HAL
open science

# Nitrogen assimilation and short term retention in a nutrient-rich tidal freshwater marsh ? a whole ecosystem 15N enrichment study

B. Gribsholt, E. Struyf, A. Tramper, L. de Brabandere, N. Brion

## To cite this version:

B. Gribsholt, E. Struyf, A. Tramper, L. de Brabandere, N. Brion. Nitrogen assimilation and short term retention in a nutrient-rich tidal freshwater marsh ? a whole ecosystem 15N enrichment study. Biogeosciences Discussions, 2006, 3 (4), pp.1081-1119. hal-00297835

**HAL Id: hal-00297835**

**https://hal.science/hal-00297835v1**

Submitted on 18 Jun 2008

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biogeosciences Discuss., 3, 1081–1119, 2006
www.biogeosciences-discuss.net/3/1081/2006/
© Author(s) 2006. This work is licensed
under a Creative Commons License.

Biogeosciences Discussions

*Biogeosciences Discussions* is the access reviewed discussion forum of *Biogeosciences*

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

**B. Gribsholt[1], E. Struyf[2], A. Tramper[1], L. De Brabandere[3,*], N. Brion[3], S. van Damme[2], P. Meire[2], F. Dehairs[3], J. J. Middelburg[1], and H. T. S. Boschker[1]**

[1]Netherlands Institute of Ecology, Center for Estuarine and Marine Ecology, P.O. Box 140, 4400AC Yerseke, The Netherlands
[2]University of Antwerp, Department of Biology, Environmental Management Research Group, Universiteitsplein 1C, 2610 Wilrijk, Belgium
[3]Vrije Universiteit Brussel, Department of Analytical and Environmental Chemistry, Pleinlaan 2, 1050 Brussel, Belgium
[*]now at: University of Florida, Department of Fisheries and Aquatic Sciences, 7922 NW 71st Street, Gainesville FL 32653, USA

Received: 18 May 2006 – Accepted: 6 June 2006 – Published: 24 July 2006

Correspondence to: B. Gribsholt (b.gribsholt@nioo.knaw.nl)

## Abstract

We conducted two (May 2002 and September 2003) pulse additions of $^{15}NH_4^+$ to the flood water inundating a tidal freshwater marsh fringing the nutrient-rich Scheldt River (Belgium) and traced the fate of ammonium in the intact ecosystem. Here we report in detail the $^{15}N$ uptake into the various marsh components (leaves, roots, sediment, leaf litter and invertebrate fauna), and the $^{15}N$ retention on a scale of 15 days. We particularly focus on the contributions of the rooted macrophytes and the microbial community in the sediment and on plant litter. Assimilation and short term retention of $^{15}NH_4^+$ was low on both occasions. Only 4–9% of the added $^{15}N$ trace was assimilated, corresponding to 13–22% and 8–18% of the processed $^{15}N$ (i.e. not exported as $^{15}NH_4^+$) in May and September, respectively. In May nitrogen assimilation rate (per hour inundated) was >3 times faster than in September. Macrophytes (above- and below ground) were of limited importance for short term $^{15}N$ retention accounting for <6% of the total $^{15}NH_4^+$ processed by the marsh. The less dominant herbaceous species were more important (on an area basis) than the dominant reed (*Phragmites australis*). The microbial community colonizing the sediment and litter surfaces were responsible for most nitrogen assimilation and short-term retention in the marsh. The large reactive surface area available for microbial colonization together with direct plant uptake, are the crucial components for nitrogen assimilation, retention and transformation in nutrient-rich tidal freshwater marshes.

## 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 at-

tenuate 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 about nitrogen dynamics in tidal freshwater marshes 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), 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 season-

ally 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 (30 and 17% in May and September, respectively), and the large reactive surface area of the marsh played a crucial role in nitrogen transformation. A significant part of the added $^{15}N\text{-}NH_4^+$ was assimilated and stored within the marsh. Collectively, the different marsh compartments (sediment, root, leaves and litter) accounted for 8–22% of the $^{15}N$-transformations (Gribsholt et al., 2005; 2006). In this paper we report in detail the $^{15}N$ uptake into these different marsh compartments and subsequent $^{15}N$ retention on a time scale of days, with particular focus on the relative importance of rooted macrophytes and the microbial community. Our results show that short term (days) 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). Although the vegetation cover is very dense with reed and Policeman's helmet growing to heights of up to 4 m, benthic microalgal mats (dominated by filamentous yellow-green algae *Vaucheria* sp.) were a conspicuous feature on the sediment surface, particularly in the creek bank and willow sites during early summer. Twelve sampling stations were placed within the study site (Fig. 1), three within each vegetation types (reed, willow and ruderal) and three in the unvegetated creek banks (four habitat types in total). Stations were chosen to represent different distances from the labelling platform as well as differences in elevation within each of the four habitats (Table 1). All stations were made accessible by walking boards, keeping disturbance of the marsh during sampling to a minimum.

## 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 $^{15}N\text{-}NH_4^+$ was added while 1.41 mol $^{15}N\text{-}NH_4^+$ 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 $(^{15}NH_4)_2SO_4$

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.

## 2.3 Marsh sampling and analysis

### 2.3.1 $^{15}N$ and total nitrogen

Marsh stations were sampled before labelling ($T_{-2}$) to establish natural abundance levels of $^{15}N$ and just after labelling ($T_0$). In May samples were also collected after two subsequent tides ($T_5$ and $T_{31}$), while $T_1$, $T_2$, $T_4$ and $T_{29}$ were sampled in September. The subscript denotes the tide relative to tracer addition. 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 $^{15}N$ content.

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. In May, a sub-sample (~4 g) of the surface sediment was immediately transferred to 10 ml 2 M KCl and 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 was inferred from $N_{tot}=N_{sob}+N_{org}$; as-

suming $N_{org}$ equals nitrogen remaining after KCl extraction, where $N_{tot}$ is total nitrogen determined in untreated samples, $N_{sob}$ and $N_{org}$ are sorbed and organic nitrogen (assuming that all immobilization is due to microbial incorporation), respectively.

