area represented by each station) in the first tide (T₀) normalized to per hours inundation (hᵢ) was relatively similar in May (11.8 μmol ¹⁵N m⁻² h⁻¹, Fig. 8a) and September (12.6 μmol ¹⁵N m⁻² h⁻¹, Fig. 8b). On both occasions the total ¹⁵N uptake rate was higher in ruderal >reed>willow habitats. The creek bank habitat revealed the highest ¹⁵N uptake rates in May, but the lowest in September. This discrepancy was due to a high average sediment uptake rate (14.9 μmol ¹⁵N m⁻² h⁻¹) caused by high (39 μmol ¹⁵N m⁻² h⁻¹) uptake in the algal covered St. 10. Excluding St. 10 the total creek bank sediment uptake rate is reduced from 14.9 to 2.8 μmol ¹⁵N m⁻² h⁻¹ (and the average marsh uptake rate to 10.2 μmol ¹⁵N m⁻² h⁻¹), revealing a ranking in total uptake rate similar to September among habitat types (ruderal>reed>willow>creek bank). Within each habitat type, however, the relative importance of the four compartments (sediment, roots, leaves, and litter) varied among habitats as well as sampling occasions. While ¹⁵N assimilation by roots only occurred in the ruderal habitat in May, roots of both reed and ruderal assimilated ¹⁵N at a similar rate in September (the root compartment in the willow habitat was omitted for logistic reasons, as described previously). Uptake rate into litter in the reed habitat was similar among sampling occasions, but 2–6 times higher in September compared to May in the other habitats. Macrophyte uptake rate was generally low (<1.2 μmol ¹⁵N m⁻² h⁻¹), except in the ruderal habitat. On average (weighted according to habitat distribution) the relative contribution of the different compartments

Fig. 7. Isotopic value (δ¹⁵N) in (a) hand-picked invertebrates excluding gastropods (all stations), (b) gastropods collected from the 12 sampling stations, and (c) invertebrate infauna (all stations) on the four sampling occasions in September.

Fig. 8. Total ¹⁵N uptake rate (μmol m⁻² h⁻¹) (a, b) and corresponding absolute N uptake rate (c, d) normalized to per hour inundated in May and September. Results are based on T₀ ¹⁵N recovery and inundation duration (see text).
Table 4. $^{15}$N recovery in the marsh compartments after T$_{0}$, T$_{1}$, T$_{2}$ T$_{4/5}$ and T$_{29/31}$ in May and September. Numbers in parenthesis are percentage of the total amount of $^{15}$N added at T$_{0}$.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>T$_{0}$</th>
<th>T$_{1}$</th>
<th>T$_{2}$</th>
<th>T$_{4/5}$</th>
<th>T$_{29/31}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>May Marsh</td>
<td>79 (4.0)</td>
<td>nd</td>
<td>nd</td>
<td>135 (6.8)</td>
<td>98 (5.0)</td>
</tr>
<tr>
<td>Sediment</td>
<td>32 (1.6)</td>
<td>nd</td>
<td>nd</td>
<td>70 (3.5)</td>
<td>46 (2.3)</td>
</tr>
<tr>
<td>Roots</td>
<td>6 (0.3)</td>
<td>nd</td>
<td>nd</td>
<td>17 (0.9)</td>
<td>10 (0.5)</td>
</tr>
<tr>
<td>Leaves</td>
<td>8 (0.4)</td>
<td>nd</td>
<td>nd</td>
<td>20 (1.0)</td>
<td>19 (1.0)</td>
</tr>
<tr>
<td>Litter</td>
<td>33 (1.7)</td>
<td>nd</td>
<td>nd</td>
<td>27 (1.4)</td>
<td>23 (1.2)</td>
</tr>
<tr>
<td>Fauna</td>
<td>nd$^a$</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>September Marsh</td>
<td>56 (3.9)</td>
<td>126 (8.9)</td>
<td>53 (3.8)</td>
<td>57 (4.1)</td>
<td>68 (4.8)</td>
</tr>
<tr>
<td>Sediment</td>
<td>9 (0.7)</td>
<td>6 (0.4)</td>
<td>6 (0.4)</td>
<td>8 (0.6)</td>
<td>7 (0.5)</td>
</tr>
<tr>
<td>Roots</td>
<td>16 (1.1)</td>
<td>38 (2.7)</td>
<td>7 (0.5)</td>
<td>11 (0.8)</td>
<td>19 (1.4)</td>
</tr>
<tr>
<td>Leaves</td>
<td>5 (0.3)</td>
<td>18 (1.3)</td>
<td>14 (1.0)</td>
<td>12 (0.9)</td>
<td>21 (1.5)</td>
</tr>
<tr>
<td>Litter</td>
<td>26 (1.8)</td>
<td>64 (4.5)</td>
<td>26 (1.9)</td>
<td>25 (1.8)</td>
<td>20 (1.4)</td>
</tr>
<tr>
<td>Fauna</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Not determined.
$^b$ In May T$_{5}$ was sampled, while T$_{4}$ was sampled in September.
$^c$ In May T$_{31}$ was sampled, while T$_{29}$ was sampled in September.

