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^{15}N MEASUREMENTS OF AMMONIUM AND NITRATE UPTAKE BY *ULVA FENESTRATA* (CHLOROPHYTA) AND *GRACILARIA PACIFICA* (RHODOPHYTA): COMPARISON OF NET NUTRIENT DISAPPEARANCE, RELEASE OF AMMONIUM AND NITRATE, AND ^{15}N ACCUMULATION IN ALGAL TISSUE[1]

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Ammonium and nitrate uptake rates in the macroalgae *Ulva fenestrata* (Postels and Ruprecht) (Chlorophyta) and *Gracilaria pacifica* (Abbott) (Rhodophyta) were determined by ^{15}N accumulation in algal tissue and by disappearance of nutrient from the medium in long-term (4–13 days) incubations. Nitrogen-rich algae (total nitrogen > 4% dry weight [dw]) were used to detect isotope dilution by release of inorganic unlabeled N from algal thalli. Uptake of NH_4^+ was similar for the two macroalgae, and the highest rates were observed on the first day of incubation (45 μmol N·g dw^{-1}·h^{-1} in *U. fenestrata* and 32 μmol N·g dw^{-1}·h^{-1} in *G. pacifica*). A significant isotope dilution (from 10 to 7.9 atom % enrichment) occurred in *U. fenestrata* cultures during the first day, corresponding to a NH_4^+ release rate of 11 μmol N·g dw^{-1}·h^{-1}. Little isotope dilution occurred in the other algal cultures. Concurrently to net NH_4^+ uptake, we observed a transient free amino acid (FAA) release on the first day in both macroalgal cultures. The uptake rates estimated by NH_4^+ disappearance and ^{15}N incorporation in algal tissue compare well (82% agreement, defined as the percentage ratio of the lower to the higher rate) at high NH_4^+ concentrations, provided that isotope dilution is taken into account. On average, 96% of added $^{15}NH_4^+$ was recovered from the medium and algal tissue at the end of the incubation. Negligible uptake of NO_3^- was observed during the first 2–3 days in both macroalgae. The lag of uptake may have resulted from the need for either some N deprivation (use of NO_3^- pools) or physiological/metabolic changes required before the uptake of NO_3^-. During the subsequent days, NO_3^- uptake rates were similar for the two macroalgae but much lower than NH_4^+ uptake rates (1.97–3.19 μmol N·g dw^{-1}·h^{-1}). Very little isotope dilution and FAA release were observed. The agreement between rates calculated with the two different methods averaged 91% in *U. fenestrata* and 95% in *G. pacifica*. Recovery of added $^{15}NO_3^-$ was virtually complete (99%). These tracer incubations show that isotope dilution can be significant in NH_4^+ uptake experiments conducted with N-rich macroalgae and that determination of ^{15}N atom % enrichment of the dissolved NH_4^+ is recommended to avoid poor isotope recovery and underestimation of uptake rates.

Key index words: **ammonium; *Gracilaria*; isotope dilution; macroalgae; ^{15}N; nitrate; *Ulva*; uptake**

Abbreviations: **dw, dry weight; DON, dissolved organic nitrogen; FAA, free amino acid; PN, particulate nitrogen; TN, total nitrogen**

Nitrogen is an important factor controlling algal growth in marine environments (Lobban and Harrison 1994), and numerous studies have examined nitrogen utilization by marine macroalgae. Many studies have focused on uptake physiology (e.g. determination of kinetics parameters, interactions between ammonium and nitrate uptake, influence of algal nitrogen content on uptake). Other investigations have examined the impact of macroalgal nitrogen requirements and uptake capacity on nitrogen cycling in the environment.

The traditional method for determining uptake rates has been to measure changes in nitrogen concentration in the incubation medium over time (Harlin and Wheeler 1985). More recently, the incorporation of the nitrogen stable isotope ^{15}N into algal tissue has been used to calculate uptake rates. Isotope techniques have been extensively used in phytoplankton studies, but relatively fewer tracer studies of nitrogen uptake exist for macroalgae and macrophytes (Owens and Stewart 1983, Short and McRoy 1984, Williams and Fisher 1985, O'Brien and Wheeler 1987, Döhler et al. 1995, McGlathery et al. 1997). Nitrogen dynamics within macroalgal mats have also been studied with ^{15}N (Thybo-Christesen and Blackburn 1993, McGlathery et al. 1997, Krause-Jensen et al. 1999).

Advantages and problems of the ^{15}N technique have been reviewed by Glibert and Capone (1993). Isotope measurements have higher sensitivity and allow shorter incubation times, identification of critical steps in ni-

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trogen uptake (Fujita et al. 1988), determination of inorganic N regeneration rates by isotope dilution (O'Brien and Wheeler 1987), and determination of rates of nitrogen assimilation into specific organic molecules (Haxen and Lewis 1981, Döhler et al. 1995). On the other hand, in many phytoplankton studies not all added isotope is recovered at the end of the experiment (Glibert et al. 1982, Laws 1984). Release of ^{15}N in organic molecules, adsorption of the isotope to the incubation container walls, and nitrification (when labeled ammonium is used) are among the explanations hypothesized to account for the missing isotope. To our knowledge, only one study with macroalgae has attempted to trace the possible fate of ^{15}N during laboratory incubations (O'Brien and Wheeler 1987). Macroalgae can accumulate cellular pools of inorganic nitrogen (Chapman and Craigie 1977, Fujita et al. 1988), and dilution of the isotope can occur if inorganic nitrogen is released from these pools. In such cases it is necessary to monitor the isotope enrichment of the dissolved nutrient throughout the experiment for an accurate calculation of uptake rates through isotope incorporation in algal cells (Glibert et al. 1982).

Although the use of isotopes allows for short experimental times, thus avoiding problems related to long-term incubations, studies of the impact of macroalgal nitrogen metabolism on nitrogen cycling in the environment often require that laboratory or field enclosures are maintained for several hours or even days. For example, McGlathery et al. (1997) studied $^{15}NH_4^+$ dynamics in *Chaetomorpha linum* mats incubated in continuous flow-through systems for 8 days. Under these conditions, significant variations of algal biomass and total nitrogen (TN) content can occur, and the models normally used to calculate uptake rates (Glibert et al. 1982) should take these changes into consideration.

