Vol. 219: 99–107, 2001 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published September 10

The role of benthic vegetation as a sink for elevated inputs of ammonium and nitrate in a mesotrophic estuary

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ABSTRACT: Benthic vegetation plays an important role in determining the fate of nitrogen inputs to estuaries, thus influencing their degree of eutrophication. This study investigated the role of benthic vegetation as a sink for anthropogenic inputs of nitrate and ammonium into Wilson Inlet, a mesotrophic estuary in southwestern Australia. The dominant aquatic angiosperm in Wilson Inlet is *Ruppia megacarpa* Mason. We examined: (1) whether *R. megacarpa* leaves remove inorganic N (as nitrate and/or ammonium) from the water column, despite the presence of a layer of epiphytes; (2) whether the macrophyte and its epiphytes are equally important in the removal of inorganic N from the water column; and (3) whether inorganic N taken up by leaves is translocated to other plant parts. We added inorganic ^{15}N nitrogen, as ammonium, nitrate, or both, to aquaria containing intact cores of sediment, *R. megacarpa* and attached epiphytes, and unfiltered estuary water. We measured depletion of nitrogen species from the water column and incorporation of ^{15}N into components of the core. Epiphytes removed more nitrate and ammonium from the water column than *R. megacarpa*, despite having 25% of the biomass of the macrophyte. Maximum rates of nitrate uptake were 4.6 (for epiphytes) and 2.0 µmol h^{-1} g^{-1} DW (for *R. megacarpa*), and maximium rates of ammonium uptake were 35 (for epiphytes) and 23 µmol h^{-1} g^{-1} DW (for *R. megacarpa*). The presence of ammonium reduced rates of nitrate uptake, indicating that benthic vegetation prefers ammonium as a nitrogen source. Using mass spectrometry, we recovered between 37 and 45% of the added ^{15}N nitrogen. The remainder was transformed to either organic nitrogen in the water column by algal epiphytes or nitrogen gas via coupled nitrification-denitrification in the sediment. This experiment indicates ecosystem-scale responses to dissolved inorganic nitrogen which would not have been observable from experiments conducted with isolated plants. Benthic vegetation in Wilson Inlet removes nitrate and ammonium quickly from the water column. Depending on water mixing, it may reduce transient increases in the concentration of these nutrients to background levels within 30 h. This process may be responsible for maintaining low water-column concentrations and reducing the likelihood of algal blooms.

KEY WORDS: Benthic vegetation · Eutrophication · Estuary · ^{15}N · Nutrient sink

INTRODUCTION

Benthic vegetation may play an important role in determining the fate of nitrogen inputs into estuaries, thus influencing their degree of eutrophication. Aquatic angiosperms, benthic macroalgae and epiphytic algae are all components of benthic vegetation, and all utilise inorganic nitrogen from the water column (Short & McRoy 1984, Fujita 1985, Sand-Jensen & Borum 1991). Aquatic angiosperms, unlike the other organisms mentioned, can also utilise nitrogen from the sediment, but are still capable of leaf uptake and translocation to below-ground organs (Thursby & Har-

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lin 1982, 1984, Short & McRoy 1984, Paling & McComb 1994, Romero et al. 1994, Pedersen et al. 1997). Removal of inorganic nitrogen from the water column reduces its availability to floating macroalgae and planktonic microalgae and reduces the potential for growth of these organisms, which are often limited by nitrogen availability (Jones 1985, Sand-Jensen & Borum 1991).

Wilson Inlet is a microtidal, seasonally closed estuary on the southwestern coast of Australia. It receives inorganic nitrogen (the ions nitrate, nitrite and ammonium) from its farmed catchment and from organic-rich sediment. Inputs of inorganic nitrogen are lower than eutrophic estuaries elsewhere in the world (e.g. Chesapeake Bay; Magnien et al. 1992, Boynton et al. 1995), but large compared to nearby, pristine estuaries. Despite increased inputs, concentrations of inorganic nitrogen species are relatively low in the water column. Inputs of inorganic nitrogen (mostly as nitrate) from the catchment are on the order of 50 to 100 t yr^{-1} (Lukatelich et al. 1986, 1987), which equates to 1–2 g m^{-2} yr^{-1} across the whole estuary. Additionally, ammonium is released from the sediment at about 25 g m^{-2} yr^{-1} (Fredericks & Heggie 2000). Catchment inputs are associated with rainfall and are highly seasonal, with most arriving in the estuary between July and October (winter to spring) (Kalnejais & Robb 1999), but sediment inputs are more constant (Fredericks & Heggie 2000). Despite these sources of ammonium and nitrate, water-column concentrations are generally low throughout the year. The median recorded concentration of ammonium over 3 yr from January 1995 was 0.4 µM, and the median concentration of nitrate during the same period was 1 µM (Kalnejais & Robb 1999). The estuary has been classified as mesotrophic, based both on nutrient concentrations (Lukatelich et al. 1987) and on primary productivity (Nixon 1995).