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 three specimens (per station) out of the sediment. Triplicates were pooled after separating leaves and roots. Reed top shoots were collected separately (St. 1–3; May only), while the two most dominant species were sampled separately from ruderal stations (St. 7–9). 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 sp.*) 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 $\mu$m, filled with local litter at $T_{-2}$) were collected at stations 2, 4 and 7 at $T_{-2}$, $T_0$ and $T_5$.

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 $m^2$ at each station were identified to taxonomic class or order and analyzed separately for $^{15}N$. Abundances were not quantified.

All samples described above were analyzed for isotopic composition and total nitrogen. Sub-samples of (freeze) dried leaves, roots, and sediment were grinded to a fine powder before total nitrogen analysis on a Carlo Erba Elemental Analyzer EA following Nieuwenhuize et al. (1994), and $^{15}N$ analysis on a Fisons elemental analyzer (EA-1500) coupled on line, via a Finnigan CONFLO 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$^{PLUS}$XL mass spectrometer connected on line to an elemental analyzer (EA, Flash series 1112) via a continuous flow interface (Finnigan Conflo 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 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, unpubl.). 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 analy-

sis. 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

Advective water fluxes in and out of the study area were determined for all the tides except Sept $T_{29}$ (Gribsholt et al. 2005, 2006). Creek water nitrogen concentrations and $^{15}N$ in dissolved ($NH_4^+$, $NO_3^-$+$NO_2^-$, $N_2O$ and $N_2$) 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. (May 2005; September 2006).

### 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 $T_{(-2)}$. 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 ($\mu mol\,^{15}N\,m^{-2}\,h_i^{-1}$) during $T_0$ flooding were calculated by dividing the amount of $^{15}N$ recovered ($\mu mol\,^{15}N\,m^{-2}$) 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 ($\mu mol\,N\,m^{-2}\,h_i^{-1}$) were determined by dividing the $^{15}N$ uptake rate by the average percentage of $^{15}N$ labelling of the floodwater ammonium pool.

## 3 Results

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 1m. 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 were found on the sediment surface es-

pecially on creek bank and willow sites, and creek banks were covered by watercress. 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$^{-2}$.

### 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). Thus, only the main differences and similarities between the two campaigns will be highlighted here 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 $\mu$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 May (0.64±0.16%) and September (0.61±0.14%), and surface sediment pool size (0–0.5 cm) was similar between 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 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 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 between habitats.

### 3.3 Marsh $^{15}N$ labelling

Isotopic enrichment ($\Delta\delta^{15}N$, Fig. 3), as well as the absolute amount of $^{15}N$ assimilated (excess $^{15}N$) 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 $^{15}N$ 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 top shoots 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 $^{15}N$-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 $^{15}N$ content per unit area was one order of magnitude higher in ruderal (24.3±11.0 $\mu$mol $m^{-2}$) compared to reed habitats (2.2±2.2 $\mu$mol $m^{-2}$) in May (Fig. 4a). Patterns were similar but less clear in September, due to large heterogeneity in the reed $^{15}N$ content between 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 $^{15}N$ 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

$^{15}N$ in *in situ* litter and litter incubated in litterbags (data not shown). Litter $^{15}N$ 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 between tides in both May and September. No $^{15}N$ enrichment was detected in deeper sediment layers and only data for the top layer are reported. While the sediment $^{15}N$ enrichment and total $^{15}N$ was similar (and low) in all habitats in September (Figs. 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 $^{15}N$ 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 $T_0$ the $^{15}N$ enrichment to the sediment was largely due to sorption while almost all of the $^{15}N$ was found in the organic N pool (remaining after KCl treatment, see methods) after $T_5$ (Fig. 5). Following $T_{31}$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 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 $T_0$ (Figs. 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 (SPN) (Gribsholt et al. 2005, 2006). Enrichment ($\Delta\delta^{15}$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 $\delta^{15}$N-values were only observed after $T_4$ in the sap-sucking aphids (Aphididae) 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, Lumibricidae 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 $\Delta\delta^{15}$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 increase) due to very high (total) sediment uptake in the willow St. 4 and creek bank St. 10, where a thick algal mat covered the sediment surface. Initially most (81%) 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 $T_5$ than after $T_0$ in May. In September, the highest total marsh enrichment was observed after $T_1$. This was mainly due to a high enrichment in the litter, accounting for 54% of the increase compared to $T_0$.

In May the sediment and the litter were the most important sinks for $^{15}N$ accounting for 40–52% and 20–40% of the assimilated $^{15}N$, respectively. In September the litter was the most important pool accounting for 29–50% of marsh $^{15}N$ assimilation, while only 5–16% was assimilated by the sediment and associated microbes. In total 1.2 mmol $^{15}N$ settled on the marsh surface (September $T_0$ filter traps), corresponding to 0.1% of the added label or 13% of the $T_0$ sediment $^{15}N$ content. Eight times more $^{15}N$ was exported as suspended particulate matter (9.5 mmol) during $T_0$-September, than settled on the marsh surface (data not shown).

Average marsh $^{15}N$ uptake rate (weighted by factors proportional to the area represented by each station) in the first tide ($T_0$) normalized to per hours inundation ($h_i$) was relatively similar in May (11.8 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$, Fig. 8a) and September (12.6 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$, Fig. 8b). On both occasion the total $^{15}N$ uptake rate was higher in ruderal > reed > willow habitats. The creek bank habitat revealed the highest $^{15}N$ uptake rates in May, but the lowest in September. This discrepancy was due to a high average sediment uptake rate (14.9 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$) caused by high (39 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$) 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 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$ (and the average marsh uptake rate to 10.2 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$), 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 $^{15}N$ assimilation by roots only occurred in the ruderal habitat in May, roots of both reed and ruderal assimilated $^{15}N$ 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 $\mu$mol $^{15}N\,m^{-2}\,h_i^{-1}$), except in the ruderal habitat. On average (weighted according to habitat distribution) the relative contribution of the different compartments 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 (Figs. 8c, d). Thus in May average marsh nitrogen uptake rate (908 $\mu$mol $N\,m^{-2}\,h_i^{-1}$) was more than 3 times faster than in September (280 $\mu$mol $N\,m^{-2}\,h_i^{-1}$).

