Uptake of dissolved free amino acids (DFAA) by microphytobenthic communities

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ABSTRACT: Although it is established that labile fractions of dissolved organic nitrogen (DON), such as dissolved free amino acids (DFAA), can be utilized by microalgae, few studies have considered their quantitative importance for microphytobenthos (MPB). Mixed MPB communities, from 1 and 20 m water depth and treated in 2 ways (semi-natural and isolated), and axenic diatom cultures (*Cylindrotheca fusiformis*) were examined for the uptake of DFAA. Uptake was measured using naturally low concentrations (<1 µmol l^{-1}) of a mixture of ^{3}H-labeled DFAA (^{3}H-AA) under different light conditions and varying levels of dissolved inorganic nitrogen (DIN). Prokaryotic and eukaryotic uptake were differentiated using chloramphenicol. Biomass (chl *a*) and production (^{14}C uptake) were measured as well. ^{3}H-AA was taken up during the incubations in all experiments: 20 to 40% in the culture experiments, and 2 to 18% in the mixed MPB experiments. Of the total adsorption-corrected uptake, prokaryotic uptake accounted for 45 to 85% (0.3 to 2.2 µmol N m^{-2} h^{-1}), while eukaryotic uptake accounted for 15 to 55% (0.3 to 0.7 µmol N m^{-2} h^{-1}). Short-term (hours) light changes had no effect on ^{3}H-AA uptake, and ammonium availability lowered ^{3}H-AA uptake only marginally. Shade-adapted MPB communities (20 m) took up ^{3}H-AA more efficiently than light-adapted communities (1 m). The estimated contribution from DFAA to the microalgal N-demand was 6 to 12 and 55 to 100% (1 and 20 m sediment, respectively). Our findings suggest that DON, specifically DFAA, may be an important source of N for MPB communities, particularly where DIN and light are limiting. Moreover, tentative estimations suggest that DFAA can provide MPB communities with a considerable portion of their total N-demand (10 to 100%).

KEY WORDS: Dissolved free amino acids · DFAA · Nitrogen · Sediment · Microphytobenthos · DON

INTRODUCTION

Dissolved organic nitrogen (DON) is a dynamic component of the nitrogen (N) pool in marine coastal waters (Zehr & Ward 2002), where its fractions are used not only by heterotrophic bacteria, but also by photoautotrophs (Antia et al. 1991, Mulholland et al. 2002). Because coastal sediments function as important sites for mineralization, pore water DON concentration is often high and hence DON flux often dominates the sediment-water exchange of dissolved N (Lomstein et al. 1998, Tyler et al. 2001, 2003, Sundbäck et al. 2004). In shallow waters where light reaches the sediment surface, benthic primary producers (both macroscopic and microscopic) largely control the N flux (McGlathery et al. 2004).

Among these, microphytobenthos (MPB) form dynamic microbial mats at the sediment surface, contributing ≥50% of the primary production in shallow estuaries and embayments (Underwood & Kromkamp 1999, McGlathery et al. 2004). These communities can affect the sediment-water exchange of inorganic nutrients, directly by uptake, and indirectly by oxygen production (Henriksen et al. 1980, Sundbäck et al. 2000, for further references see McGlathery et al. 2004). MPB can also control bacterial processes such as nitrification and denitrification by producing oxygen and competing for N with bacteria (Risgaard-Petersen 2003). Recent studies suggest that MPB, and other benthic primary producers, are also important in controlling the turnover of DON in shallow areas (Anderson et al. 2003, Tyler et al. 2003).

Little is known about the quantitative importance of DON fractions for the growth of natural MPB communities. One obvious reason is that controlled uptake experiments are difficult to do in natural sediments, mainly because of the indigenous pool of inorganic and organic nutrients in the sediment pore water (cf. Nilsson & Sundbäck 1991). In addition, MPB has been generally thought to have an inexhaustible pool of available nutrients in the interstitial waters (Underwood & Kromkamp 1999). Dissolved free amino acids (DFAA) constitute a labile component of DON that is readily available both as a N and carbon (C) source for growth (Antia et al. 1991, Mulholland et al. 2002). Culture experiments have shown that growth of benthic diatoms can be stimulated by the addition of DON, such as DFAA (Admiraal & Peletier 1979), and that benthic diatoms take up DFAA occurring at low, natural concentrations (Admiraal et al. 1984, 1987). While the quantitative role of DFAA has been studied to some extent for phytoplankton (Mulholland et al. 2002, Veuger et al. 2004) and diatoms freshly isolated from low-light benthic communities (Rivkin & Putt 1987), only a few studies have tried to estimate the role of DFAA in natural MPB communities in sediments (Nilsson Sundbäck 1996, Linares in press).