The estuary supports a large biomass of the aquatic angiosperm *Ruppia megacarpa* Mason, which hosts diverse algal epiphytes, including *Polysiphonia infestans* (Rhodophyta), *Polyphysa peniculus* (Chlorophyta) and many diatoms (Bacillariophyta) (Wilshaw 1996). *R. megacarpa* meadows can be as dense as 200 g (dry wt) m^{-2} and are persistent throughout the year (Carruthers et al. 1999). The meadows are distributed around the shallow periphery of the estuary (in <2.5 m water depth), but are denser at the eastern end, where the main streams of the catchment enter (Carruthers et al. 1999). In contrast, phytoplankton abundance is low. The median concentration of chlorophyll from 3 yr of sampling was 1.5 µg l^{-1}, and blooms have been recorded only occasionally (Kalnejais & Robb 1999).

Studies of nitrogen uptake generally focus on isolated shoots of plants removed from their environment (Thursby & Harlin 1982, 1984, Brix & Lyngby 1985, Paling & McComb 1994, Pedersen et al. 1997, Lee & Dunton 1999). Whilst these studies tell us much about macrophyte physiology and nutrition on the plant scale, they are limited in explaining the fate of nitrogen in a marine or estuarine ecosystem. Many epiphytic algae, microbes and animals are present in benthic vegetation (Penhale 1977, Silberstein et al. 1986, King et al. 1990), and these are excluded in experiments on isolated plants. The existence of biota on the exterior of leaves may affect the macrophyte's ability to extract nutrients from the water column. Additionally, these organisms may increase the overall capacity of the vegetation for nutrient uptake. There is a need for studies that observe nutrient fluxes without disrupting seagrass epiphytes.

Benthic vegetation in Wilson Inlet may be behaving as a sink, taking up dissolved inorganic nitrogen as it enters the estuary and converting it to organic particulate forms. We suggest that the uneven distribution of vegetation around the periphery of the estuary and the disparity between large inputs and low water-column concentrations of inorganic nitrogen are evidence of such a situation

We examined the role of *Ruppia megacarpa* and its epiphytes in nitrogen uptake in Wilson Inlet by testing the following: (1) *R. megacarpa* leaves remove inorganic N (as nitrate and/or ammonium) from the water column, despite the presence of a layer of epiphytes; (2) the macrophyte and its epiphytes are equally important in the removal of inorganic N from the water column; and (3) inorganic N taken up by leaves is translocated to other plant parts.

MATERIALS AND METHODS

We added inorganic ^{15}N nitrogen to aquaria containing intact cores of sediment, the macrophyte *Ruppia megacarpa* and attached epiphytes, and unfiltered estuary water. After adding nitrogen as ammonium, nitrate, or both, we measured depletion of those nitrogen species from the water column and incorporation of ^{15}N into various components of the core. This enabled us to partition nitrogen uptake between seagrass leaves, epiphytes, sediment and suspended material.

Collection and transport of cores. We collected intact seagrass and sediment cores from an area no larger than 100 m^2 in Wilson Inlet (117° 24′ E, 35° 00′ S) in 1 m water depth. We extracted the cores using cylindrical PVC pipe sections with a diameter of 86 mm and a length of 78 mm. Cores were then held in place with PVC caps secured on the core bottoms. The cores were placed in plastic tubs filled with estuarine water, and baffles of PVC tubes were placed around each core of

seagrass to reduce disturbance during transport. We also collected 60 l of well-mixed surface water from a nearby deeper part of the estuary (3 m). The plastic tubs were aerated overnight and transported back to the laboratory the following day. Cores were collected on 19 January and the experiment was run between 22 and 23 January 1999.

^{15}N uptake experiment. Sixteen cores were each placed in individual aquaria containing 1.4 l of estuary water, in a temperature-regulated growth room (18°C). Aquaria were aerated to ensure oxygenation and mixing of the water and illuminated with Philips 36 W fluorescent lights, providing about 420 µmol quanta m^{-2} s^{-1}. The aquaria were illuminated during the first 8 h and the final 4 h of the experiment. Each aquarium was randomly assigned 1 of 4 nutrient treatments: addition of *Ammonium*, addition of *Nitrate*, addition of *Both* ammonium and nitrate, or *Control*. Each treatment was represented by 4 replicate aquaria.