Here we present the results of uptake experiments with the macroalgae *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) designed to compare ammonium and nitrate uptake rates determined by ^{15}N accumulation in algal tissue and by disappearance of nutrient from the medium in long-term (4–13 days) incubations. We assessed the recovery of the tracer by measuring ^{15}N enrichment of both algae and dissolved nutrients. Macroalgal growth was monitored throughout the experiment. Nitrogen-rich algae were used to detect possible isotope dilution by release of inorganic unlabeled nitrogen from algal thalli and to assess if nitrogen uptake is inhibited by high thallus N levels.

MATERIALS AND METHODS

Ulva fenestrata and *G. pacifica* samples were collected at low tide from the Yaquina River estuary mud flats in Newport, Oregon, USA. The thalli were rinsed with seawater and gently scrubbed to remove sediment and epiphytes. Before the experiment, the macroalgae were maintained in the laboratory at 15° C on a 16:8-h light:dark cycle at about 100 μmol photons·m^{-2}·s^{-1}; the same temperature and light conditions were maintained during the subsequent experiment. During the 3-week acclimation, macroalgae density was about 2 g dry weight (dw)·L^{-1}. Seawater was changed every 2 days and enriched to 1 mM N (0.5 mM KNO_3 and 0.5 mM NH_4Cl) and 44 μM KH_2PO_4. Aeration was provided by bubbling with compressed air.

For the uptake experiments, 12 glass jars were filled with 1.5 L of filtered seawater and approximately 0.9–1.4 g (dw) of *U. fenestrata* or *G. pacifica*. For each species, seawater was enriched to 1 mM N with labeled (10 atom % ^{15}N) NH_4Cl or KNO_3 and 70 μM KH_2PO_4. Each combination of alga and nitrogen source was tested in triplicate. Two control jars without algae were prepared for each nutrient. We used a high nitrogen concentration mainly to ensure that we could conduct the experiment for several days without further additions of the labeled nutrient. Ammonium and nitrate uptake experiments lasted 4 and 13 days, respectively. For the ammonium experiment, water and macroalgae were sampled at the beginning (t_0) of the incubations and then once every day. For the nitrate experiment, water and macroalgae were sampled at t_0 and then on days 1, 2, 3, 6, 9, and 13.

Thallus samples were briefly rinsed with deionized water, dried (70° C, 24 h), powdered with a Spex mixer (Spex CertiPrep, Inc., Edison, NJ), and analyzed for TN and ^{15}N enrichment with a mass spectrometer. Water samples were filtered (GF/F) and immediately analyzed for ammonium, nitrite, and nitrate. Subsamples were frozen for later analysis of free amino acids (FAA), proteins, and atom % ^{15}N of ammonium and nitrate. Ammonium concentration was determined with the indophenol-blue method (Koroleff 1970). Nitrite and nitrate concentrations were determined by diazotization before and after reduction with Cd columns (Grasshoff 1976). FAA concentration was determined by fluorescence, with the o-phthalaldehyde method using glycine as the standard (Parsons et al. 1984); fluorescence values were corrected for the ammonium contribution. Protein concentration was determined with the Coomassie Blue method (Bradford 1976). Atom % enrichment of dissolved ammonium was determined with a mass spectrometer after a 6-day diffusion of the sample (with the addition of MgO to raise the pH above 9) on an acidified paper filter (Brooks et al. 1989). The same method was used for the determination of atom % enrichment of dissolved nitrate, after preliminary diffusion of the unlabeled ammonium and reduction of labeled nitrate to ammonium with Devarda's alloy (Brooks et al. 1989). Isotope enrichment of dissolved nitrate was not determined on days 1, 2, and 3 of the nitrate experiment, because little or no variation of dissolved nitrate concentration was observed. Isotope enrichment of the dissolved inorganic N was also determined throughout the incubations in one of the two control cultures.

Growth rates were determined on algal subsamples. For each nutrient and at each sampling time, three 5-disk subsamples of *U. fenestrata* were collected from one of the three replicate jars, briefly rinsed with deionized water, dried (70° C, 24 h), and weighed. For *G. pacifica* growth determinations, five thallus pieces were enclosed in a small net cage inside one of the three replicate jars for each nutrient. The same five pieces were weighed (fresh) at each sampling time. Fresh weight was then converted to dry weight with the % dw estimated, on another subsample, at each sampling time. For both macroalgae an average specific growth rate (d^{-1}) was calculated for the entire incubation assuming exponential growth. The amount of algal biomass present in each jar at each sampling time was calculated from initial biomass corrected for growth and biomass removed at every previous sampling.

Uptake rates were expressed in μmol N·g dw^{-1}·h^{-1} and were calculated, for each time interval, in three different ways.

Net uptake rates were calculated from the difference between initial and final amounts of nutrient (NH_4^+ or NO_3^-) in the medium according to Eq. 1:

$$\text{net uptake} = \frac{\mu\text{mol}N_i - \mu\text{mol}N_f}{t \cdot B} \quad (1)$$

where $\mu molN_i$ and $\mu molN_f$ are the initial and final amounts, respectively, of ammonium or nitrate in the medium (concentration × volume), t is time (h), and B is the exponential average between the initial and final algal biomass (g dw).

^{15}N incorporation rate was calculated from appearance of the isotope in algal tissue:

$$^{15}\text{N incorporation} = \frac{(B_f \cdot TN_f \cdot \text{atom}\%\,^{15}N_f) - (B_i \cdot TN_i \cdot \text{atom}\%\,^{15}N_i)}{R \cdot t \cdot B} \quad (2)$$

where B_i and B_f are, respectively, the initial and final algal biomass (g dw); atom%$^{15}N_i$ and atom%$^{15}N_f$ are, respectively, the initial and final isotopic enrichment of algal TN (%); TN_i and TN_f are, respectively, the initial and final TN content of algae (μmol N·g dw^{-1}); and R (%) is the calculated exponential average of the initial and final atom % enrichment of dissolved NH_4^+ or NO_3^- (Glibert et al. 1982). Equation 2 differs from isotope incorporation rate equations used in most studies of macroalgae because it takes into account algal growth and variations of TN content.

