Vol. 100: 283–286, 1993 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published October 19

Internal N-cycling, measured by $^{15}NH_4^+$ dilution, in *Cladophora sericea* in a shallow Danish bay

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ABSTRACT: $^{15}NH_4^+$ was added to *Cladophora sericea* mats to give ca 30% enrichment. The mats were incubated in the dark and the dilution of the $^{15}NH_4^+$ was followed. Some of the $^{15}NH_4^+$ label was not recovered in the algal cells nor in the water at time zero. This loss amounted to ca 40% of the $^{15}NH_4^+$ label. The lost label reappeared during the incubation. $^{15}NH_4^+$ was incorporated into the cells in the dark. The incorporation rate was greater when the cells had previously been exposed to strong sunlight. There was little change in NH_4^+ concentration during incubation, but the calculated rate of NH_4^+ production was 3.82 mmol m^{-2} d^{-1} This was presumed to be the amount available for resynthesis into cells.

INTRODUCTION

A nitrogen budget was made for the filamentous algae *Cladophora sericea* in a shallow Danish bay with different sampling methods (Thybo-Christesen et al. 1993). Net changes in NH_4^+ concentration in the water column gave net incorporation and growth rates. The total NH_4^+ incorporation, consisting of recycled NH_4^+ and NH_4^+ coming from external sources, could not be measured by these methods. In this study, the $^{15}NH_4^+$ dilution technique was used to measure NH_4^+ production within the mat and to infer that NH_4^+ was available for algal growth. The technique was described by Blackburn (1979a, b) and Caperon et al. (1979). The ^{15}N technique has previously been used to study ecosystems containing bacteria, phytoplankton and microzooplankton. This study applied the technique to filamentous algal mats for the first time.

The $^{15}NH_4^+$ dilution technique is a relatively complicated and expensive methodology and is probably restricted to experimental research, unlike simpler methods as described in Thybo-Christesen et al. (1993).

MATERIALS AND METHODS

The archipelago of southern Fyn, Denmark, is a 415 km^2 large shallow water area. In one-third of the area the water depth is < 2 m. The Lunkebugten Bay (54° 59' N, 10° 39' E) was chosen as a representative shallow water area for the study of *Cladophora sericea* algal mats (hereafter mats). This species was the most abundant filamentous green algae in May–June (Thybo-Christesen et al. 1993).

Mats were incubated *in situ* in 10 dark bottles for every experiment. Incubation was done in the dark as it was assumed that N-mineralization was unaffected by light, but rapid uptake of NH_4^+ in the light would have depleted the labelled NH_4^+ pool (Thybo-Christesen et al. 1993). New 1 l polyethylene bottles with an opening internal diameter of 5.5 cm, closed by screw cap, were washed with 10% HCl and rinsed with deionized water. Five bottles were incubated with mat (ca 0.29 g l^{-1}), and 5 bottles were used as controls. All the bottles were wrapped in aluminium foil. In every bottle, 1 µmol $^{15}NH_4^+$ was added to give ca 30% enrichment. All bottles were placed in open boxes *in situ* at a water depth of 0.8 to 1.2 m. After 0, 0.5, 1.0, 2.0 and 4.0 h respectively, 2 of the 10 bottles (1 containing mat and 1 control) were collected and 6 ml water was sampled from each bottle and frozen for later nutrient analysis. All biological activity in the bottles was then stopped by adding $ZnCl_2$ to 0.5% concentration. The water and the mat were separated and frozen. The water samples (246 ml) were analyzed for $^{15}NH_4^+$ by

using a modification of Kristiansen & Paasche (1989). A NH_4^+ recovery efficiency of 83% was obtained in bottles without mat during diffusion by shaking for 65 h at 20°C. This high efficiency precluded isotope fractionation during diffusion in the analysis (Dugdale & Wilkerson 1986). The mat was thawed, dried, homogenized, weighted, enclosed in tin capsules and analyzed for ^{15}N content on a mass spectrometer (VG-isogas). The C:N ratios were determined on a Carlo-Erba NA-1500 analyzer.

One experiment was carried out in June 1990, and 6 in June 1991 (2 each on 24, 26 and 29 June).

RESULTS

The algal cells were examined for their ^{15}N content (Fig. 1). There was a linear uptake of $^{15}NH_4^+$ in the dark for 2 h. The rates were variable and there was evidence that the rate of uptake was dependent on the previous exposure of the mat to light. The photon flux was 1100, 400 and 800 µE m^{-2} s^{-1} and corresponded to uptake rates of 1.89, 0.53 and 1.25 µmol g^{-1} h^{-1} on 24, 26 and 29 June respectively. The recovery of ^{15}N in the cells and in the water, on these dates in June 1991, showed an increasing recovery with time of incubation (Fig. 2). Disappearance and reappearance of $^{15}NH_4^+$ with time was never observed in the control incubations without mat (n = 30). In the controls there was no dilution of ^{15}N label. In bottles containing seawater and mat, there was a dilution of $^{15}NH_4^+$, indicating NH_4^+ production (Table 1).