was relatively similar between May and September, except that the root uptake was more important in September.

Total nitrogen uptake rate per hour inundated, estimated from the $^{15}$N uptake rate and taking the average degree of labelling in the ammonium pool (1.3% and 4.5% in May and September, respectively) and flooding duration at each station into account, varied greatly between May and September, mainly because of the differences in the degree of labelling of ammonium (Fig. 8c, d). Thus in May average marsh nitrogen uptake rate (908 µmol N m$^{-2}$ h$^{-1}$) was more than 3 times faster than in September (280 µmol N m$^{-2}$ h$^{-1}$).

4 Discussion

4.1 Whole ecosystem $^{15}$N labelling

Several studies have used deliberate $^{15}$N additions to trace nitrogen flow in freshwater (e.g., Kling, 1994; Peterson et al., 1997; Hamilton et al., 2001; Webster et al., 2003 and references therein) and estuarine (e.g. Hughes et al., 2000; Holmes et al., 2000; Tobias et al., 2003) ecosystems. In fringing marshes stable isotopes have been applied to elucidate the effects of ground water discharge on marsh nitrogen cycling (Tobias et al., 2001); however our study is the first to use this approach to elucidate the fate of watershed derived nitrogen in tidal marshes. Here, the label was added in a short pulse, mainly due to the constraints of periodic, two-directional water-flows and the complete drainage of the marsh between tides. While adequate for tracing short term processes such as nitrification (Gribsholt et al., 2005, 2006), the feasibility to trace transfer into higher trophic levels and to investigate long term (years) retention is limited. However, even within this relatively short period of labelling, a significant amount (4–9%) of the added tracer was assimilated and retained by the marsh biota. Moreover, the $^{15}$N label addition method allowed us to identify the microbial community (bacteria, algae, fungi) colonizing the surfaces of the sediment and plant litter as the main sink for watershed derived $^{15}$NH$^+$.$^4$. While higher organisms were less important for short term nitrogen retention, considerable species specific uptake was revealed, with ruderal vegetation being more important than reed per unit surface area.