Nitrate was added as a 10 ml aliquot of 8.4 mM $NaNO_3$ and ammonium as 10 ml of 0.91 mM NH_4Cl, to make final concentrations of 6.5 µM $NaNO_3$ and 60 µM NH_4Cl. In the *Both* treatment, 10 ml of both nitrate and ammonium solutions were added, and in the *Control*, 10 ml of distilled water was added. Concentrations were chosen to simulate fluxes observed in Wilson Inlet. Both nitrate and ammonium solutions were enriched in ^{15}N by 15 at.% ^{15}N. This level of enrichment was chosen based on preliminary uptake experiments, sensitivity of mass spectrometry, and cost of ^{15}N compounds.

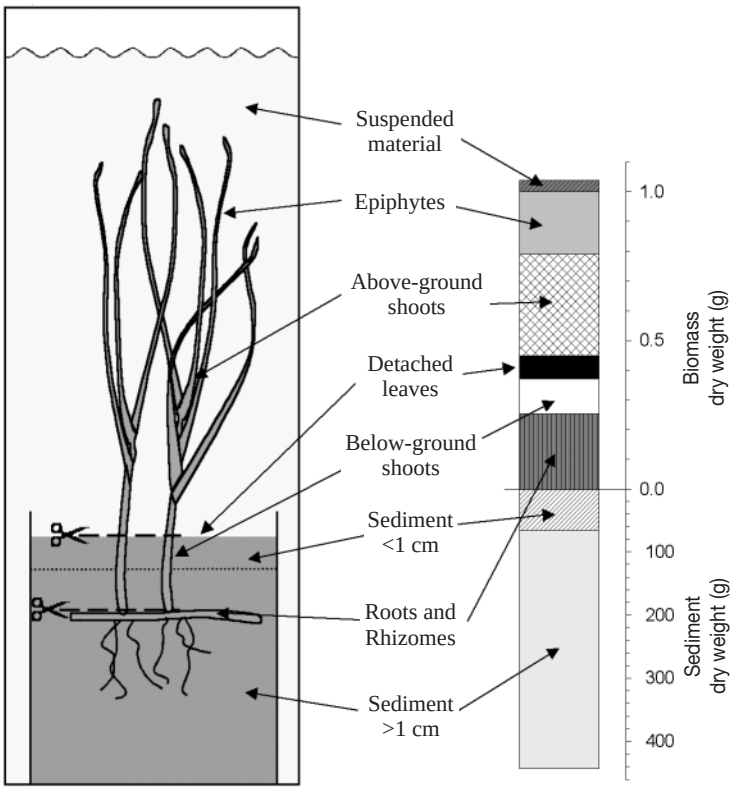

Fig. 1. Components of aquaria containing cores of *Ruppia megacarpa* and sediment, used in ^{15}N uptake experiment and dry weights determinations. Scissor symbols show where plant parts were separated. Values are averages of all 16 cores used in the experiment. Standard deviations were within 20% of the mean for sediment, 120% of the mean for detached leaves, and 40% of the mean for all other components

Sampling. To measure depletion of nitrate and/or ammonium, water was sampled from each aquarium 6 times: immediately after nutrient (or water) addition, then after approximately 0.5, 2, 6, 12 and 24 h. At each sampling time, 10 ml of water was collected in a syringe, filtered through a 0.45 µm cellulose nitrate filter (Whatman) and frozen until further analysis.

After 24 h, we terminated the experiment and separated the contents of each aquarium into the following components: *Suspended* solids, *Epiphytes*, *Above-ground shoot*, *Below-ground shoot*, *Root* and *Detached leaves* of *Ruppia megacarpa*, and *Sediment* (Fig. 1).

We obtained *Suspended* material by filtering 200 ml of water from each aquarium through pre-ashed (combusted for 1 h at 450°C, to remove possible nitrogenous contaminants), pre-weighed Whatman GF/C filters. The remaining water in the aquaria was then discarded.

Any *Detached leaves* were removed from the water column and sediment surface, then we cut *Above-ground shoots* of *Ruppia megacarpa* at the level of the sediment surface with scissors. One 20 mm diameter sediment sample was removed from each core. This was separated into upper (*Sediment < 1 cm*) and lower (*Sediment > 1 cm*) components, then sorted to remove any plant material. Plant material remaining in the sediment was removed and classified as *Below-ground shoot* or *Root* (root material included rhizome).

We removed *Epiphytes* from a subsample of each of the leaf samples with a razor blade. Weights of the epiphytes and leaf from this subsample were used to determine the total weights of *Leaf* and *Epiphyte* material.

Mass spectrometry analysis. Sediment samples were freeze-dried, to ensure no loss of dissolved ammonia. All other samples were dried at 60°C for approximately 24 h. Filters containing suspended material were weighed and then cut into small pieces and placed directly into tin canisters for mass spectrometry analysis. All other samples were weighed and ground, then subsamples were weighed for mass spectrometry.