Release rates of NH_4^+ or NO_3^- were calculated from isotope dilution in the medium:

$$\text{release} = \frac{\ln(R_f/R_i)}{\ln(\mu\text{molN}_f/\mu\text{molN}_i)} \cdot \frac{(\mu\text{molN}_i - \mu\text{molN}_f)}{t \cdot B} \quad (3)$$

where R_i and R_f are, respectively, the initial and final atom % enrichment of dissolved NH_4^+ or NO_3^- corrected, by subtraction, for the natural ^{15}N abundance of algae (algal ^{15}N determined at t_0). Equation 3 corresponds to the dilution rate d of Blackburn (1979) and Glibert et al. (1982), except that in Eq. 3 N amounts, instead of concentrations, are used, to take into account volume variations due to evaporation.

Finally, a "release corrected" uptake rate was calculated from the decrease of the dissolved NH_4^+ or NO_3^- corrected for NH_4^+ or NO_3^- released, and it is equivalent to the sum of net uptake and release:

$$\text{release corrected uptake} = \text{release} + \frac{\mu\text{molN}_i - \mu\text{molN}_f}{t \cdot B} \quad (4)$$

If no significant isotope dilution occurs, the net uptake rate is expected to be equal to the ^{15}N incorporation rate. If, on the contrary, isotope dilution is significant, the ^{15}N incorporation rate is expected to be equal to the release corrected uptake rate, because one of the model's assumptions is that no recently taken up ^{15}N is released.

We refer to the calculated rates as uptake rates, even though they represent both uptake and assimilation processes due to the long incubation times. Hourly rates represent the integrated measure of nitrogen uptake and assimilation over 24 h, or longer, time periods.

RESULTS

Ammonium uptake. The time course for changes of dissolved ammonium, FAA, and nitrate concentrations in *U. fenestrata* and *G. pacifica* cultures are shown in Figure 1. Over a 4-day period, dissolved ammonium concentrations decreased continuously in both *U. fenestrata* and *G. pacifica* cultures. The macroalgae depleted dissolved ammonium from 1 mM to less than 50 μM (Fig. 1a). The decrease was fastest on the first day of incubation, when about 50% of initial ammonium disappeared from the medium. Concurrently with net ammonium uptake, we observed a transient FAA release on the first day in both macroalgal cultures (Fig. 1b). The concentration of FAA-N initially increased to 40–60 μM, and on the next 2 days it declined to the initial levels. The amount of released FAA corresponds to about 6% and 12% of the ammonium taken up on the first day by *U. fenestrata* and *G. pacifica*, respectively. In *U. fenestrata*, but not *G. pacifica*, cultures, nitrate concentration increased linearly with time, up to 30 μM (Fig. 1c). The amount of nitrate released on the first day corresponds approximately to 1% of the ammonium taken up over the same time period.

Thallus TN content increased from 4.9% to 5.9% of dw in *U. fenestrata* and from 4.3% to 5.7% of dw in *G. pacifica* (Fig. 2). This TN increase occurred mostly on the first day in *U. fenestrata*, whereas it was more continuous in *G. pacifica*. For each sampling time, cumulative TN was calculated by multiplying algal TN (as % of dw) and algal biomass in each jar at that time and adding all previously sampled algal TN. In both algae, cumulative TN increases followed trends similar to those of TN content (Fig. 2).

A large significant isotope dilution occurred in *U. fenestrata* cultures during the first day, when atom % enrichment of dissolved ammonium decreased from 10% to approximately 7.9% (Fig. 3a). Little isotope dilution

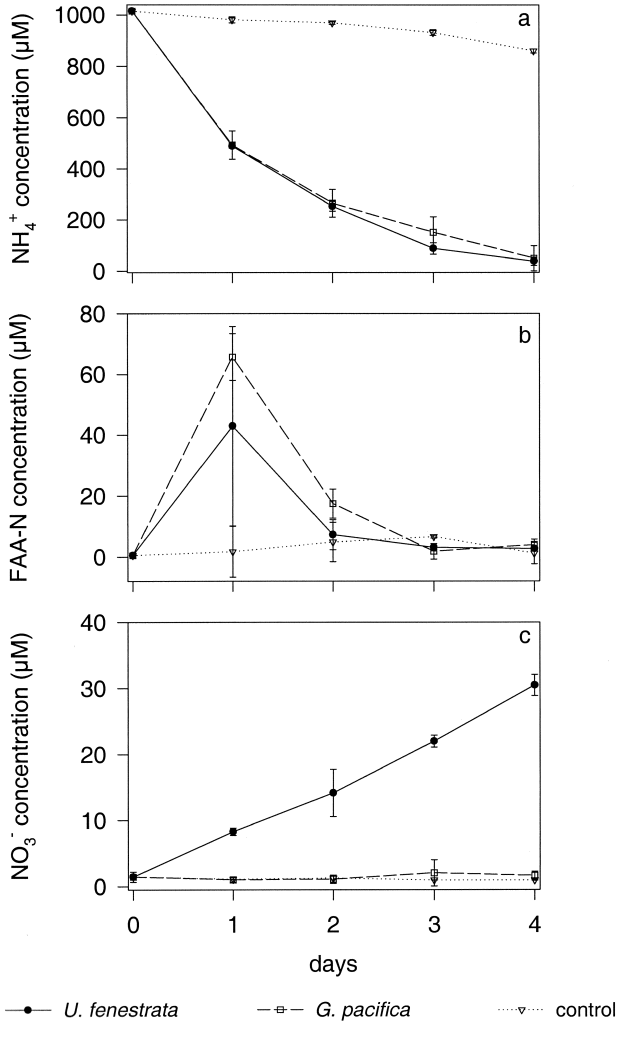

FIG. 1. Ammonium uptake experiment. Time variations of dissolved ammonium (a), free amino acid (b), and nitrate (c) concentrations in *Ulva fenestrata*, *Gracilaria pacifica*, and control cultures. Averages and standard deviations of three (macroalgae) or two (controls) replicates are shown.