The timing of the label addition was carefully selected based on the predicted tidal heights, and while there was very little difference in predicted and observed inundations in May, the September tides were much lower than predicted. Unfortunately this resulted in a significantly shorter marsh inundation time in September compared to May, and less contact between surfaces of standing vegetation, litter and sediment and labelled floodwater. Combined with low ambient ammonium concentration this also resulted in a relatively high degree of labelling (4.5%) and a substantial (73%) increase in the total average ammonium concentration in September. Thus the basic assumption (see below) that the added $^{15}$N label does not accelerate in situ rates but merely substitute for ambient $^{14}$N may not be entirely met in the September experiment and ammonium process rates may have been slightly accelerated (Gribsholt et al., 2006). We expect, however, any perturbation caused by this rela-
tively excessive label addition in September to be of minor importance, since ammonium is likely not limiting in this very nutrient-rich system. Furthermore, our assimilation estimates are prone to errors due to 1) heterogeneity in labelling degree owing to temporal heterogeneity in in situ ammonium concentrations (Fig. 2a); 2) within compartment heterogeneity in $^{15}\text{N}$ natural abundance values (which are subtracted to estimate enrichment ($\Delta\delta$)); 3) heterogeneity in standing stock (biomass and N wt%) estimates and 4) uncertainties in determination of relative coverage represented by each station. Nevertheless, the value of using in situ label additions to study ecosystem nutrient dynamics is that the processes can be examined in intact systems under ambient conditions, without the artefacts resulting from stimulation of process rates by temporarily increasing nutrient concentrations (nutrient enrichment studies) or artefacts associated with the use of enclosures (microcosm studies) (Schindler, 1998; Mulholland et al., 2000). Moreover, no a priori assumptions about the relative importance of compartments are required. Thus, rather than providing highly accurate process rates of a few quantitatively more or less important compartments, our study delivers semi-quantitative knowledge of the important ecosystem processes and compartments simultaneously.

Our results clearly revealed that microbial communities on the sediment surface and on plant litter contribute similarly to $^{15}\text{N}$ assimilation despite the predominance of macrophyte biomass (reed, ruderal and willow) and expected high nitrogen demand. The relatively low uptake by the vegetation likely reflects that nitrogen is not limiting their growth in these marshes fringing the heterotrophic, nutrient-rich Scheldt River (Van Damme et al., 2005; Soetaert et al., 2006). Plant nutrient uptake is usually also not the major pathway of nitrogen removal in most natural wetlands (Verhoeven and Van der Toorn, 1990) and especially not in high-nutrient treatment wetlands where it often accounts for only 1–4% of nutrient removal (e.g., Peterson and Teal, 1996; Huttunen et al., 1996; Brix, 1997). However, while macrophytes derive their nitrogen from different sources, our study only elucidates the direct assimilation and dependence on flood-water ammonium of marsh ecosystem compartments, and not the indirect, eventual nitrogen resources. Especially deeper-rooted species, such as the dominant reed, likely assimilate $\text{N}$ from deeper layers, which because of the short term nature of our study is much less enriched than the overlying water. Thus macrophyte retention is likely underestimated and macrophyte and tree tissues may be more important for long-term (months) retention (Drake et al., 2006).

Although direct uptake by vegetation generally played a minor role in short-term retention of watershed derived $\text{NH}_4^+$, the marsh plants are crucial for nitrogen cycling and marsh ecosystem functioning. Plants provide a large surface area for microbial growth, as well as a source of carbohydrates for microbial consumption (Brix, 1997). They release $\text{O}_2$ into the sediment promoting coupled nitrification-denitrification (Bodelier et al., 1996; Gribsholt and Kristensen, 2002), influence hydrology and promote sedimentation of particles and subsequent retention. Furthermore, most plant material produced is retained and decomposed by microbes within the marsh system. The presence of higher plants therefore has a significant but indirect impact on nitrogen cycling in tidal freshwater marshes.

### 4.2 Species composition and nutrient retention

Many wetlands are dominated by one or a few vascular plant species, and while the capacity of the strongest competitors such as reed ($P. \text{australis}$, the dominant plant of many European marshes) (Cronk and Fennessy, 2001) to extract nutrients from its environment has been the subject of numerous studies (e.g. Meulman et al., 2002), the importance of less abundant species is often overlooked as nutrient sinks in input-output studies of wetlands. Although direct $^{15}\text{N}$ uptake by vegetation was lower than expected given the high biomass, the isotopic $^{15}\text{N}$-tracer technique revealed interesting differences in species functionality. Both the limited importance of direct uptake (leaves and roots) in total $^{15}\text{N}$-processing and the species-specific $^{15}\text{N}$ enrichments of macrophytes confirm previous findings on a low order, forested stream (Ashkenas et al., 2004).