Weights of between 5 and 50 mg were used, depending on nitrogen content. We used a 20/20 Europa Scientific ANCA-SL mass spectrometer to measure percent nitrogen content and at.% ^{15}N.

The water samples were analysed for nitrate plus nitrite (Johnson 1983), and ammonium (Switala 1993) using a Lachat Quick-Chem 8000 Automated Flow Injection Analyser.

Data analysis and interpretation. For each component in the 3 ^{15}N addition treatments, we calculated the total amount of nitrogen incorporated during the 24 h incubation. We also calculated rates of nitrate and ammonium uptake by *Ruppia megacarpa* and its epiphytes, based on the incorporation results and rates of depletion of the nutrients observed from water samples.

For each component in each nutrient treatment, mean (of 4 cores) ^{15}N content (at.%^{15}N) was calculated. If a component's mean at.%^{15}N was found to be significantly (*t*-test: $p < 0.05$) higher than in the corresponding control, ^{15}N enrichment was calculated as

$$^{15}N \text{ enrichment} = \text{Treatment at.\%}^{15}N - \text{Control at.\%}^{15}N$$

The total nitrogen content of each component was calculated as

$$\text{Total N content} = \text{percent N} \times \text{Dry Weight}$$

Total N incorporation was then calculated:

$$\text{Total N incorporation} = {}^{15}N \text{ enrichment} \times \text{total N content} \times {}^{100}/_{15}$$

The last factor provided correction for the ^{15}N enrichment of the addition solution (15 at.% ^{15}N). The mean total N incorporation of the 4 replicate cores was then calculated.

Ammonium and nitrate uptake rates (V) were calculated from changes in the water-column concentrations (depletion), and ^{15}N incorporation, based on a modification of the following formula:

$$V = \frac{\Delta S}{\Delta T \times DW}$$

where ΔS is the change in substrate concentration (depletion), ΔT is the time interval, and DW is the dry weight of the organism under investigation (Harrison et al. 1989). Here, with multiple components causing depletion of the substrate, we have used the N incorporation value obtained above to partition this depletion. Our formula for the uptake rate of a component (V_c) is

$$V_c = \frac{P_c \times \Delta S}{\Delta T \times DW_c}$$

where P_c is the proportion of total recovered N incorporated in the component and DW_c is the dry weight of the component (V is in units of µmol h^{-1} g^{-1} DW).

RESULTS

^{15}N enrichment of components

The greatest enrichment (up to 1.3 at.% ^{15}N) was measured in the *Both* treatment (Fig. 2). Greater enrichment was measured in the *Ammonium* treatment than in the *Nitrate* treatment. In each treatment, the order of decreasing enrichment in components was the same. The greatest ^{15}N enrichment was found in

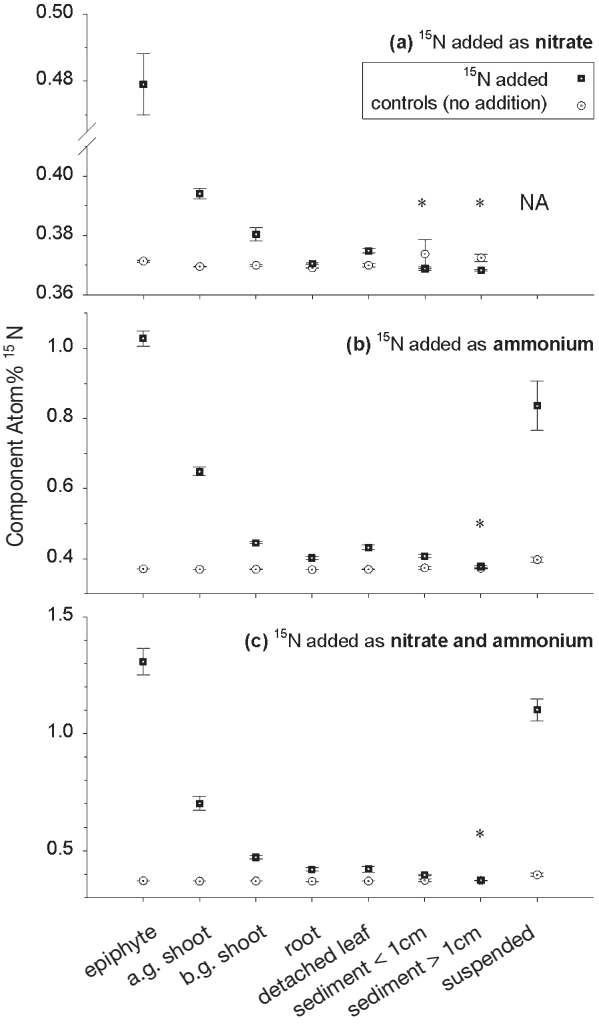

Fig. 2. ^{15}N content (at.%^{15}N) of components of *Ruppia megacarpa* sediment cores 24 h after addition of ^{15}N as (a) nitrate (6.5 µM 15 at.% $K^{15}NO_3$), (b) ammonium (60 µM 15 at.% $^{15}NH_4Cl$), or (c) both ammonium and nitrate. Values are averages of 4 replicates ± standard error. Circles represent at.%^{15}N values from *Control* treatment (the unamended ^{15}N abundance). Components in which average at.%^{15}N values are not significantly different (*t*-test: $p < 0.05$) from controls are marked with an asterist. b.g.: below ground; a.g.: above ground; NA: value not available due to loss of sample during mass spectrometry