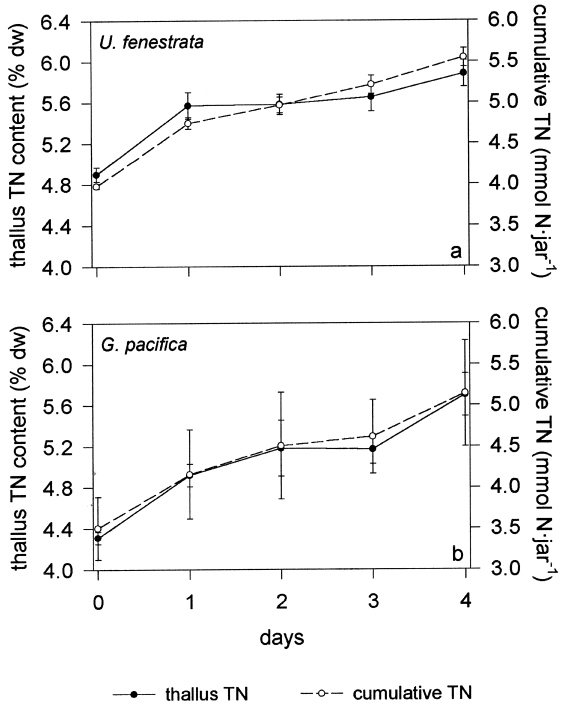

FIG. 2. Ammonium uptake experiment. Time variations of algal TN content and of cumulative algal N in *Ulva fenestrata* (a) and *Gracilaria pacifica* (b) cultures. Cumulative algal N is calculated by multiplying algal TN (as % of dw) and algal biomass in each jar and adding all sampled algal TN. Averages and standard deviations of three replicates are shown.

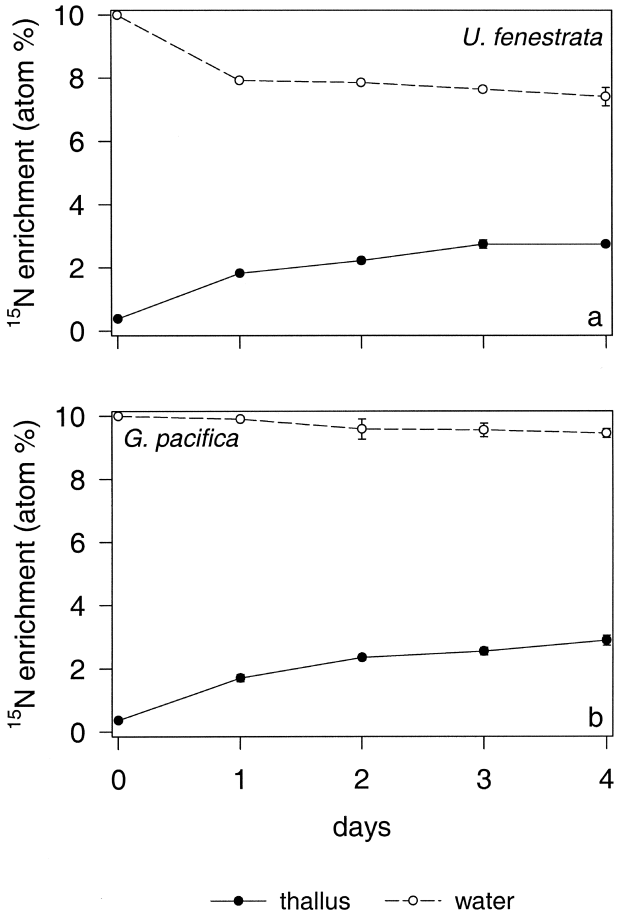

FIG. 3. Ammonium uptake experiment. Time variations of ^{15}N enrichment of algal TN and of dissolved ammonium in *Ulva fenestrata* (a) and *Gracilaria pacifica* (b) cultures. Averages and standard deviations of three replicates are shown.

occurred in the other algal cultures and the control culture. Nitrogen-15 atom % enrichment of thallus TN increased from natural levels to approximately 2.8% in both macroalgae over the entire 4-day period (Fig. 3, a and b). The increase was fastest on the first day (1.44 and 1.34 atom % d^{-1} in *U. fenestrata* and *G. pacifica*, respectively), possibly due to fast isotope exchange between internal and external ammonium pools. Subsequently, ^{15}N atom % enrichment rate of thalli slowed to approximately 0.33 atom % d^{-1} in *U. fenestrata* and 0.37 atom % d^{-1} in *G. pacifica*.

Ammonium uptake rates were similar for the two macroalgae, and the highest uptake rates were observed on the first day of incubation, averaging 45 μmol N·g $dw^{-1}·h^{-1}$ in *U. fenestrata* and 32 μmol N·g $dw^{-1}·h^{-1}$ in *G. pacifica* (Table 1). Uptake rates decreased to between 5 and 15 μmol N·g $dw^{-1}·h^{-1}$ for days 2–4. Ammonium release rates were also highest during the first day of incubation for *U. fenestrata*, which released 11 μmol N·g $dw^{-1}·h^{-1}$ or about 25% of gross uptake. Later ammonium release rates by *U. fenestrata* and all release rates by *G. pacifica* ranged from 0.1 to 0.8 μmol N·g $dw^{-1}·h^{-1}$ and accounted for 1%–6% of gross uptake. Because of the large initial ammonium release and isotope dilution of dissolved ammonium for *U. fenestrata*, net disappearance of ammonium is not a good measure of ammonium uptake (considered as movement of ammonium across cell membranes). After this initial period for both macroalgae there is fairly good agreement (except on the last day of incubation) between uptake rates measured as net ammonium disappearance from the medium and ^{15}N incorporation (Table 1).

Nitrate uptake. Trends of nitrate concentration were very different from the changes in ammonium concentrations. During the first 2–3 days of incubation, little or no significant nitrate decrease was observed in either *U. fenestrata* or *G. pacifica* cultures. During the subsequent days, both macroalgae took up nitrate, and nitrate concentrations decreased steadily from 1 mM to approximately 400 μM (Fig. 4a). Very little FAA release occurred at the end of the experiment (Fig. 4b).

In both macroalgae the highest growth rates were measured over the first 9 days of incubation (0.020 d^{-1} in *U. fenestrata*, 0.035 d^{-1} in *G. pacifica*); between day 9 and the end of the experiment growth decreased to about 50% of the initial values. The TN content of *U. fenestrata* declined throughout the experimental period, from 4.9% to 4.3% of dw (Fig. 5a). The decrease was faster during the first 3 days, when little significant uptake occurred. Even if thallus TN (as % dw) decreased,

TABLE 1. Comparison of ammonium uptake rates.