In our study the ruderal vegetation proved to be more important for (short term) nitrogen retention than previously assumed. On both occasions $^{15}\text{N}$ uptake into both leaves and roots was largely due to uptake by the tall, fast growing annual Policeman’s helmet. Other ruderal species were also enriched, while reed uptake was undetectable or low (Fig. 3). Apparently reed relies less on external and more on internal nitrogen resources than ruderal species, and/or nitrogen turnover rate is much slower in reed compared to ruderal. In addition to differences in life-history strategies, we speculate that higher $^{15}\text{N}$ uptake by ruderal vegetation (annuals) are influenced by a shallower root system in these species compared to reed, thus promoting contact with labelled nitrogen from the flood water. With their large, permanent root-rhizome system, reed assimilate and recycle nutrients from much deeper sediment layers throughout the year, while ruderals have to build up their entire root system from the sediment surface down during the growing season.

While a positive relation between the species richness of macrophytes and phosphorous retention has e.g. been reported for experimental ponds by Engelhardt and Ritchie (2001), it remains to be demonstrated whether species diversity enhances the long term nutrient retention in tidal freshwater marshes. Clearly, species diversity has a role in the short term assimilation of watershed-derived ammonium, but differences in internal recycling and release processes, litter decomposition and long term burial needs further attention. Furthermore, Policeman’s helmet is an exotic, invasive species and is expected to reduce species diversity and to out-compete native light-demanding species in riparian habitats (Naiman and Decamps 1997).
Table 5. $^{15}$N mass balance budget. Recovery in the various pools after $T_0$ in May 2002 and September 2003. Numbers in parenthesis are percentage of the total $^{15}$N added.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>May (mmol)</th>
<th>September (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracer input</td>
<td>1976 (100)</td>
<td>1409 (100)</td>
</tr>
<tr>
<td>$^{15}$N exported unchanged (as $^{15}$NH$_4$)</td>
<td>1370 (69)</td>
<td>715 (51)</td>
</tr>
<tr>
<td>$^{15}$N transformed</td>
<td>607 (31)</td>
<td>694 (49)</td>
</tr>
<tr>
<td>$^{15}$NO$_3$+$^{15}$NO$_2$</td>
<td>172 (8.7)</td>
<td>109 (7.7)</td>
</tr>
<tr>
<td>$^{15}$N$_2$O</td>
<td>0.13 (0.01)</td>
<td>0.2 (0.01)</td>
</tr>
<tr>
<td>$^{15}$N$_2$</td>
<td>0.11 (0.01)</td>
<td>7.9 (0.5)</td>
</tr>
<tr>
<td>SP$^{15}$N</td>
<td>9.6 (0.5)</td>
<td>9.5 (0.7)</td>
</tr>
<tr>
<td>Stored</td>
<td>79 (4.0)</td>
<td>56 (3.9)</td>
</tr>
<tr>
<td>Sediment</td>
<td>32 (1.6)</td>
<td>9 (0.7)</td>
</tr>
<tr>
<td>Leaves</td>
<td>8 (0.4)</td>
<td>5 (0.3)</td>
</tr>
<tr>
<td>Roots</td>
<td>6 (0.3)</td>
<td>16 (1.1)</td>
</tr>
<tr>
<td>Litter</td>
<td>33 (1.7)</td>
<td>26 (1.8)</td>
</tr>
<tr>
<td>Fauna</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Balance not accounted for</td>
<td>345 (17)</td>
<td>512 (36)</td>
</tr>
</tbody>
</table>