In marine waters, DFAA concentrations are usually below 1 µM (Mulholland et al. 2003). In shallow-water sediments, DFAA can constitute between 10 and 30 % of the DON pool (Lomstein et al. 1998, Guldberg et al. 2002). The mixture of frequent light-limiting conditions, and the high concentrations of dissolved organic compounds found in sediments should imply that the capacity to use organic substrates is a selective advantage for algae in sediments. This could particularly apply to conditions of low availability of dissolved inorganic nitrogen (DIN), such as shallow sandy sediment areas, where DIN often appears to be limiting (Nilsson et al. 1991, Flothman & Werner 1992). It has been hypothesized that MPB can sustain a high production rate under low DIN conditions, because nutrients (particularly DON) are rapidly recycled and reused by the microbial mats at the sediment surface (Kristensen 1993, Lomstein et al. 1998, Sundbäck et al. 2000), thereby efficiently retaining N within the sediment system. A recent study showed that DFAA indeed stimulates MPB biomass and production in sandy sediments (Linares in press). The aim of our study was to estimate uptake of DFAA by mixed MPB communities. Our hypothesis was that MPB could make use of a large fraction of DFAA, even when available at naturally low concentrations, particularly when DIN was limiting. A second hypothesis was that shade-adapted MPB communities could take up DFAA more efficiently than light-adapted communities. The approach was to measure uptake of a ^{3}H-labeled DFAA (^{3}H-AA) mixture under different light conditions and in the presence of DIN.

MATERIALS AND METHODS

General approach. Measuring nutrient uptake in sediments under *in situ* conditions is difficult. For instance, DON can be adsorbed onto sediment particles (Christensen & Blackburn 1980, Aufdekampe et al. 2001), to high molecular weight dissolved organic matter (Schuster et al. 1998), to the surfaces of microorganisms (Carman & Guckert 1994), or it can be used by microbiota such as bacteria. In addition, nutrient availability is difficult to control because of the indigenous nutrient pool of the pore water. In order to minimize these sources of error, we used 3 types of microalgal material in our experiments: (1) an axenic unialgal diatom culture, (2) MPB communities isolated from natural sediment onto chemically cleaned sand (isolated communities, IC), and (3) MPB communities in natural, but gently sieved, sediment (semi-natural communities, SNC). A series of experiments was carried out (Table 1) using a ^{3}H-AA mixture (Amersham TRK-440, Table 2). Two treatments were made, one with the ^{3}H-AA mixture plus an addition of ammonium (NH$_4$) (+NH$_4$ treatment), and one with the ^{3}H-AA mixture alone (–NH$_4$ treatment). Killed material was used as an adsorption control, and a protein synthesis inhibitor (chloramphenicol, CAP) was used to discriminate between prokaryotic and eukaryotic uptake. See Table 1 for experiment specifications.

Setup of culture experiments. An axenic culture of the pennate diatom *Cylindrotheca fusiformis* Reimann and Lewin Culture Collection of Algae and Protozoa (CCAP) was used in 4 experiments to test washing, adsorption, and tracer additions (Table 1). Since the culture was axenic, we assume ^{3}H-AA was taken up only by live cells or adsorbed onto algal cells and the filter used. To create N-limiting conditions, a known amount of cells was transferred to a 50 ml Nunc flask with artificial seawater (ASW) with no added nutrients, and were kept for 4 d prior to the uptake experiments. In the first 3 experiments, only live cells were used to test washing and addition of ^{3}H-AA. In Expt 4, cells killed by heat (5 min at 120°C) were also incubated to assess the proportion of label adsorbed to surfaces (Table 1).

Setup of mixed community experiments. Uptake of ^{3}H-AA in mixed MPB communities was measured in 4 experiments, with an additional washing test (Table 1). Sandy sediment (top 0.5 to 1 cm) was collected from 2 sites: from ≤1 m depth in a shallow bay in the NE Kattegat (57° 40′ N, 11° 55′ E), and from 1 and 20 m depth in the Gullmar Fjord (58° 22′ N, 11° 37′ E), on the Swedish west coast. The sediment was mixed in a plastic tray and spread evenly. Filtered seawater (Whatman GF/F) from the sampling site was added, and the sediment was left to settle overnight at 10°C.