## 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 at 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 relatively 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

$^{15}N$ 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. Our results clearly revealed that microbial communities on the sediment surface and on plant litter contribute similarly to $^{15}N$ 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, 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, 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 for carbohydrates for microbial consumption (Brix, 1997). They release $O_2$ 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. 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. Meulenman et al., 2002), the importance of less abundant species is often overlooked as nutrient sinks in input-output studies of wetlands. Although direct $^{15}$N uptake by vegetation was lower than expected given the high biomass, the isotopic $^{15}$N-tracer technique revealed interesting differences in species functionality. Both the limited importance of direct uptake (leaves and roots) in total $^{15}$N-processing and the species-specific $^{15}$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 $^{15}$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 $^{15}$N uptake by ruderal vegetation are influenced by a shallower root system in these species compared to reed, thus promoting contact with labelled nitrogen from the flood water.

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).

### 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 micro-organisms in short-term nitrogen retention confirms previous findings on low order streams (Webster et al., 2003, and references therein; Ashkenas et al., 2004). But while 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 areas 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.

## 5 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 nitrogen retention. 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.

*Acknowledgements.* This research was supported by the Flemish-Dutch co-operation on Coastal Research (VLANEZO) under the Flemish and Dutch Science Foundations FWO-NWO, and by the "Scientific Research Community" FWO project "Ecological characterization of European estuaries, with emphasis on the Scheldt estuary". We thank the staff at the NIOO-CEME analytical laboratory and at the Testing Laboratory for Chemical Water Quality, Department of Biology, University of Antwerp for their assistance. This is publication nr 3883 from the NIOO-KNAW, Yerseke.

## References

Ashkenas, L. R., Johnson, S. L., Gregory, S. V., Tank, J. L., and Wollheim, W. M.: A stable isotope tracer study of nitrogen uptake and transformation in an old-growth forest stream, Ecology, 85, 1725–1739, 2004.

Bodelier, P. L. E., Libochant, J. A., Blom, C. W. P. M., and Laanbroek, H. J.: Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats, Appl. Environ. Microbiol., 62, 4100–4107, 1996.

Bowden, W. B.: Nitrification, nitrate reduction, and nitrogen immobilization in a tidal freshwater marsh sediment, Ecology, 67, 88–99, 1986.

Bowden, W. B., Peterson, B. J., Hobbie, J. E., Steudler, P. A., and Moore III, B.: Transport and processing of nitrogen in a tidal freshwater wetland, Water Resour. Res., 27, 389–408, 1991.

Brix, H.: Do macrophytes play a role in constructed treatment wetlands?, Water Sci. Technol., 35, 11–17, 1997.

Cronk, J. K. and Fennessy, M. S.: Wetland plants. Biology and Ecology, Lewis Publisher, Boca Raton, USA, 2001

Drake, D. C., Naiman, R. J., and Bechthold, J. S.: Fate of nitrogen in riparian forest soils and trees: An $^{15}$N tracer study simulating salmon decay, Ecology, 87, 1256–1266, 2006.

Engelhardt, K. M. A. and Ritchie, M. E.: Effects of macrophyte species richness on wetland ecosystem functioning and services, Nature, 411, 687–689, 2001.
Gribsholt, B. and Kristensen, E.: Effects of bioturbation and plant roots on salt marsh bioturbation: a mesocosm study. Mar. Ecol. Prog. Ser., 241, 71–87, 2002.
Gribsholt, B., Boschker, H. T. S., Struyf, E., Andersson, M., Tramper, A., De Brabandere, L., Van Damme, S., Brion, N., Meire, P., Dehairs, F., Middelburg, J. J., and Heip, C.: Nitrogen processing in a tidal freshwater marsh: a whole ecosystem $^{15}$N labeling study, Limnol. Oceanogr., 50, 1945–1959, 2005.
Gribsholt, B., Struyf, E., Tramper, A., Andersson, M. G. I., Brion, N., De Brabandere, L., Van Damme, S., Meire, P., Middelburg, J. J., Dehairs, F., and Boschker, H. T. S.: Ammonium transformation in a nitrogen-rich tidal freshwater marsh, Biogeochem., in press, 2006.
Hamilton, S. K., Tank, J. L., Raikow, D. F., Wollheim, W. M., Peterson, B. J., and Webster, J. R.: Nitrogen uptake and transformation in a midwestern U.S. stream: A stable isotope enrichment study, Biogeochem., 54, 297–340, 2001.
Hansson, L-L., Brönmark, C., Nilsson, P. A., and Åbjörnsson, K.: Conflicting demands on wetland ecosystem services: nutrient retention, biodiversity or both?, Freshw. Biol., 50, 705–714, 2005.
Holmes, R. M., Peterson, B. J., Deegan, L. A., Hughes, J. E., and Fry, B.: Nitrogen biogeochemistry in the oligohaline zone of a New England estuary, Ecology, 81, 416–432, 2000.
Hughes, J. E., Deegan, L. A., Peterson, B. J., Holmes, R. M., and Fry, B.: Nitrogen flow through the food web in the oligohaline zone of a New England estuary, Ecology, 81, 433–452, 2000.
Huttunen, A.: Heikkinen, K., and Ihme, R.: Nutrient retention in the vegetation of an overland flow treatment system in northern Finland, Aquat. Bot., 55, 61–73, 1996.
Jeffrey, S. W. and Humphrey, G. F.: New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton, Biochem. Physiol. Pflanzen, 167, 191–194, 1975.
Kling, G. W.: Ecosystem-scale experiments. The use of stable isotopes in fresh waters, in: Environmental Chemistry of lakes and reservoirs, edited by: Baker, L. A., Am. Chem. Soc., 91–120, 1994.
Merrill, J. Z. and Cornwell, J.: The role of oligohaline marshes in estuarine nutrient cycling, in: Concepts and controversies in tidal marsh ecology, edited by: Weinstein, M. P. and Kreeger, D. A., Dordrecht, The Netherlands, 425–441, 2000.
Meuleman, A. F. M., Beekman, J. P., and Verhoeven, J. T. A: Nutrient retention and nutrient-use