	Uptake rates							
	Net NH_4^+ uptake (μmol N·g^{-1}·h^{-1})		^{15}N incorporation (μmol N·g^{-1}·h^{-1})		Release corrected (μmol N·g^{-1}·h^{-1})		Release rate (μmol N·g^{-1}·h^{-1})	
Time (day)	Average	SD	Average	SD	Average	SD	Average	SD
				Ulva fenestrata				
0–1	33.70	0.23	38.59	0.50	44.97	0.22	11.27	0.19
1–2	12.12	0.87	10.59	2.69	12.28	0.95	0.16	0.08
2–3	8.10	1.41	14.59	6.19	8.32	1.46	0.22	0.07
3–4	2.56	0.29	6.02	5.71	2.64	0.27	0.08	0.05
				Gracilaria pacifica				
0–1	31.30	0.38	23.38	2.34	31.76	0.62	0.47	0.24
1–2	11.76	1.75	12.44	2.92	12.53	2.63	0.77	0.89
2–3	5.37	0.42	4.66	4.48	5.60	0.29	0.23	0.14
3–4	5.00	1.03	11.09	0.75	5.12	1.06	0.12	0.03
				Control (μmol N·L^{-1}·h^{-1})				
0–1	1.61	0.48	—	—	—	—	—	—
1–2	0.48	0.72	—	—	—	—	—	—
2–3	1.46	0.76	—	—	—	—	—	—
3–4	2.80	0.79	—	—	—	—	—	—

Net NH_4^+ uptake refers to uptake calculated from the difference between initial and final amounts of NH_4^+ in the medium (Eq. 1); ^{15}N incorporation refers to uptake calculated from appearance of the isotope in algal tissue (Eq. 2); release corrected refers to uptake calculated from dissolved NH_4^+ decrease corrected for NH_4^+ release (Eq. 4); NH_4^+ release is calculated from isotope dilution (Eq. 3). Averages and standard deviations (SD) of three (macroalgae) or two (controls) replicates are shown.

cumulative TN remained relatively constant during the first 3 days, due to macroalgal growth; later, when uptake started, cumulative TN did increase (Fig. 5a). Thallus TN content of *G. pacifica* decreased from 4.3% to 3.9% of dw during the first 3 days and then it increased to approximately the initial level (Fig. 5b). The cumulative TN increased steadily between day 3 and the end of the experiment.

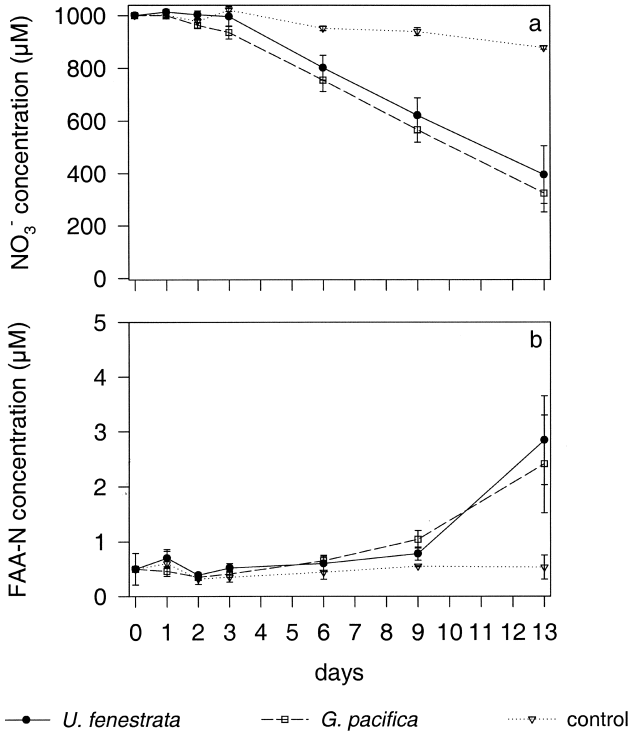

FIG. 4. Nitrate uptake experiment. Time variations of dissolved nitrate (a) and free amino acid (b) concentrations in *Ulva fenestrata*, *Gracilaria pacifica*, and control cultures. Averages and standard deviations of three (macroalgae) or two (controls) replicates are shown.

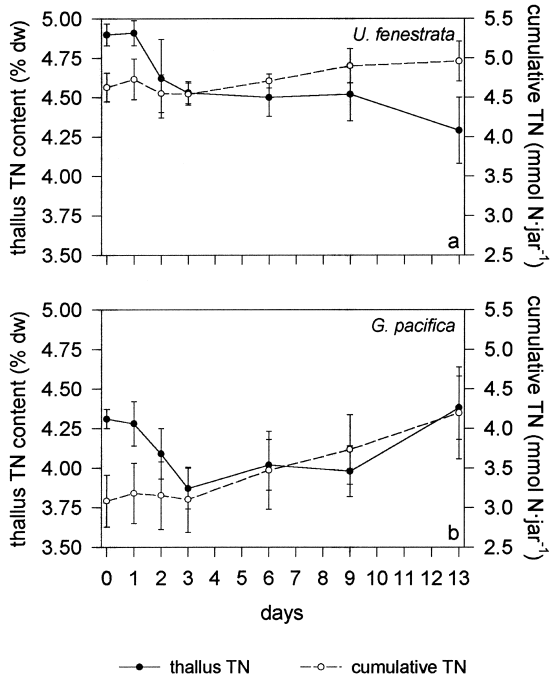

FIG. 5. Nitrate uptake experiment. Time variations of algal TN content and of cumulative algal N in *Ulva fenestrata* (a) and *Gracilaria pacifica* (b) cultures. Cumulative algal N is calculated by multiplying algal TN (as % of dw) and algal biomass in each jar and adding all sampled algal TN. Averages and standard deviations of three replicates are shown.

In both macroalgae, the lag of nitrate uptake may have resulted from the need for either some N deprivation (use of nitrate pools) or physiological/metabolic changes required before the uptake of nitrate. During the first 3 days of incubation, the growth of new biomass was sustained by internal TN reserves, as shown by decreases of thallus TN. Nitrogen-15 atom % enrichment of thallus TN increased from natural levels to approximately 2% in both macroalgae (Fig. 6). Very little isotope dilution occurred in either macroalgal culture throughout the incubation (Fig. 6). In the control culture, isotope enrichment decreased slightly from 9.57 atom % to 9.05 atom %.

Nitrate uptake rates were similar for the two macroalgae but much lower than ammonium uptake rates, averaging 2.50 and 2.61 μmol N·g dw^{-1}·h^{-1} in *U. fenestrata* and *G. pacifica*, respectively (Table 2). Uptake rates calculated by nitrate disappearance (corrected for nitrate release) and ^{15}N incorporation in algal tissue were statistically equivalent (*t*-tests). The agreement (defined as the percentage ratio of the lower to the higher rate) between rates calculated in the two different ways averaged 91% in *U. fenestrata* and 95% in *G. pacifica*. Nitrate release was generally less than 0.4 μmol N·g dw^{-1}·h^{-1} in both macroalgae (Table 2).