Table 1. Experimental design and specifications for all of the experiments in the study. Type/Depth: IC, isolated microphyto-benthic community; SNC, semi-natural microphytobenthic community. Treatments: L, light; D, dark (numbers in parentheses are light irradiance, in µmol photons m^{-2} s^{-1}); CAP, chloramphenicol prokaryotic inhibitor. Variables measured: AA, amino acid uptake; Chl, chlorophyll a; CC, cell counts; Sp, species composition; PP, primary productivity (^{14}C)

Expt	Type/ Depth (m)	Temp. (°C)/ Salinity	^{3}H-AA (nmol l^{-1})	Treatment	Cell condition	Variables measured	Comment
1	Culture/Sed. (method test)/ 0.5	10/26	128	L (250)	Live cells	AA, CC	Washing experiment
2	Culture (method test)/ 0.5	10/26	32–640	L (250)	Live cells	AA, CC	Different DFAA concentrations
3	Culture (method test)/ 0.5	10/26	32 and 128	L (250)	Live cells	AA, CC	Different cell concentrations
4	Culture (method test)/ 0.5	10/26	128	L+D (100, 250)	Live and killed cells	AA, CC	2 light levels
5	IC/1	10/26	128	L+D (250) 7.5 µmol l^{-1} NH_4	Live and killed cells	AA, Chl	Effect of NH_4 availability
6	IC/1	10/26	128	L+D (250) 20/100 µmol l^{-1} CAP	Live and inhibited cells	AA, Chl	Prokaryotic and eukaryotic uptake
7	IC, SNC/ 0.5	10/26	128	L (250) 7.5 µmol NH_4 20 µmol l^{-1} CAP	Live, killed and inhibited cells	AA, Chl, PP	^{14}C productivity
8	IC, SNC/ 1, 20	10/26	500	L (250, 10) 7.5 µmol NH_4 20 µmol l^{-1} CAP	Live, killed and inhibited cells	AA, Chl, PP	Light-adapted sediment (1 m), Shade-adapted sediment (20 m)

The sediment was divided into 2 portions. To remove natural pore-water nutrients, the MPB community was isolated from the sediment (IC). The sediment was stirred with filtered seawater to separate the motile microalgae (epipelic fraction) from the sediment. The remaining sediment was ultra-sonicated for 10 min, to detach algae attached to particles (epipsammic fraction). This treatment did not destroy cells (cf. Voltolina 1991). Both fractions were put in a tray with a 2 cm layer of chemically cleaned sand (MERCK Eurolab). The SNC was created by gently sieving the sediment through a 500 µm mesh to remove macrofauna. Both communities were left to acclimatize for a period of 4 d to 1 wk depending on the experiment, on a 14:10 h light: dark cycle (250 µmol photons m^{-2} s^{-1} for the 1 m sediment, and 10 µmol photons m^{-2} s^{-1} for the 20 m sediment).

Incubation for measuring DFAA uptake. All 3 types of algal material were incubated in 10 ml sterile Poly-styrene test tubes in 6 ml of ASW (Table 1). In the culture experiments, 15 000 to 60 000 cells ml^{-1} were inoculated. SNC and IC samples were taken with a 2 ml cut-off syringe (5 mm deep, area 0.567 cm^{-2}) and were transferred to tubes with ASW prior to incubation,

thereby creating N-limiting conditions. All incubations (except Expts 1 to 3 and 6) included an adsorption control in triplicate with killed cells. An amino acid mixture consisting of 15 ^{3}H-AA (Amersham Biosciences, TRK 440, Table 2) was added to each tube ($-NH_4$ treat-

Table 2. Proportions of the 15 different ^{3}H-labeled amino acids in the mixture used in the incubations (Amersham, TRK 440)

Amino acid	Proportion (%)
Alanine	7.0
Arginine	3.7
Aspartic acid	15.5
Glutamic acid	6.2
Glycine	13.8
Histidine	0.9
Isoleucine	2.0
Leucine	6.9
Lysine	2.0
Phenylalanine	8.9
Proline	6.0
Serine	3.3
Threonine	13.4
Tyrosine	4.1
Valine	6.3

ment). The composition of the mixture is close to that found in the pool of pore-water DON (Lomstein et al. 1998, Guldberg et al. 2002). To test the effect of DIN availability, NH_4 was added as NH_4Cl in Expts 5, 7 and 8 (+NH_4 treatment), to a final concentration of 7.5 µmol l^{-1}, approximately the highest concentration found in shallow waters in the study area (Sundbäck et al. 2000). Ambient NH_4 concentration in the overlying water was 1.1 µmol l^{-1}. NH_4Cl was added ca. 30 min before the addition of ^{3}H-AA.