efficiency in *Phragmites australis* stands after wastewater application, Wetlands, 22, 712–721, 2002.
Mitsch, J. M. and Gosselink, J. G.: The value of wetlands: importance of scale and landscape setting, Ecological Economics, 35, 25–33, 2000.
Mulholland, P. J., Tank, J. L., Sanzone, D. M., Wollheim, W. M., Peterson, B. J., Webster, J. R., and Meyer, J. L: Nitrogen cycling in a forest stream determined by a $^{15}$N tracer addition, Ecol. Monogr., 70, 471–493, 2000.
Neiman, R. J. and Decamps, H.:, The ecology of interfaces: Riparian zones, Annu. Rev. Ecol. Syst., 28, 621–658, 1997.
Nieuwenhuize, J., Maas, Y. E. M., and Middelburg, J. J.: Rapid analysis of organic carbon and nitrogen in particulate materials, Mar. Chem., 45, 217–224, 1994.
Odum, W. E.: Comparative ecology of tidal freshwater and salt marshes, Annu. Rev. Ecol. Syst., 19, 147–176, 1988.
Peterson, S. B. and Teal, J. M.: The role of plants in ecologically engineered wastewater treatment systems, Ecol. Eng., 6, 137–148, 1996.
Peterson, B. J., Bahr, M., and Kling, G. W.: A tracer investigation of nitrogen cycling in a pristine tundra river, Can. J. Fish. Aquat. Sci., 54, 2361–2367, 1997.
Rijstenbil, J. W.: Effects of UVB radiation and salt stress on growth, pigments and antioxidative defense of the marine diatom *Cylindrotheca closterium*, Mar. Ecol. Prog. Ser., 254, 37–48, 2003.
Schindler, D. W.: Replication versus realism: the need for ecosystem-scale experiments, Ecosystems, 1, 323–334, 1998.
Soetaert, K., Middelburg, J. J., Heip, C., Meire, P., Van Damme, S., and Maris, T.: Long-term change in dissolved inorganic nutrients in the heterotrophic Scheldt estuary (Belgium, the Netherlands), Limnol. Oceanogr., 51, 409–423, 2006.
Tobias, C. R., Anderson, I. C., Canuel, E. A., and Macko, S. A.: Nitrogen cycling through a fringing marsh-aquifer ecotone, Mar. Ecol. Prog. Ser., 210, 25–39, 2001a
Tobias, C. R., Macko, S. A, Anderson, I. C., Canuel, E. A., and Harvey J. W.: Tracking the fate of a high concentration groundwater nitrate plume through a fringing marsh: A combined groundwater tracer and an in situ isotope enrichment study, Limnol. Oceanogr., 46, 1977–1989, 2001b.
Tobias, C. R., Cieri, M., Peterson, B. J., Deegan, L. A., Vallino, J., and Hughes, J. E.: Processing watershed-derived nitrogen in a well-flushed New England estuary, Limnol. Oceanogr., 48,

1766–1778, 2003.
Van Damme, S., Struyf, E., Maris, T., Ysebaert, T., Dehairs, F., Tackx , M., Heip, C., and Meire, P.: Spatial and temporal patterns of water quality along the estuarine salinity gradient of the Scheldt estuary (Belgium and The Netherlands): results of an integrated monitoring approach, Hydrobiologia, 540, 29–45, 2005.
Verhoeven, J. T. A. and Van der Toorn, J.: Marsh eutrophication and wastewater treatment, in: Wetlands and Shallow Continental water bodies, edited by: Patten, B. C., SPB Academic Publishing, The Hague, The Netherlands, 1, 571–585. 1990.
Verhoeven, J. T .A., Whigham, D. F., van Logtestijn, R., and O'Neill, J.: A comparative study of nitrogen and phosphorous cycling in tidal and non-tidal riverine wetlands, Wetlands, 21, 210–222, 2001.
Webster, J. R., Mulholland, P. J., Tank, J. L., Valett, H. M., Dodds, W. K., Peterson, B. J., Bowden, W. B., Dahm, C. N., Findlay, S., Gregory, S. V., Grimm, N. B., Hamilton, S. K., Johnson, S. L., Marti, E., McDowell, W. H., Meyer, J. L., Morrall, D. D., Thomas, S. A., and Wollheim, W. M.: Factors affecting ammonium uptake in streams – an inter-biome perspective, Freshw. Biol., 48, 1329–1352, 2003.
White, D. S. and Howes, B. L.: Nitrogen incorporation into decomposing litter of *Spartina alterniflora*, Limnol. Oceanogr., 39, 133–140, 1994a.
White, D. S. and Howes, B. L.: Long-term $^{15}$N-nitrogen retention in the vegetated sediments of a New England salt marsh, Limnol. Oceanogr., 39, 1878–1892, 1994b.

**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.

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

[a] Above-ground biomass only and willows and benthic microalgae are excluded.
[b] Biomass of understory ruderals (e.g., Policeman's Helmet (*I. glandulifera*)).
[c] Biomass of watercress *(Rorippa sp.)* not included.