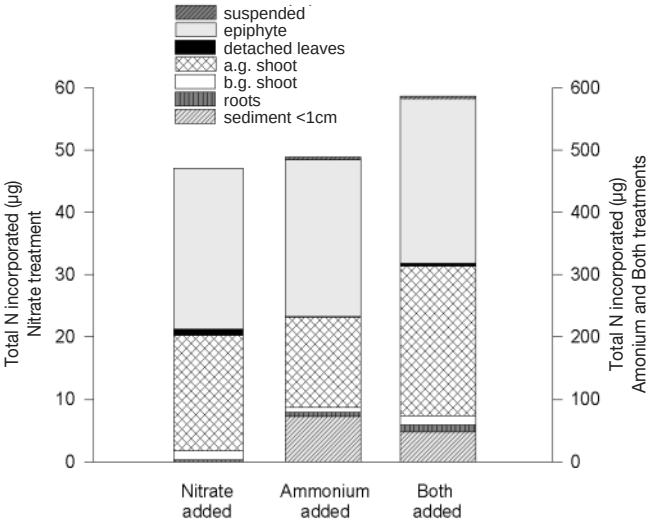

Fig. 3. Total nitrogen incorporated into components of *Ruppia megacarpa* sediment during ^{15}N uptake experiment. Values are averages of 4 cores after additions of either nitrate (6.5 µM 15 at.% $K^{15}NO_3$), ammonium (60 µM 15 at.% $^{15}NH_4Cl$) or both ammonium and nitrate. Nitrogen incorporation was calculated from ^{15}N enrichment, nitrogen content, and dry weights of each component. Only components showing significant ^{15}N enrichment have been included (see 'Materials and methods'). Standard deviations were <50% of means, except for sediment (<75% of means) and detached leaves (<150% of means). b.g.: below groud; a.g.: above ground

the *Epiphyte* component, followed by *Suspended*, *Above-ground shoot*, *Below-ground shoot*, *Detached leaf*, *Root* and *Sediment*.

With the exception of some of the sediment samples, all components showed significant enrichment in all 3 ^{15}N-addition treatments. The *Sediment < 1 cm* component showed significant enrichment in the *Ammonium* and *Both* treatments but not in the *Nitrate* treatment. There was no enrichment in the *Sediment > 1 cm* component in any of the treatments.

Total nitrogen incorporation

Of the nitrogen added 37, 42 and 45% was recovered in the *Nitrate*, *Ammonium* and *Both* treatments, respectively. In each treatment, most of the total recovered ^{15}N was incorporated in *Epiphyte* material, 55% in the *Nitrate* treatment, 51% in the *Ammonium* treatment and 45% in the *Both* treatment (Fig. 3). *Above-ground shoots* accounted for 39% of the recovered N in the *Nitrate* treatment, 30% in the *Ammonium* treatment and 41% in the *Both* treatment. Much smaller amounts were incorporated into the other components.

Inorganic N uptake rates

The presence of ammonium reduced depletion rates of nitrate, but the presence of nitrate had no effect on depletion rates of ammonium. Depletion of nitrate from the water column in the *Both* treatment was significantly slower than in the *Nitrate* treatment (Fig. 4). No such difference was observed in depletion of ammonium.