There was an insignificant change in NH_4^+ concentration during the incubation for 4 h. Therefore the turnover constant k was calculated, rather than the more complicated kinetics previously described (Blackburn 1979a, Glibert et al. 1982). The k values varied from 0.15 to 0.31 h^{-1} with a mean of 0.22. The total rate of NH_4^+ production was obtained from NH_4^+ concentration multiplied by the k value. These rates varied from 0.45 to 0.73 µmol l^{-1} h^{-1} with a mean of 0.53. Integration of these values to an areal basis gave rates of ammonium production from 2.21 to 6.10 mmol m^{-2} d^{-1} with a mean of 3.82.

The C:N molar ratio (n = 60) was 37.0 ± 4.4 for mat.

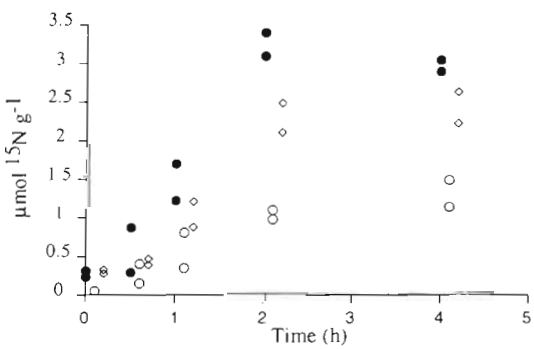

Fig. 1 *Cladophora sericea*. Changes in $^{15}NH_4^+$ content within the mat from time zero to 4 h of incubation in the dark. Values are averages of 2 measurements. Data are from 24, 26 and 29 June 1991. Photon flux was (•) 1100, (○) 400 and (◇) 800 µE m^{-2} s^{-1}, before the sample series

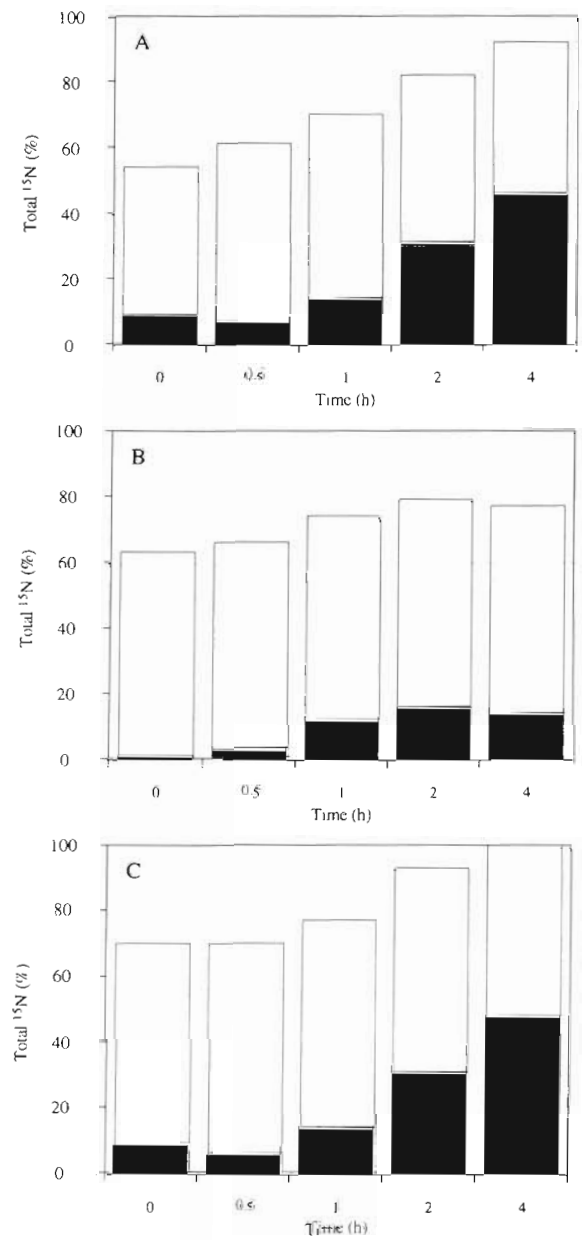

Fig. 2. *Cladophora sericea*. Changes in total $^{15}NH_4^+$ content within the mats and in the water from time zero to 4 h of incubation. Data are from (A) 24 June, (B) 26 June and (C) 29 June 1991 (■) mat, SD = 1.31 ± 0.66% (□) water, SD = 2.94 ± 0.92%

Table 1. $^{15}NH_4^+$ dilution in dark-incubated bottles containing *Cladophora sericea*. NH_4^+ concentration at time zero is an average of 10 measurements. *k*: calculated turnover constant; r: correlation coefficient. Total NH_4^+ production is calculated by multiplying $[NH_4^+]$ and *k*