The amount of label added was determined by incubating *Cylindrotheca fusiformis* cells (15 000 cells ml^{-1}) in a range of ^{3}H-AA concentrations (32 to 640 nM),
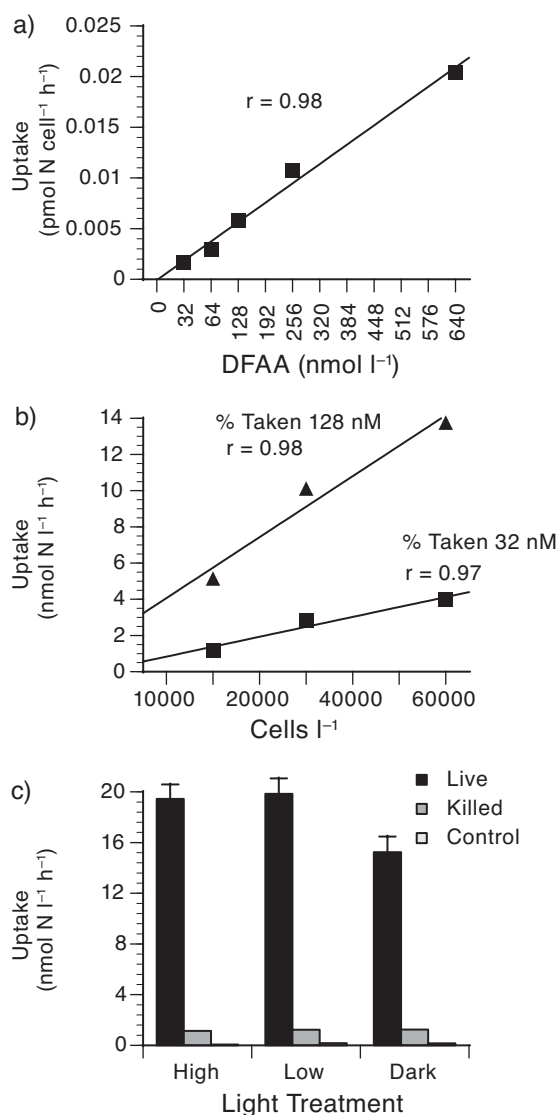

Fig. 1. *Cylindrotheca fusiformis*. Dissolved free amino acid (DFAA) uptake (+SE), Expts 2 to 4 (Table 1). (a) Uptake per cell at different DFAA concentrations; (b) DFAA taken up at different cell concentrations and 2 ^{3}H-labeled (^{3}H-AA) concentrations (32 and 128 nM); (c) uptake of DFAA under high and low light conditions and in the dark (Table 1)

and then incubating a range of cells (15 000 to 60 000 cells ml^{-1}) at 2 specific ^{3}H-AA concentrations (32 and 128 nM). A final addition of 128 nM of ^{3}H-AA was used in all experiments, except for Expt 9 (500 nM). By using these low concentrations we hoped not to enrich natural, ambient pore-water concentrations, which range from 0.3 to 30 µmol of DFAA (Admiraal et al. 1987, Antia et al. 1991).

To differentiate between prokaryotic and eukaryotic ^{3}H-AA uptake, a bacteriostatic inhibitor chloramphenicol (CAP) was used in Expts 6 to 8 (Table 1). CAP was added 30 min before incubation. Tubes were incubated for 3 to 4 h at 10°C. Positional irradiance effects from the incubation lamps were avoided by randomly moving each tube every 30 min. Irradiance ranged from 10 to 250 µmol photons m^{-2} s^{-1} (Table 1). In Expt 4, 2 light levels were used (100 and 250 photons m^{-2} s^{-1}). In Expts 4 to 6 dark incubations were also made. All treatments were run in triplicate. Samples were collected by vacuum filtration onto Polycarbonate filters (0.2 µm, Osmonics), and were washed with 20 ml of ASW. Uptake was measured in a scintillation counter, after addition of Hionic Fluor scintillation cocktail (Packard). ^{3}H-AA uptake was recalculated to uptake per m^2, using the sampled sediment surface. Uptake normalized to chlorophyll *a* (^{3}H-AA$^{chl\,a}$) was used to compare the uptake efficiency between communities with different algal biomass.