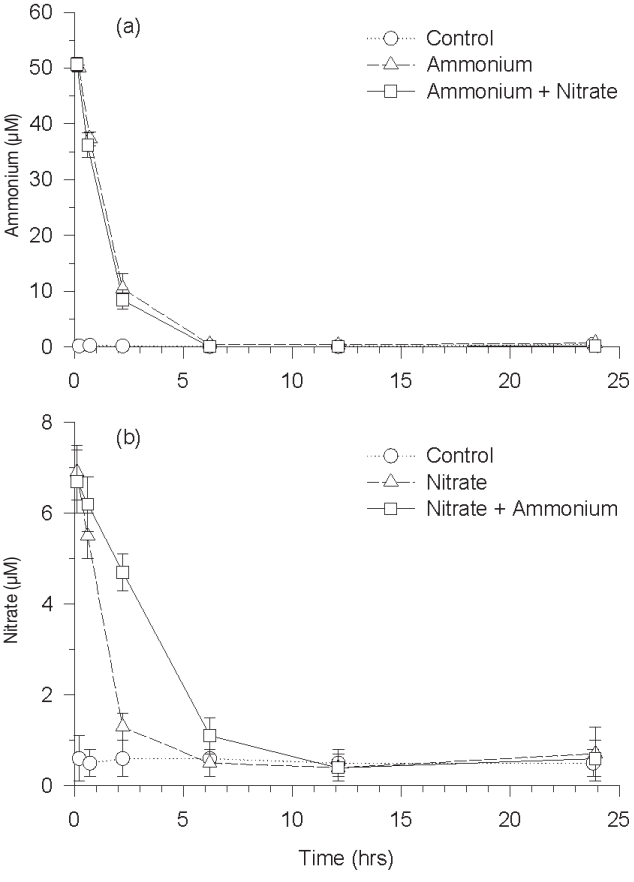

Fig. 4. Concentrations of (a) ammonium and (b) nitrate in aquaria containing cores of *Ruppia megacarpa* and sediment. Open symbols with dotted lines represent concentrations in control (no nitrate or ammonium addition) treatments, triangles with dashed lines represent treatments where (a) ammonium (60 µM NH_4Cl) or (b) nitrate (6.5 µM KNO_3) were added alone, squares with solid lines represent treatment in which both ammonium and nitrate were added. Values are averages of 4 replicates ± standard error

Epiphyte uptake rates were higher than those of *Ruppia megacarpa* (Fig. 5), due to their higher total incorporation of N and lower biomass. Maximum rates of nitrate uptake were 4.6 µmol h^{-1} g^{-1} DW for epiphytes and 2.0 µmol h^{-1} g^{-1} DW for *R. megacarpa*, and maximium rates of ammonium uptake were 35 µmol

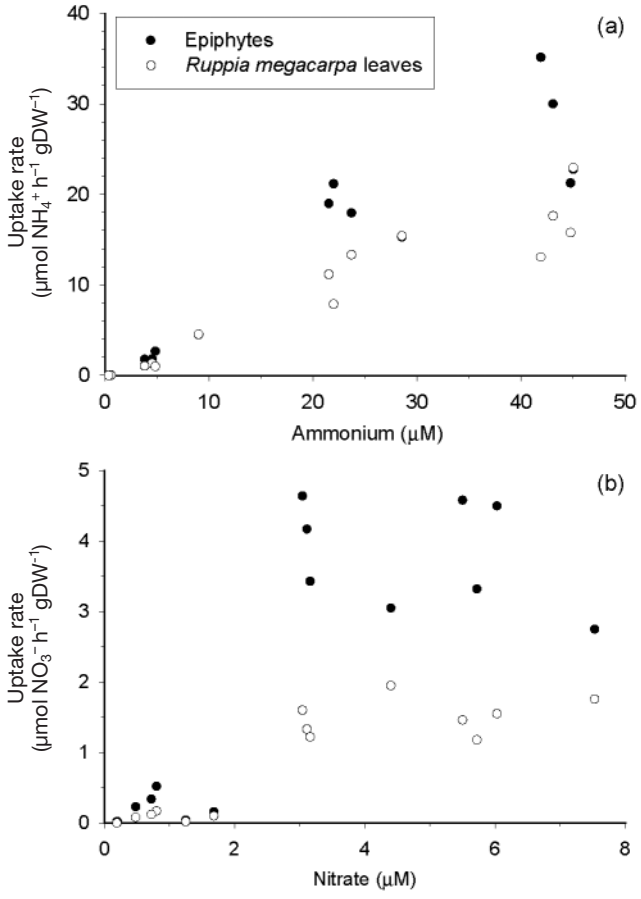

Fig. 5. (a) Ammonium and (b) nitrate uptake rates of *Ruppia megacarpa* (○) and its epiphytes (●) as a function of substrate concentration, calculated by partitioning water column depletion based on ^{15}N incorporation during a 24 h incubation with (a) ^{15}N enriched ammonium as 60 µM NH_4Cl or (b) nitrate as 6.5 µM KNO_3

h^{-1} g^{-1} DW for epiphytes and 23 µmol h^{-1} g^{-1} DW for *R. megacarpa*.

Weights and nitrogen content of core components

Mean (n = 4) component weights were not significantly different between different treatments. Cores contained 440 ± 20 g DW of sediment (average ± SE, n = 16), 0.72 ± 0.03 g DW of intact plant material, 0.08 ± 0.03 g DW of detached leaves, 0.21 ± 0.02 g DW of epiphytes and 0.036 ± 0.003 g DW of suspended solids (Fig. 1). Cores contained a total of 190 mg of nitrogen, 170 ± 10 mg in sediment (average ± SE, n=16), 15 ± 1 mg in intact plant material, 4.4 ± 0.3 mg in epiphytes, 1.5 ± 0.6 mg in detached leaves and 0.10 ± 0.01 mg in suspended solids.