**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 $T_5$, where stations were sampled at $T_4$.

| Tide | Flood (min) | | Ebb (min) | | Area inundated (%) | | Water height (cm) | | Flood ($m^3$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | May[a] | Sept[b] | May | Sept | May | Sept | May | Sept | May | Sept |
| $T_0$ | 80 | 77 | 131 | 107 | 100 | 98 | 125 | 103 | 1823 | 911 |
| $T_1$ | 77 | 71 | 119 | 103 | 100 | 95 | 117 | 95 | 1700 | 667 |
| $T_5$ | 78 | 58 | 139 | 78 | 100 | 78 | 129 | 81 | 1912 | 307 |
| $T_{31}$ | 76 | nd[c] | 106 | nd | 100 | nd | 100 | nd | 900 | nd |

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

**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.

| | | Nitrogen ($g\,m^{-2}$) | | | | |
|---|---|---|---|---|---|---|
| | Habitat | Sediment | Root | Leaves | Litter | Total |
| May | *Marsh*[a] | 12.9 (35) | 8.5 (23) | 11.3 (30) | 4.6 (12) | 37 |
| | Reed | 12.3±0.9 | 17.1±1.7 | 17.7±3.4 | 7.7 | 55 |
| | Willow | 14.0±4.3 | 1.1±0.3[b] | 2.6 ±3.8[b] | 0.8 | 18 |
| | Ruderal | 11.6±0.7 | 2.7±1.8 | 17.5±10.4 | 1.9 | 34 |
| | Creek | 13.6±4.4 | 0 | 0.3±0.0[c] | 0.8 | 15 |
| September | *Marsh*[a] | 13.0 (30) | 10.3 (24) | 15.6 (36) | 4.6 (11) | 44 |
| | Reed | 12.5±1.1 | 20.2±1.7 | 26.2±8.2 | 5.9±0.4 | 65 |
| | Willow | 13.5±3.7 | n.d | n.d | 3.4±1.2 | 17 |
| | Ruderal | 12.5±0.3 | 9.1±3.5 | 9.3±3.1 | 4.7±1.6 | 36 |
| | Creek | 12.6±4.0 | 0 | 0 | 2.6±2.0 | 15 |

[a] Area-weighted average.
[b] Policeman's Helmet (*I. glandulifera*) only.
[c] Watercress (*Rorippa sp.*).

**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 $T_0$.

| | | $^{15}N$ (mmol) | | | | |
|---|---|---|---|---|---|---|
| | Habitat | $T_0$ | $T_1$ | $T_2$ | $T^b_{4/5}$ | $T^c_{29/31}$ |
| May | *Marsh* | 79 (4.0) | nd | nd | 135 (6.8) | 98 (5.0) |
| | Sediment | 32 (1.6) | nd | nd | 70 (3.5) | 46 (2.3) |
| | Roots | 6 (0.3) | nd | nd | 17 (0.9) | 10 (0.5) |
| | Leaves | 8 (0.4) | nd | nd | 20 (1.0) | 19 (1.0) |
| | Litter | 33 (1.7) | nd | nd | 27 (1.4) | 23 (1.2) |
| | Fauna | nd[a] | nd | nd | nd | nd |
| September | *Marsh* | 56 (3.9) | 126 (8.9) | 53 (3.8) | 57 (4.1) | 68 (4.8) |
| | Sediment | 9 (0.7) | 6 (0.4) | 6 (0.4) | 8 (0.6) | 7 (0.5) |
| | Roots | 16 (1.1) | 38 (2.7) | 7 (0.5) | 11 (0.8) | 19 (1.4) |
| | Leaves | 5 (0.3) | 18 (1.3) | 14 (1.0) | 12 (0.9) | 21 (1.5) |
| | Litter | 26 (1.8) | 64 (4.5) | 26 (1.9) | 25 (1.8) | 20 (1.4) |
| | Fauna | 0 | 0 | 0 | 0 | 0 |

[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.

**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.

| Compartment | May (mmol) | | September (mmol) | |
|---|---|---|---|---|
| Tracer input | 1976 | (100) | 1409 | (100) |
| $^{15}N$ exported unchanged (as $^{15}NH_4$) | 1370 | (69) | 715 | (51) |
| $^{15}N$ transformed | 607 | (31) | 694 | (49) |
| $^{15}NO_3$+$^{15}NO_2$ | 172 | (8.7) | 109 | (7.7) |
| $^{15}N_2O$ | 0.13 | (0.01) | 0.2 | (0.01) |
| $^{15}N_2$ | 0.11 | (0.01) | 7.9 | (0.5) |
| SP$^{15}N$ | 9.6 | (0.5) | 9.5 | (0.7) |
| Stored | 79 | (4.0) | 56 | (3.9) |
| Sediment | 32 | (1.6) | 9 | (0.7) |
| Leaves | 8 | (0.4) | 5 | (0.3) |
| Roots | 6 | (0.3) | 16 | (1.1) |
| Litter | 33 | (1.7) | 26 | (1.8) |
| Fauna | – | – | 0 | – |
| Balance not accounted for | 345 | (17) | 512 | (36) |

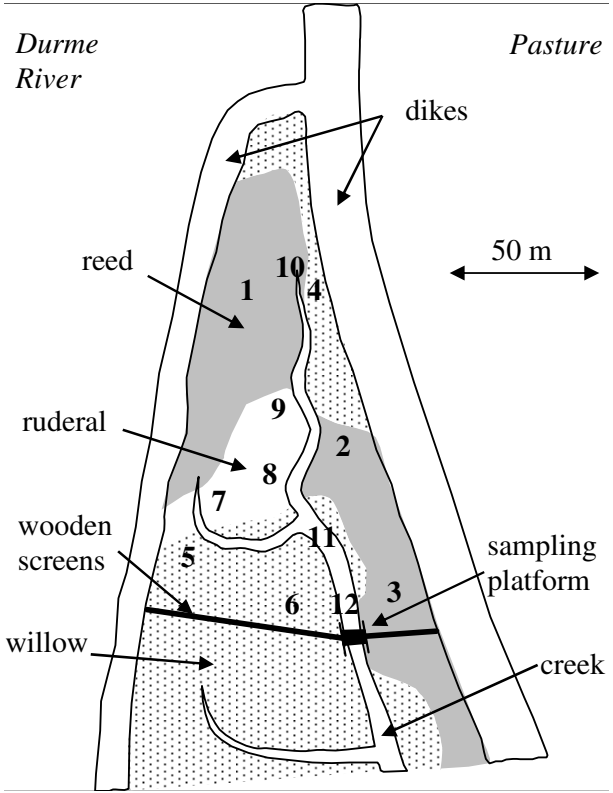


**Fig. 1.** Experimental marsh with vegetation distribution. Numbers represent sampling stations, with three station in each of the four habitats: Reed (St. 1–3), Willow (St. 4–6), Ruderal (St. 7–9) and unvegetated creek bank (St. 10–12).