4.3 Immobilization on litter and sediment surfaces

The majority of $^{15}$N assimilated by the marsh ecosystem was recovered in the litter and surface sediment compartments. These compartments are dominated by micro-organisms and account for most of the N-assimilation: 70–83% and 41–62% in May and September, respectively. Higher organisms (macrofauna and macrophytes) contributed little to the short term $^{15}$N retention. Even during the active growing season (May) uptake by vegetation (roots and leaves) was trivial compared to microbial assimilation into the surface sediment and the litter compartment. This dominance of microorganisms in short-term nitrogen retention confirms previous findings on low order streams (Webster et al., 2003 and references therein; Ashkenas et al., 2004), although the order of retention among compartments may partly reflect the vertical stratification of the marsh ecosystem, with litter and surface sediments being exposed to a higher degree of labelling compared to the deeper sediment layers and macrophyte roots. Although the importance of microbes relative to macroorganisms could be expected in relatively pristine streams where adjacent macrophyte vegetation is not subject to flooding, this is far from self-evident in nutrient rich, diurnally flooded wetlands.

Immobilization on litter was quantitatively the most important sink for $^{15}$N. Plant litter provides an excellent substratum for microbial colonization, and increases the reactive surface area manifold. Due to its refractory composition, reed litter accumulates in these marshes, providing countless surfaces for biofilm development. Tracer immobilization on litter and sediment may, however, be due to both microbial (bacteria, algae and fungi) assimilation and physical sorption. Our sediment KCl extractions suggest that active assimilation by microbes is important, and we speculated the same is true for the litter. Similarly, external N incorporation into decaying Spartina alterniflora has been demonstrated to be at least partly due to biological incorporation (White and Howes, 1994a). The fact that label recovery changes only little over the tides subsequent to addition further supports active incorporation rather than physical sorption alone. In a laboratory $^{15}$N dilution study Bowden (1986) found that litter was a net sink for ammonium with immobilization exceeding mineralization under aerobic conditions.

4.4 Whole ecosystem $^{15}$N budget

The combined data set presented in this and companion papers (Gribsholt et al., 2005, 2006) allows us to establish an integral marsh ecosystem nitrogen processing budget (Table 5). Here we present only the total budget after $T_0$, as the cumulative budgets changes only slightly after subsequent tides (Gribsholt et al., 2005, 2006). In both May and September the majority of $^{15}$NH$_4$ added was exported with the outgoing tide (69 and 51% in May and September, respectively). Nitrification was the most important transformation pathway, accounting for 8.7 and 7.7% of the added label, corresponding to 30 and 17% of the $^{15}$N-transformation in May and September, respectively. A comparison between whole-system nitrification estimates and water-column nitrification rates revealed that most (>70%) of the nitrification was associated with the marsh surface. Moreover, sedimentary denitrification was identified to be significant in September, while short term assimilation accounted for a minor fraction (~4% after $T_0$) of added label (Table 5). Consequently, in terms of nitrogen processing marsh surfaces appear more
important as habitats for nitrifiers and denitrifiers than for nitrogen assimilating organisms.

The relative importance of the litter and surface sediments for $^{15}$N assimilation (see above) are consistent with these findings. Rather than direct uptake by macrophytes (leaves and roots), it is the large reactive surface area (and carbon source) provided by the tidal freshwater marsh vegetation (standing or litter) that is most crucial for the functioning of these ecosystems both when it comes to nitrogen transformation and short term retention of flood water derived ammonium. The transformation rates and pathways of flood water derived nitrate may, however, differ substantially (Gribsholt et al., 2006). And although we clearly identified microbes to govern short-term nitrogen retention in tidal marshes, our whole ecosystem labelling study does not allow us to elucidate in detail the dynamics within the microbial compartments; e.g. we do not know whether eukaryotes (benthic algae or fungi) or prokaryotes contribute most to nitrogen retention. Our next step will be to quantify the relative roles of benthic algae and bacteria in marsh nitrogen retention and to study the long-term retention of nitrogen in tidal marsh systems.

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