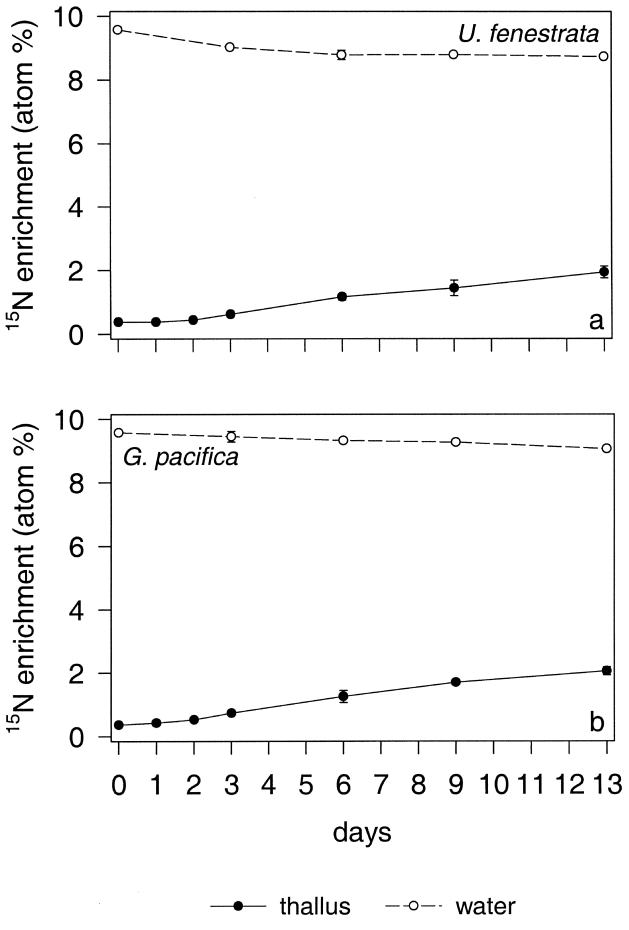

FIG. 6. Nitrate uptake experiment. Time variations of ^{15}N enrichment of algal TN and of dissolved nitrate in *Ulva fenestrata* (a) and *Gracilaria pacifica* (b) cultures. Averages and standard deviations of three replicates are shown.

Recovery of ^{15}N. A mass balance of ^{15}N is presented in Table 3. In *U. fenestrata* and *G. pacifica* cultures an average of 96% (SD = 3%, $n = 6$) of added $^{15}NH_4^+$ was recovered from the medium and algal tissue at the end of the incubation. Recovery of added $^{15}NO_3^-$ was virtually complete, with an average value of 99% (SD = 3%, $n = 6$). In the control cultures, isotope recovery was 88% and 91% in ammonium and nitrate incubations, respectively.

DISCUSSION

Net release of inorganic and organic N. We did not observe a net release of NH_4^+ by either algal species. As shown below, this resulted from uptake rates that exceed release rates. In contrast, we did observe net release of NO_3^- by *U. fenestrata* in NH_4^+ medium. Nitrification is unlikely because NO_3^- did not increase in the control cultures or with *G. pacifica*. Corzo and Niell (1992) found net efflux of both NH_4^+ and NO_3^- from *Ulva rigida* with maximum release rates of about 12 and 2 μmol N·g dw^{-1}·h^{-1}, respectively. Nitrate release by *U. fenestrata* in our experiments was slower (0.4 μmol N·g dw^{-1}·h^{-1}) but represented a net release of about 7% per day from the internal nitrate pool of 133 μmol N·g dw^{-1} (determined according to Naldi and Wheeler 1999). Absence of release in *G. pacifica* despite a similar internal NO_3^- pool suggests a better retention capacity for NO_3^- by *G. pacifica*. A complementary explanation could be the different thallus morphology of the two species. *Ulva fenestrata* has a sheet-like thallus just two cells thick, with a high surface area:volume quotient, so that all cells have access to the external medium, whereas *G. pacifica* thallus is several cells thick (lower surface area:volume quotient), so that most cells are not in contact with the medium.

We measured very little organic N release in our experiments (but see discussion below). FAA concentration only increased significantly in the ammonium experiment on the first day; proteins were never detectable (data not shown). Studies on dissolved organic matter (mostly dissolved organic carbon) release by macroalgae have produced contrasting results in the past. Up to 45% of the photosynthetically fixed carbon was released as dissolved organic matter by several macroalgal species (Sieburth 1969). Other authors, however, found lower dissolved organic matter release rates, usually representing less than 10% of fixed carbon (Fankboner and de Burgh 1977, Pregnall 1983, Carlson and Carlson 1984). Our isotopic results and mass balance (below) confirm the low organic N release for *U. fenestrata* and *G. pacifica*.

Isotopic measurements of release rates. Although neither algal species showed a net release of NH_4^+, the isotopic measurements clearly showed significant release of both NH_4^+ and NO_3^-. The release of unlabeled NH_4^+ and NO_3^- must be considered for the accurate determi-

TABLE 2. Comparison of nitrate uptake rates.

	Uptake rates							
	Net NO_3^- uptake (μmol N·g^{-1}·h^{-1})		^{15}N incorporation (μmol N·g^{-1}·h^{-1})		Release corrected (μmol N·g^{-1}·h^{-1})		Release rate (μmol N·g^{-1}·h^{-1})	
Time (day)	Average	SD	Average	SD	Average	SD	Average	SD
				Ulva fenestrata				
3–6	2.73	0.31	2.95	0.07	3.09	0.53	0.36	0.26
6–9	2.37	0.18	2.35	1.59	2.35	0.17	−0.02	0.17
9–13	2.03	0.30	1.97	0.86	2.06	0.31	0.03	0.02
				Gracilaria pacifica				
3–6	2.98	0.20	2.84	0.80	3.19	0.41	0.22	0.23
6–9	2.55	0.32	2.56	0.45	2.60	0.32	0.06	0.02
9–13	1.98	0.33	2.10	0.61	2.07	0.33	0.09	0.02
				Control (μmol N·L^{-1}·h^{-1})				
3–6	0.97	0.04	—	—	—	—	—	—
6–9	0.06	0.08	—	—	—	—	—	—
9–13	0.56	0.10	—	—	—	—	—	—