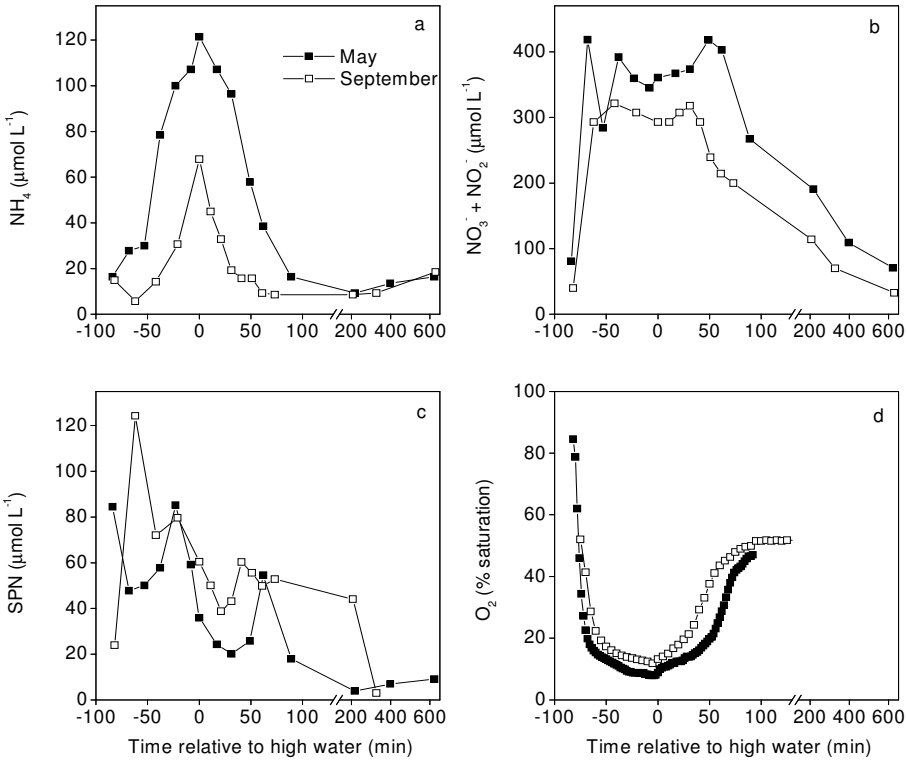


**Fig. 2.** **(a)** dissolved ammonium **(b)**, dissolved nitrate + nitrite, **(c)** suspended particulate nitrogen (SPN), and $O_2$ saturation during $T_0$ (tracer addition) in May and September. Only data from $T_0$ are shown, as the temporal patterns of all parameters were quite similar among tides on both occasions. (For details see Gribsholt et al., 2005, 2006).

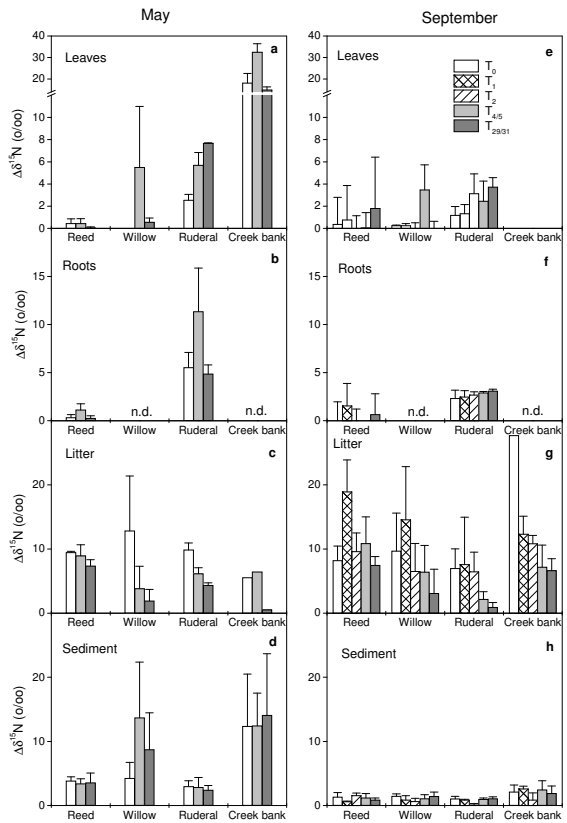


**Fig. 3.** Isotopic enrichment ($\Delta\delta$) (above natural abundance levels) in the main marsh compartments **(a, e)** leaves, **(b, f)** roots, **(c, g)** litter and **(d,h)** sediment in the four habitats (reed, willow, ruderal and creek bank) during May and September (Mean ± SE, n=3; n.d.: not determined). $T_1$ and $T_2$ were only sampled in September. In May $T_5$ and $T_{31}$ were sampled, while $T_4$ and $T_{29}$ were sampled in September. Note the different scales on the y-axis.