Primary production. Primary production (PP) was measured as ^{14}C-uptake during Expts 7 and 8. Eighty µl of H^{14}CO$_3$ (activity 20.54 µCi ml^{-1}, ^{14}C Agency Denmark) were added to each tube. Samples were incubated for 4 h under the same conditions as the corresponding ^{3}H-AA tubes. A mixture of unlabeled DFAA was added to all tubes to mimic the conditions in the ^{3}H-AA tubes, and NH_4Cl was added to a final concentration of 7.5 µmol l^{-1}. The ^{14}C-samples were processed as described by Sundbäck et al. (1990). Total carbon dioxide (CO_2) concentration was calculated using the equations described by Aertebjerg Nielsen & Bresta (1984).

Chlorophyll *a*. Chl *a* was used as a measure of MPB biomass. Three sediment samples (5 mm deep) were taken from the IC and SNC sediments before the start of each experiment using a 2 ml cut-off syringe, and were frozen at –80°C. Pigment content was extracted by adding 3 ml of 100 % acetone to each sample. The tubes were ultra-sonicated for 10 min (while cooling with ice). Samples were left for 24 h at 7°C in darkness, after which they were centrifuged for 10 min at 2000 rpm (750 × *g*). Samples were analyzed with a spectrophotometer (Shimadzu UV-2401 PC), correcting for phaeopigment degradation according to Lorenzen (1967).

Analysis of dissolved nitrogen. A TRAACS autoanalyzer (Bran & Lubbe) was used for the analysis of NH_4, nitrate plus nitrite and total dissolved nitrogen (TDN) from pore water and overlying water in Expt 8.

Table 3. Summary of results from Expts 7 and 8. All values have been corrected for DFAA adsorption. Percent of the added label taken up, measured uptake rate of ^{3}H-DFAA including amount and proportion of prokaryotic (Prok.) and eukaryotic (Euk.) uptake, primary production (PP) and chlorophyll-normalized production (PP/Chl), estimated DFAA uptake and percent of estimated N-demand (SNC in Expt 8) assuming 10 and 20% of DON pool is DFAA. IC: isolated community: SNC: semi-natural community

Expt	Type	Depth (m)	Treatment	Uptake of added label (%)	Measured uptake (μmol m^{-2} h^{-1})			PP (mg C m^{-2} h^{-1})	PP/Chl (mg C chl^{-1} h^{-1})	Actual N-uptake (μmol m^{-2} h^{-1})		% of N-demand[3]	
					Total	Prok.	Euk.			10%	20%	10%	20%
7	IC	0.5	$-$NH$_4$	7.6	1.1	0.7 (65)	0.4 (35)	24.8	1.4				
			$+$NH$_4$	8.9	1.0	0.7 (65)	0.3 (35)	24.0	1.4				
	SNC	0.5	$-$NH$_4$	8.9	1.2	0.6 (46)	0.6 (54)	64.0	0.7				
			$+$NH$_4$	9.1	1.2	0.7 (56)	0.5 (44)	61.4	0.7				
8	IC	1	$-$NH$_4$	3.7	2.0	1.6 (80)	0.4 (20)	8.3	0.7				
			$+$NH$_4$	6.2	1.7	1.5 (85)	0.3 (15)	4.8	0.4				
	SNC	1	$-$NH$_4$	3.2	1.7	1.2 (73)	0.5 (27)	6.1	0.1	5.6	11.2	6.0	12.1
			$+$NH$_4$	2.9	1.6	1.2 (75)	0.4 (25)	5.2	0.1	4.9	9.8	6.0	12.0
	IC	20	$-$NH$_4$	4.9	2.6	2.2 (84)	0.4 (16)	0.6	0.4				
			$+$NH$_4$	3.4	1.8	1.5 (84)	0.3 (16)	1.3	0.9				
	SNC	20	$-$NH$_4$	2.6	1.5	1.1 (77)	0.4 (23)	0.4	0.02	3.4	8.0	56.8	113.6
			$+$NH$_4$	1.1	0.6	0.3 (52)	0.3 (48)	0.3	0.01	3.5	7.0	53.6	107.3

Samples were collected in triplicate by centrifuging a known amount of sediment, and freezing the supernatant at -80°C. TDN was analyzed using the persulfate oxidation method (Valderrama 1981, Bronk et al. 2000). DON was calculated by subtracting DIN from TDN.