Net NO_3^- uptake refers to uptake calculated from the difference between initial and final amounts of NO_3^- in the medium (Eq. 1); ^{15}N incorporation refers to uptake calculated from appearance of the isotope in algal tissue (Eq. 2); release corrected refers to uptake calculated from dissolved NO_3^- decrease corrected for NO_3^- release (Eq. 4); NO_3^- release is calculated from isotope dilution (Eq. 3). Averages and standard deviations (SD) of three (macroalgae) or two (controls) replicates are shown.

nation of N uptake by N-rich macroalgae, particularly when ammonium is supplied as the N source. In the NH_4^+ experiment, *U. fenestrata* released on average 11 μmol NH_4^+-N·g dw^{-1}·h^{-1} on the first day and 0.2 μmol NH_4^+-N·g dw^{-1}·h^{-1} thereafter. The initial NH_4^+ release corresponds to 25% of gross NH_4^+ uptake, similar to the 30% reported by O'Brien and Wheeler (1987) for *Enteromorpha prolifera*. Very little release of NH_4^+ occurred after the first day in *U. fenestrata* cultures and in most of the NH_4^+ cultures with *G. pacifica*.

The release of inorganic nitrogen can result from the isotopic equilibration of internal and external pools (Fujita et al. 1988). In short-term experiments with *U. rigida*, Fujita et al. (1988) found that the cellular NH_4^+ pool became labeled rapidly and reached isotopic equilibrium with the medium during the first 30 min of the incubation. The high NH_4^+ release rate measured on the first day for *U. fenestrata* in our experiment is a conservatively low estimate if isotopic equilibrium was reached in the first few minutes or hours. The pool exchange mechanism could also explain why NH_4^+ release decreased with time.

Regeneration of NH_4^+ can also contribute to the observed isotope dilution of dissolved NH_4^+. McGlathery et al. (1997) found that decomposition and regeneration from *Chaetomorpha linum* mats resulted in a 35%–45% dilution of the added $^{15}NH_4^+$. Regeneration rates ranging between 20% and 189% of daily gross inorganic

TABLE 3. Recovery of ^{15}N in NH_4^+ and NO_3^- incubations with *Ulva fenestrata* and *Gracilaria pacifica* and in control incubations.

	Initial ^{15}N			Final ^{15}N				Recoverd ^{15}N
Replicate jars	Dissolved (μmol)	Algal (μmol)	Total (μmol)	Dissolved (μmol)	Algal (μmol)	Removed subsamples (μmol)	Total (μmol)	(% of initial)
				$^{15}NH_4^+$ incubation (t_f = 4 days)				
				Ulva fenestrata				
1	152	15	167	1	110	49	161	96
2	152	15	167	3	108	47	158	95
3	152	15	167	4	103	48	155	93
				Gracilaria pacifica				
1	152	11	164	10	101	40	152	93
2	152	14	166	1	132	32	165	99
3	152	13	165	4	121	36	162	98
				Control				
1	152	—	152	89	—	45	134	88
				$^{15}NO_3^-$ incubation (t_f = 13 days)				
				Ulva fenestrata				
1	144	18	162	21	60	72	153	95
2	144	17	161	29	57	73	159	99
3	144	17	161	37	50	75	162	100
				Gracilaria pacifica				
1	144	11	155	28	64	62	155	100
2	144	13	156	18	82	62	163	104
3	144	10	154	28	53	66	148	96
				Control				
1	144	—	144	67	—	64	131	91

N uptake have been observed in several studies with phytoplankton (e.g. Dickson and Wheeler 1995, Bronk and Ward 1999). However, because no dissolved NH_4^+ increase was observed in our controls or NO_3^- incubations, NH_4^+ regeneration was insignificant, unless the regenerated NH_4^+ was immediately taken back up by the algae.

In the NO_3^- incubations with both algal species, NO_3^- release was low (0.2–0.4 μmol N·g dw^{-1}·h^{-1}), but during the first half of the experiment it amounted to 7%–18% of gross NO_3^- uptake. No NH_4^+ release occurred in the incubations with NO_3^-. It is interesting that these rates were similar to the net NO_3^- release of *U. fenestrata* in NH_4^+ medium. We suggest that the internal NO_3^- pools are slowly released (over a period of several days) after preincubation with high external NO_3^-.

As discussed above, we saw little net release of FAA by *U. fenestrata* and *G. pacifica*. High dissolved organic nitrogen (DON) release rates (ranging between 25% and 68% of uptake) have been measured in studies with phytoplankton (Collos et al. 1992, Bronk et al. 1994, Slawyk and Raimbault 1995, Bronk and Ward 1999), and the unmeasured release of ^{15}N in the DON pool can lead to low isotope recovery. However, Slawyk et al. (1998) found that losses of dissolved inorganic ^{15}N to the DON pool were negligible in laboratory uptake experiments with phytoplankton and represented on average only 15% of gross dissolved inorganic ^{15}N uptake in the open ocean. In our study, the high recovery of ^{15}N in algal tissue and in the dissolved inorganic nitrogen pools suggests that release of DON by these macroalgal species is not significant.

Recovery of ^{15}N and comparison of uptake rates. The recovery of ^{15}N at the end of the incubations was very good for $^{15}NH_4^+$ (96%) and virtually complete (99%) for $^{15}NO_3^-$. Similar $^{15}NH_4^+$ recovery (90%–93%) was obtained by O'Brien and Wheeler (1987) and McGlathery et al. (1997) in experiments with macroalgae. The almost complete recovery of the tracer, at the end of the experiment, between the dissolved inorganic pool and algal tissue confirms that the net release of labeled DON must have been low in the long term. Agreement between ^{15}N uptake rates and net uptake plus release was good for both macroalgae at high ammonium concentrations and for nitrate. At the end of the experiment with lower NH_4^+ concentrations, however, ^{15}N incorporation rates exceeded net uptake plus release. We attribute this discrepancy to the variability in estimates of thallus N in longer incubations. The utility of ^{15}N isotopes in long incubations is limited by the degree to which accurate measurement of changes in thallus nitrogen is possible.