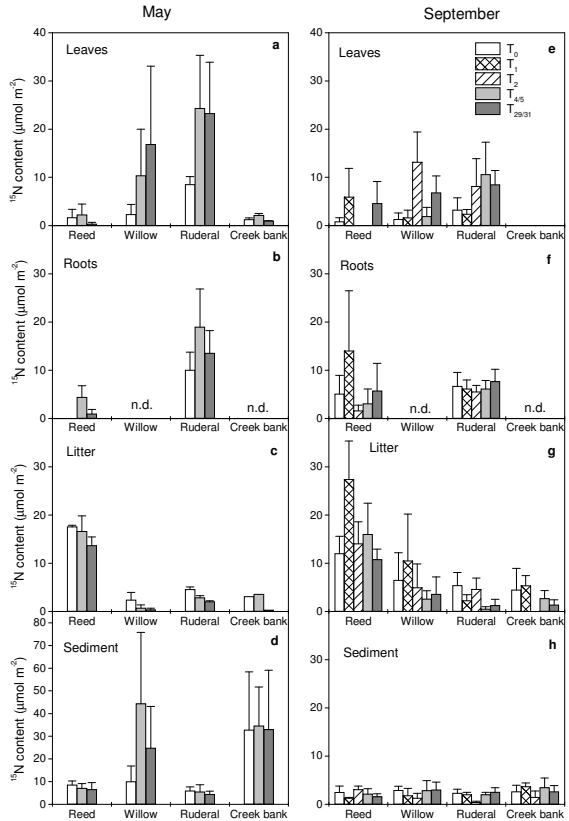


**Fig. 4.** Amount of $^{15}$N (excess $^{15}$N) per unit surface area ($\mu$mol m$^{-2}$) recovered in the four main compartments **(a, e)** leaves, **(b, f)** roots, **(c, g)** litter and **(d, h)** sediment in the four marsh habitats (reed, willow, ruderal and creek bank) during May and September (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 panels d and h.

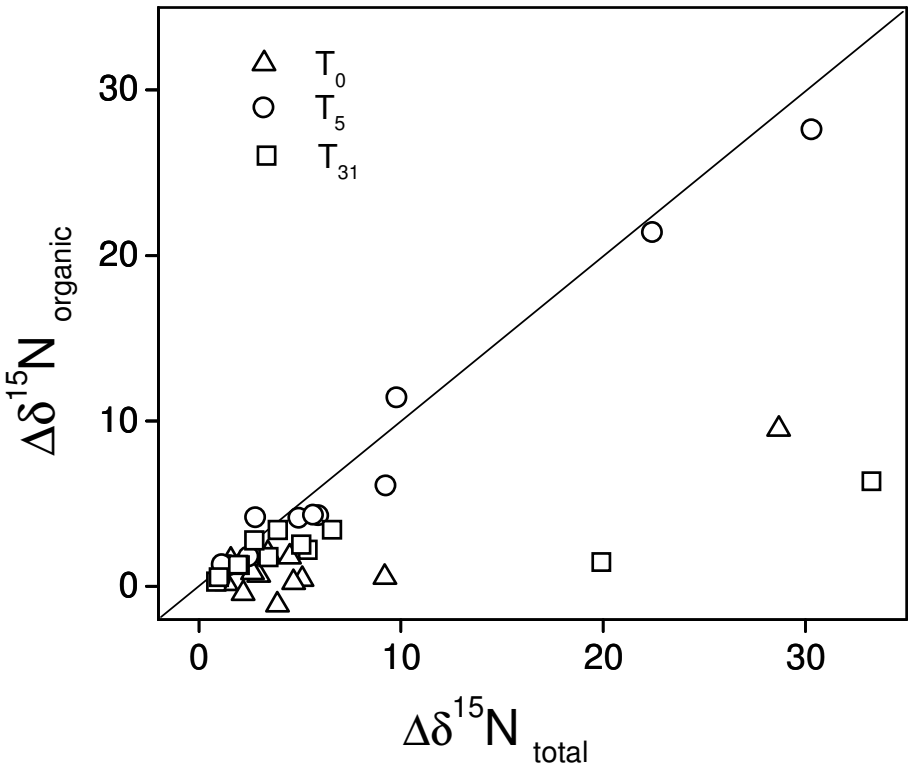


**Fig. 5.** Isotopic enrichment ($\Delta\delta$) in the sediment due to microbial assimilation ($\Delta\delta^{15}N_{org}$) in relation to total sediment enrichment ($\Delta\delta^{15}N_{tot}$) at all 12 sampling station after $T_0$, $T_5$ and $T_{31}$ in May.

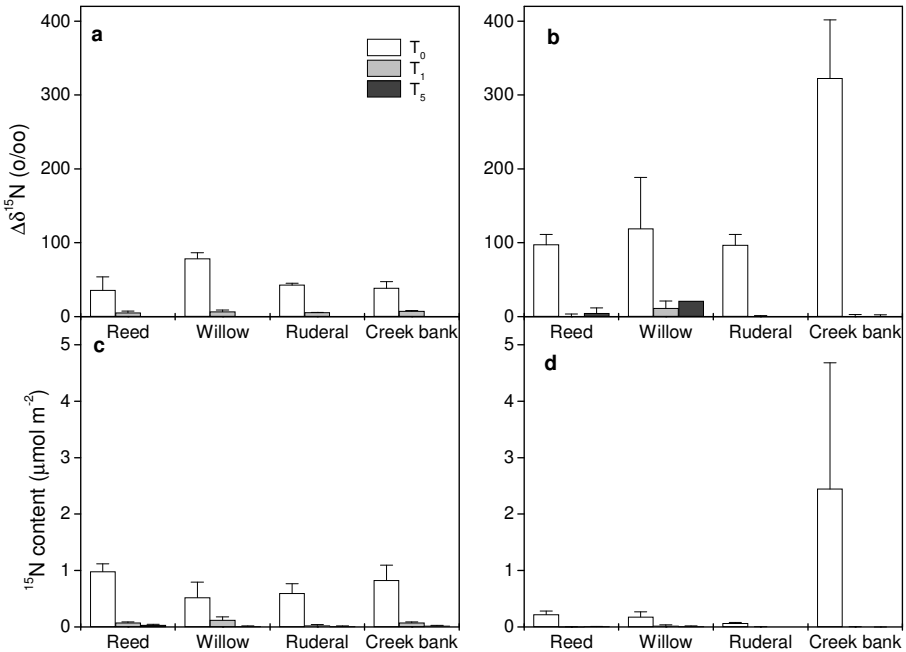


**Fig. 6.** **(a, b)** Isotopic enrichment ($\Delta\delta^{15}$N) and **(c, d)** amount of $^{15}$N (excess $^{15}$N) per unit surface area ($\mu$mol m$^{-2}$) in material deposited on the sediment surface (filter traps).