Date	Expt	$[NH_4^+]$ ($\mu mol\ l^{-1}$)	*k* (h^{-1})	Total NH_4^+ production ($\mu mol\ l^{-1}\ h^{-1}$)	NH_4^+ production ($mmol\ m^{-2} d^{-1}$)
10 Jun 1990	1	2.98 ± 0.13	0.15, r = 0.85	0.45	2.21
24 Jun 1991	1	2.36 ± 0.68	0.31, r = 0.81	0.73	4.19
	2	2.08 ± 0.35	0.27, r = 0.87	0.56	3.21
26 Jun 1991	1	2.70 ± 0.73	0.27, r = 0.92	0.73	6.10
	2	2.82 ± 0.93	0.17, r = 0.90	0.48	4.01
29 Jun 1991	1	2.36 ± 0.68	0.17, r = 0.91	0.40	3.42
	2	2.08 ± 0.38	0.20, r = 0.90	0.42	3.60
Mean		2.48	0.22	0.53	3.82

DISCUSSION

Although the main purpose of these experiments was to measure $^{15}NH_4^+$ dilution, it was observed that some ammonium uptake occurred in the dark (Fig. 1), as has previously been observed for *Cladophora* spp. (Gordon et al. 1981, Thybo-Christesen et al. 1993). This uptake is discussed in some detail as it has relevance to $^{15}NH_4^+$ disappearance and probable reappearance during the experiment. The ^{15}N uptake ceased after 2 h of incubation (Fig. 1) as has been observed in phytoplankton and microzooplankton ^{15}N studies (Goldman et al. 1981, Glibert et al. 1982, Paasche & Kristiansen 1982, Laws 1985).

Earlier experiments (Thybo-Christesen et al. 1993) had shown that there was a pool of NH_4^+ which was loosely bound to the mat and was released at the end of the light period. This NH_4^+ was subsequently taken up by the mat in the dark. There was evidence (Fig. 2) that there might also be some NH_4^+ loosely bound in the dark, as a portion of the $^{15}NH_4^+$ was not recovered at time zero. A probable explanation was that $^{15}NH_4^+$ was adsorbed to the mat and washed off in the preparation of the algal cells for analysis. This loosely bound $^{15}NH_4^+$ was incorporated into the mat with time (Fig. 2), consistent with the earlier observations of NH_4^+ uptake in the dark. An initial adsorption of $^{15}NH_4^+$ to the cell surface prior to absorption has previously been demonstrated in *Zostera marina* (Short & McRoy 1984).

The total recovery of $^{15}NH_4^+$ rose from 75 to 83% during the experiment in bottles with algae. In ^{15}N experiments, constant, or increasing net loss of tracer had previously been found and explained as systematic error in the field samples, adsorption to the bottle wall, nitrification, or incorporation into a third pool (dissolved organic nitrogen) (Glibert et al. 1982, Laws 1984, Williams & Fischer 1985, LaRoche & Harrison 1987). The decreasing loss in our study indicated that other explanations were needed.

The reappearance of $^{15}NH_4^+$ with time would underestimate the $^{15}NH_4^+$ dilution. The mean value of NH_4^+ production of 3.82 mmol m^{-2} d^{-1} is probably a minimal estimate. As there was no marked change in the NH_4^+ concentration with incubation time, it was concluded that production of NH_4^+ equalled uptake. Thus, 3.82 mmol m^{-2} d^{-1} was a minimum value for uptake, when no external NH_4^+ source was available. The net rate of NH_4^+ incorporation, however, was 1.33 mmol m^{-2} d^{-1} (Thybo-Christesen et al. 1993), indicating that NH_4^+ was supplied from outside the mat in addition to being recycled. The most likely source of NH_4^+ in the (semi-) open systems was the sediment. Though the mean value was relatively low (Thybo-Christesen et al. 1993), an experiment in June 1991 (data not presented) indicated that the sediment could export as much as ca 10 mmol NH_4^+ m^{-2} d^{-1}, which was more than adequate to meet this demand.

The net daily production in the mat represented only 3% of the total mat biomass. Thus, the mat would have net growth for several days before the increase would be detectable and might be exported laterally or vertically.

The rate of NH_4^+ uptake in the dark depended on the extent of previous exposure of the mat to light. Possibly, a high photon flux prior to the incubation would result in a higher N-depletion of the mat and thus a higher N-demand in the dark. The C:N (molar) ratio was higher (37.0 ± 4.4) in *Cladophora sericea* compared to a ratio of 7 for other *Cladophora* spp. (Atkinson & Smith 1983).

Acknowledgements. Financial support was provided through the ISHTAR project by grant DPP-B605659 from the Division of Polar Programs, National Science Foundation.

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This article was submitted to the editor

Manuscript first received: February 15, 1993
Revised version accepted: July 27, 1993