DISCUSSION

In this study we used *Ruppia megacarpa* sediment cores as mimics of the Wilson Inlet ecosystem, and measured the fate of nitrogen introduced into the water column, simulating inputs of nitrate from stream flow and ammonium from sediment release. Results of this experiment suggest ecosystem-scale responses to dissolved inorganic nitrogen, which would not have been observable from experiments conducted with isolated plants.

Changes in the relative biomass of epiphytes are likely to alter the proportion of nitrogen that they remove from the water column. Epiphytes had higher uptake rates than *Ruppia megacarpa* and accounted for the bulk of ammonium and nitrate recovered as ^{15}N. In our study, epiphyte biomass was about 25% of *R. megacarpa* biomass, but previous work has shown that epiphyte biomass can change both in space and time (Carruthers 1998). Relative biomass of epiphytes can vary from 10 to 120% of macrophyte biomass, depending on location within the estuary, and from an average (over many sites) of 20 to 80% seasonally (Carruthers 1998). Given that epiphyte cover can have a 10-fold spatial variation and a 4-fold temporal variation, the proportion of inorganic N removed by epiphytes may vary substantially. Changes in the relative abundance of epiphytes can be triggered by changes in salinity (Wilshaw 1996), nutrient availability (Morand & Briand 1996) or light availability. Nitrogen uptake by the epiphyte assemblage may also be affected by changes in species composition, as uptake kinetics vary between different algal and microbial species (Lobban & Harrison 1994).

The potential effects of a change in epiphyte abundance on inorganic nitrogen uptake may be very important to nitrogen cycling within the estuary. Epiphytes have higher uptake rates and faster turnover rates (Sand-Jensen & Borum 1991) than their angiosperm hosts. They are more readily grazed (Duarte 1995) and are likely to decompose faster, due to their higher carbon:nitrogen ratio (Twilley et al. 1986). In contrast, angiosperms grow, decompose and are grazed more slowly. They are also able to immobilise nitrogen by translocating nitrogen sourced from the water column to below-ground organs (Romero et al. 1994). An increase in relative abundance of epiphytes would result in more rapid uptake of nutrients, but also more rapid remobilisation of nutrients. We suggest that a system in which epiphytic algae dominate is likely to have more variation in inorganic nitrogen availability, than a system in which seagrass dominate.

Ruppia megacarpa leaves were able to take up inorganic nitrogen despite a layer of epiphytes. *R.*

megacarpa leaves accounted for 40% of the ^{15}N nitrate and 30% of the ^{15}N ammonium recovered in our experiment. Since diatomaceous epiphytes appeared to cover *R. megacarpa* leaves uniformly, the leaves must be able to access the water column through the layer of epiphytes. We expect that the degree of accessibility would change as a result of variation in epiphyte cover, altering the ability of *R. megacarpa* to remove nitrate and ammonia from the water column, and creating a greater dependence on the sediment for nitrogen supply. Increases in epiphyte cover are also likely to result in slower growth rates of the macrophyte because of decreased light availability. This effect has been suggested as the cause of macrophyte decline in marine (Bulthuis & Woelkerling 1983, Cambridge et al. 1986, Silberstein et al. 1986) and freshwater systems (Phillips et al. 1978).

The rate of nitrate depletion from the water column was reduced in the presence of ammonium. This indicates that benthic vegetation as a whole prefers ammonium to nitrate when both are available, which is consistent with other studies (Short & McRoy 1984, Thursby & Harlin 1984). Uptake and assimilation of nitrate requires more energy than that of ammonium. Nitrate assimilation in vascular plants involves transformation to ammonium by the nitrate reductase enzyme (Clarkson 1985), prior to formation of amino acids. This additional step is energetically expensive (Lobban & Harrison 1994) and may well lead to a preference for ammonium over nitrate. On an ecosystem scale, this phenomenon could result in an increase in nitrate availability when ammonium is plentiful.

Significant enrichment of root/rhizome tissues in all 3 treatments demonstrated basipetal translocation of ammonium and nitrate or their derivatives, as has been demonstrated for other species (*Zostera marina*; Thursby & Harlin 1982). This signifies that the plant is capable of supplying below-ground tissues with nitrogen sourced from leaves and that it need not rely on roots for nutrition. An alternate explanation for elevated ^{15}N content of below-ground plant parts is that this nitrogen was taken up by roots after transfer from the water column to the sediment interstitial waters. This seems unlikely for 2 reasons: Firstly, there was no detectable enrichment of sediment below 1 cm depth, and enrichment above 1 cm depth was very small compared to root enrichment. Secondly, the root and rhizome material was located predominantly >1 cm below the sediment surface in our cores (Fig. 1).