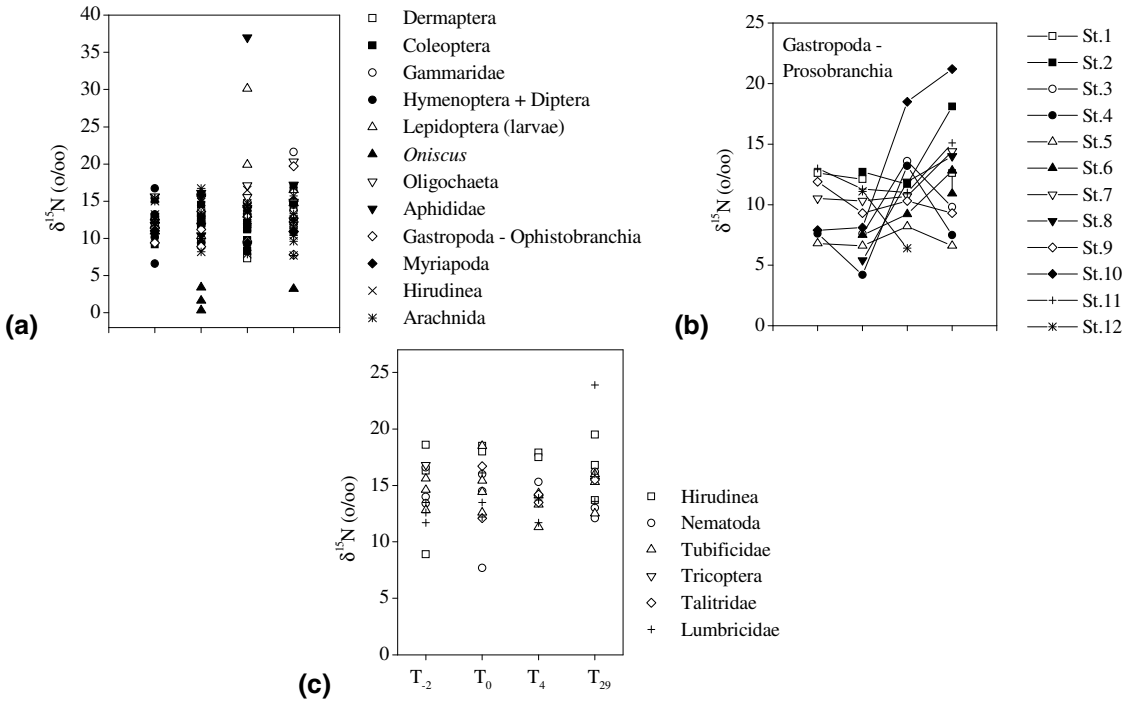


**Fig. 7.** Isotopic value ($\delta^{15}$N) in **(a)** hand-picked invertebrates excluding gastropods (all stations), **(b)** gastropods collected from the 12 sampling stations, and **(c)** invertebrate infauna (all stations) on the four sampling occasions in September.

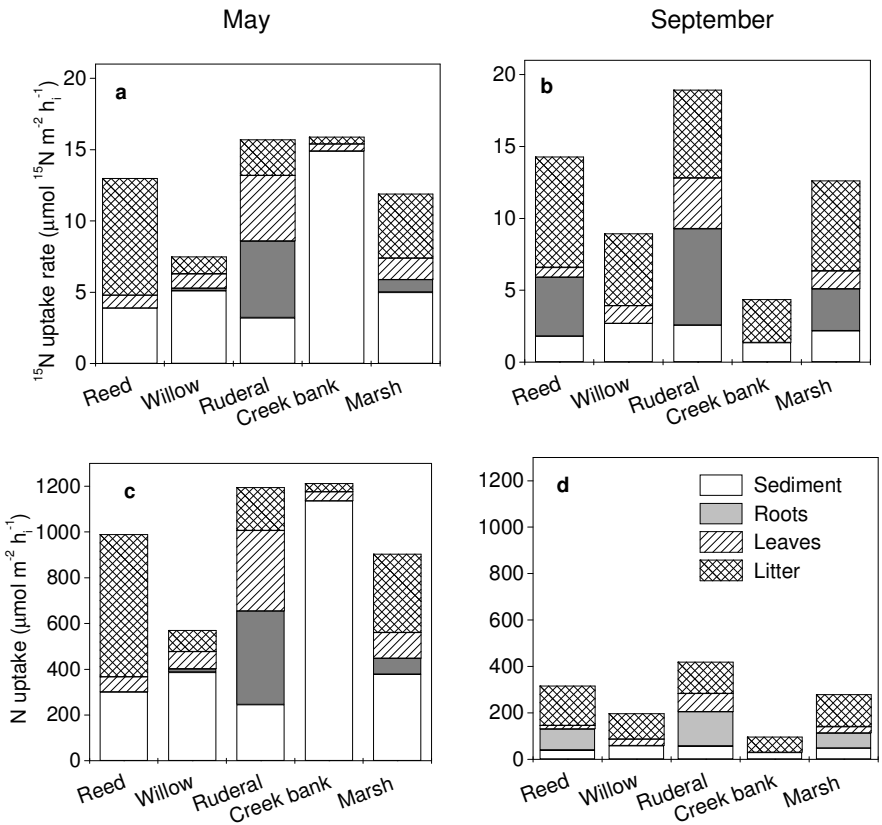


**Fig. 8.** Total $^{15}$N uptake rate ($\mu$mol m$^{-2}$ h$_i^{-1}$) **(a, b)** and corresponding absolute N uptake rate **(c, d)** normalized to per hour inundated in May and September. Results are based on $T_0^{15}$N recovery and inundation duration (see text).