Species composition. Sediment samples collected using a 2 ml cut-off syringe, and preserved with glutaraldehyde (final concentration of 2.5%), were inspected semi-quantitatively using epifluorescence microscopy and phase contrast (Zeiss Axiophot Plan II) at 40× magnification. Both the epipelic and epipsammic fractions were separated and identified to major microalgal groups.

Statistical analysis. The results were analyzed by 1-way ANOVA with treatment ($-$NH$_4$ and $+$NH$_4$, and light level, respectively) and community type (prokaryotic and eukaryotic) as the fixed factors in their respective cases. Differences between treatments were considered significant when $p < 0.05$.

RESULTS

We found uptake of ^{3}H-AA (Figs. 1 to 3, Table 3) during the incubations in all experiments: 20 to 40% in the culture experiments, and 2 to 18% in the mixed MPB experiments. Of the total uptake, 4 to 19% was due to adsorption to surfaces (cells, sediment particles), i.e. non-biological uptake (Figs. 1c & 2a). Initial method tests using different concentrations of *Cylindrotheca fusiformis* cells showed that uptake increased linearly with increasing ^{3}H-AA additions, and increasing cell numbers (Fig. 1a,b), suggesting

that uptake was mainly biological. Adsorption-corrected uptake for the culture experiments varied between 1.2 and 14 nmol l^{-1}, or 2 and 15 nmol ^{3}H-AA cell^{-1} h^{-1}. Total adsorption-corrected ^{3}H-AA uptake rates per m^2 (eukaryotic + prokaryotic) were within the same range (0.6 and 2.6 μmol m^{-2} h^{-1}) for both IC and SNC (Fig. 3, Table 3). However, on one occasion (Gullmar Fjord, 20 m) the total uptake by IC was significantly higher (2.6 μmol m^{-2} h^{-1}) when compared with the SNC at the same depth (1.5 μmol m^{-2} h^{-1}, Fig. 3).

The addition of CAP at 2 concentrations (20 and 100 μmol l^{-1}) resulted in a similar percentage of inhibition, and 20 μmol l^{-1} was used in all subsequent experiments (Fig. 2b). Prokaryotic uptake was proportionally higher than eukaryotic uptake (ANOVA, $p < 0.05$) in most cases (Figs. 2b & 3). Prokaryotic uptake ranged from 45 to 85% of the total adsorption-corrected uptake, or 0.3 to 2.2 μmol N m^{-2} h^{-1} (Table 3). Eukaryotic uptake ranged from 15 to 55%, or 0.3 to 0.7 μmol N m^{-2} h^{-1} (Table 3, Fig. 3).

Chl *a* measurements showed that MPB biomass varied between the different communities (Fig. 4, text value). Therefore, to compare uptake 'efficiency' between communities, eukaryotic uptake was normalized for chl *a* (^{3}H-AA$^{chl\ a}$). Since diatoms dominated, cyanobacteria being rare, we assumed that eukaryotic uptake represented the main part of algal uptake. ^{3}H-AA$^{chl\ a}$ accentuated the difference in eukaryotic uptake between the IC and SNC, as well as between sampling depths (Fig. 4). Algae in the IC took up significantly more ^{3}H-AA per unit chl *a* than those in the SNC (ANOVA, $p < 0.05$). In the 20 m sediment,

^{3}H-AA$^{chl\,a}$ for the IC was an order of magnitude higher than in the 1 m sediment (see below). A similar magnitude of difference also existed between IC and SNC for the 20 m sediment.

Short-term light effects

Irradiance during the incubation had an effect only in the culture experiments. Uptake in the dark by *Cylindrotheca fusiformis* was slightly, but significantly (ANOVA, $p < 0.05$), lower than in the light (Fig. 1c). No significant difference in ^{3}H-AA uptake was seen when cells were incubated at 2 different light levels (100 and 250 µmol photons m^{-2} s^{-1}) (Fig. 1c). Incubation of IC sediment in light and dark resulted in no significant light effects (Fig. 2a). Based on this, we decided not to measure dark uptake in the subsequent community experiments.