Advantages of isotopic methods. Isotopic measurements can be more involved than following net disappearance of nutrients but are necessary for estimates of actual fluxes. The release of ammonium would not have been detected without the use of ^{15}N. Furthermore, without the determination of $^{15}NH_4^+$ of the dissolved pool, the ^{15}N incorporation uptake rates would have been underestimated by 25%. Determination of ^{15}N atom % enrichment of the dissolved NH_4^+ is thus recommended when N-rich thalli are used; otherwise, isotope dilution by released NH_4^+ from algal tissue will result in poor recovery and underestimation of uptake rates. Ammonium uptake kinetics estimated in short-term experiments with the classical net nutrient decrease method may be particularly sensitive to this problem. In contrast, isotope dilution seems less significant in incubations with $^{15}NO_3^-$, even if nitrate-rich thalli are used. Nitrate uptake rates were low in our experiments, however, and NO_3^- release rates may be more significant at higher NO_3^- uptake rates.

A further consideration for experiments with macroalgae concerns growth measurements. For phytoplankton uptake studies with ^{15}N, Collos (1987) recommended that final particulate nitrogen (PN) concentration should be used for calculations only if PN variation (ΔPN) is lower than 10%. In longer incubations required for macroalgae, however, both biomass and algal TN content can vary. In our experiments, the daily growth rates were lower than 10% (data not shown), but growth combined with TN variations resulted in ΔPN values higher than 10% d^{-1} in most cases. It is thus important to determine growth rates in long-term experiments with macroalgae, particularly if the culture conditions are favorable for high growth rates.

Effect of algal nutrient status on uptake rates. The high TN content of thalli used in these experiments influenced nitrate uptake rates in both macroalgae. Negligible uptake of nitrate was observed during the first 2–3 days. Apparently, the utilization of part of the internally accumulated tissue N was necessary before the onset of NO_3^- uptake. Growth conditions probably influenced nitrate utilization too. Nitrate metabolism has higher light requirements than ammonium (Davison and Stewart 1984, O'Brien and Wheeler 1987), and it is possible that the light conditions maintained during the experiment (100 μmol photons·m^{-2}·s^{-1}) were not favorable for maximum rates of cellular nitrate reduction and of external nitrate uptake.

Inhibition of nitrate uptake in algae with high TN content has been reported in several macroalgal species (Rosenberg et al. 1984, Fujita 1985, O'Brien and Wheeler 1987, Duke et al. 1989). Inhibition of ammonium and nitrate uptake by cellular pools of inorganic nitrogen in short-term experiments has been shown for *Porphyra perforata* (Thomas and Harrison 1985), *Ulva lactuca* (Pedersen 1994), and *Chaetomorpha linum* (McGlathery et al. 1996). To our knowledge, only McGlathery et al. (1997) described a prolonged (48 h) lag period before the occurrence of NH_4^+ uptake in field incubations with *C. linum*. The authors attributed the delay of uptake to the turnover of existing internal N pools. For *U. fenestrata* and *G. pacifica* we observed a lag period only in nitrate incubations, whereas NH_4^+ was taken up immediately. Thus, NH_4^+ uptake on the first 2 days may have been passive influx at very high external concentrations.

Implications for physiological and ecological studies. Our comprehensive analysis of nitrogen uptake and release by *U. fenestrata* and *G. pacifica* provide important insights for further work with macroalgae. Measurements of multiple internal and external nitrogen pools ensured complete recovery of added isotopes and clearly showed that release of labeled DON was not a significant process for these two algae under nitrogen-rich conditions. This contrasts sharply with the previous studies with phytoplankton (Glibert et al. 1982, Laws 1984, Collos et al. 1992, Bronk et al. 1994, Slawyk and Raimbault 1995, Bronk and Ward 1999). Recently, however, Flynn and Berry (1999) demonstrated that the ^{15}N protocol may significantly overestimate the net loss of DON from phytoplankton cells in field incubations. Although some DON may be released by both phytoplankton and macroalgae under certain conditions, our results suggest that accurate measurement of external and internal nitrogen pools and good isotope recovery are essential to evaluate the significance of DON release. The absence of sustained DON release in *U. fenestrata* and *G. pacifica* during nitrogen-rich incubations may result from the relatively small internal FAA pools (Naldi and Wheeler 1999) and from the close coupling of rates of amino acid and protein synthesis demonstrated in *Gracilariopsis lemaneiformis* (Vergara et al. 1995).

Ammonium efflux was most significant with *U. fenestrata* but for both algae could only be detected with isotopes because uptake rates always exceeded release rates. Nonetheless, internal NH_4^+ can be large, and isotope exchange with external pools causes a significant isotope dilution and potential underestimate of NH_4^+ uptake rates with ^{15}N tracers. The faster NH_4^+ efflux for *U. fenestrata* probably results from a greater surface area:volume quotient compared with *G. pacifica.* Net NO_3^- efflux for *U. fenestrata* could result from the inhibition of NO_3^- uptake by high availability of NH_4^+. It is clear from our experiments that higher tissue nitrogen was obtained by both algal species with NH_4^+ as the primary nitrogen source. Moreover, the lag period before NO_3^- utilization indicates the requirement for a major shift in metabolism prior to NO_3^- supported growth.

Ulva and *Gracilaria* thrive in eutrophic environments. In coastal lagoons, nitrate concentrations can exceed 100 μM and DON concentrations range from 50 to 120 μM (Viaroli et al. 1993). Dissolved inorganic nitrogen can be inversely related to nitrogen in macroalgal thalli and during the growing season most of the nitrogen is accumulated in the algal biomass (Viaroli et al. 1993, Valiela et al. 1997, McGlathery et al. 2001). Macroalgal blooms in such regions are followed by release of DON to the water column (Viaroli et al. 1993, Tyler et al. 2001); our results suggest that the release of DON is not from live algal tissue but is more likely due to degradation of detritus within these systems. The potential significance of release of inorganic and organic nitrogen by nitrogen-rich macroalgae seems relatively low for *U. fenestrata* and *G. pacifica.* The only sustained net release was for NO_3^- by *U. fenestrata* when switched from a high $NO_3^- + NH_4^+$ medium to a high NH_4^+ medium. Our results combined with metabolic studies by Vergara et al. (1995) suggest that a combination of efficient transport systems and coupled assimilatory rates prevent major net losses of inorganic nitrogen and organic metabolites from macroalgae. Our results do indicate, however, that net disappearance of NO_3^- and/or NH_4^+ must be combined with release measurements and changes in thallus N content to assess the contribution of each N source to algal growth in laboratory and field experiments with macroalgae.

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