Translocation was observed to a lesser extent when ^{15}N was added as nitrate. This is evidence that either nitrate is translocated less readily than ammonium or that nitrate reduction occurs in the shoot before translocation can occur. Nitrate reduction occurs in the shoots of many vascular plants (Lambers et al. 1998), and this extra step would increase the time taken for nitrogen obtained as nitrate to be transferred to below-ground organs.

In our experiment, we recovered between 37 and 45% of the nitrogen that was added to the aquaria as ^{15}N. Previous studies of ^{15}N uptake have not attempted to reconcile amounts of ^{15}N recovered with amounts added (e.g. Short & McRoy 1984, Pedersen et al. 1997), and it is difficult to deduce the fate of the missing ^{15}N. Nitrogen components not measured in this study were N_2 gas, dissolved organic nitrogen and fine particulate nitrogen (particles <1.2 µm). This discrepancy between added and recovered ^{15}N has also been observed to a lesser extent in split chamber experiments (Gahnström 2000). In that study, where *Ruppia megacarpa* plants were present, but sediment was not, 65% of ^{15}N added as $NaNO_3$ was recovered from plant and epiphyte tissue by mass spectrometry. Aquarium effects were discounted, as this phenomenon was not observed when plants were absent. Gahnström (2000) concluded that the discrepancy resulted from transformation of nitrate to dissolved or particulate organic nitrogen by epiphytic microalgae, as described by Bronk & Glibert (1993).

In the present study, the discrepancy between added and recovered ^{15}N was greater, and we conclude that the unrecovered amounts can be accounted for by 2 co-occurring processes. They are the transformation of inorganic nitrogen to some form of organic nitrogen as described above and coupled nitrification-denitrification processes occurring in the sediment.

Coupled nitrification-denitrification results in transformation of inorganic nitrogen into N_2 gas. These processes have been associated with boundaries between oxidised and reduced regions of sediment, at the sediment surface and in the rhizosphere (Boynton et al. 1995, Koottatep & Polprasert 1997). Denitrification in the sediment of Wilson Inlet has been inferred by Fredericks et al. (1999), who measured fluxes of ammonium, inorganic carbon and silica using *in situ* benthic chambers and then applied stoichiometric methods to calculate denitrification rates. Fredericks et al. (1999) estimated that up to half of the ammonium remineralised in sediment was lost through denitrification to the atmosphere as N_2 gas.

When water-column concentrations of ammonium and nitrate were elevated in our experiment to values representative of flux events in Wilson Inlet, depletion was rapid. When added alone, concentrations of both nitrate and ammonium were reduced to initial concentrations within 6 h. The water:biomass ratio in our microcosms (approximately 1.4 l g^{-1} DW) was lower than that in Wilson Inlet. Given a total water volume of 7.2×10^{10} l and an estimated biomass of 10^{10} g DW (Lukatelich et al. 1986), the ratio for the estuary is

about 7.2 l g^{-1} DW. We estimate that an estuary-wide increase in either nitrate or ammonium, of the same magnitude as those simulated in this experiment, could be removed by *Ruppia megacarpa* vegetation within 30 h, 5 times as long as in our experiment. This estimate assumes either mixing throughout the inlet or uniform distribution of benthic vegetation. *R. megacarpa* biomass is higher (by up to an order of magnitude) in the eastern basin of Wilson Inlet (Carruthers et al. 1999), where inputs of inorganic nitrogen are also higher (Kalnejais & Robb 1999); this would ameliorate the effect of lower water mixing.

We suggest that benthic vegetation in Wilson Inlet is behaving as a sink, taking up dissolved inorganic nitrogen as it enters the estuary and converting it to organic particulate forms. A reduction in the abundance of *Ruppia megacarpa* or an increase in the relative abundance of its epiphytes is likely to result in increased rates of nitrogen cycling, increased fluctuations in the concentration of inorganic nitrogen, and increased frequency and intensity of micro- and macroalgal blooms.

Acknowledgements. Thanks to the Western Australian Water and Rivers Commission for providing funding and valuable resources, including the use of a boat and office space in Denmark. This study was conducted as part of The National Eutrophication Management Program, co-funded by the Land and Water Resources Research and Development Corporation (Australia). Water samples were analysed by Murdoch University's Marine and Freshwater Research Laboratory. We are also very grateful to Sue Williams and Marion Cambridge for their reviews of the manuscript.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: July 27, 2000; Accepted: January 9, 2001
Proofs received from author(s): August 24, 2001