DFAA uptake in shade- and light-adapted sediment communities

Eukaryotic uptake (^{3}H-AA$^{chl\,a}$) in the IC was an order of magnitude higher for shade-adapted sediment (20 m), ranging from 200 to 300 nmol N mg chl a^{-1} h^{-1}, than the 1 m IC (ca. 25 nmol N mg chl a^{-1} h^{-1}) (Fig. 4). For the SNC, the difference was smaller, but still significant (ANOVA, $p < 0.05$), ca. 8 vs. 14 nmol mg chl a^{-1} h^{-1} (Fig. 4). The chl *a*-normalized production (P$^{chl\,a}$) was higher for the IC than SNC, following the pattern of ^{3}H-AA$^{chl\,a}$ (Fig. 5). P$^{chl\,a}$ was an order of magnitude lower for the SNC (Fig. 5).

Effect of DIN availability

The effect of DIN on ^{3}H-AA uptake was only measured in the community experiments. No effects were found in Expts 5 and 7 (Figs. 2a, 3a & 4a). In Expt 8, the presence of NH$_4$ significantly lowered eukaryotic ^{3}H-AA uptake in the IC but not in the SNC (Fig. 3b,c). This effect was most obvious for the ^{3}H-AA$^{chl\,a}$ uptake rates, where NH$_4$ addition decreased tracer uptake by as much as 30% (Fig. 4b,c). In Expt 8, NH$_4$ addition also affected P$^{chl\,a}$, but only in the IC and with different direction of effects (Fig. 5).

Estimated DFAA uptake and N-demand of MPB

Our DFAA uptake rates are probably underestimations of the actual uptake, particularly for the sediment communities. Measuring true DFAA uptake

rates with tracer techniques requires that the indigenous DFAA pool in the pore water be taken into account (Mulholland et al. 2002). In Expt 8 we roughly estimated the actual uptake of DFAA by microalgae, as well as its contribution to the algal N-demand in the SNC. Uptake was calculated according to the formula: % label taken up by eukaryotes × DFAA concentration in the pore water (Mulholland et al. 2002). We assumed the DFAA pool to be either 10 or 20% of the measured DON pool in the pore water (Guldberg et al. 2002, their Table 1). Mean DON concentration was 63 µM in the pore water (1 and 20 m sediments), giving an estimated DFAA concentration of 12.6 µM (20%) or 6.3 µM (10%). Uptake of ^{3}H-AA was 3.5 to 5.6 µmol m^{-2} h^{-1} (10% DFAA in the DON pool) and 7 to 11.3 µmol m^{-2} h^{-1} (20% DFAA in the DON pool) (Table 3). The algal N-demand was calculated from primary production, assuming a C/N ratio of 6.6 (Redfield). Assuming that 10% of the available DON pool is DFAA, the contribution to the algal N-demand that comes from DFAA was 6% for the 1 m sediment, and 55% for the 20 m sediment (Table 3). If 20% of the available DON pool is assumed to be DFAA, the contribution is 12% for the 1 m sediment, and 110% for the 20 m sediment (Table 3).
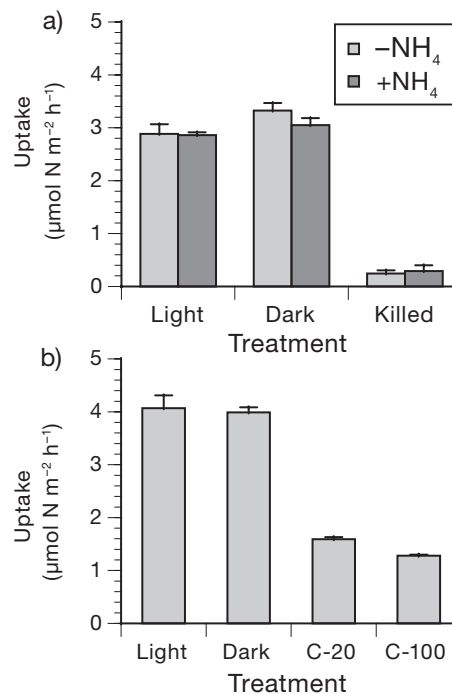

Fig. 2. DFAA uptake by isolated microphytobenthos communities (+SE), Expts 5 and 6 (Table 1). (a) Uptake of DFAA in the light and in the dark (–NH$_4$ = only ^{3}H-DFAA, +NH$_4$ = non-labeled DFAA and NH$_4$ addition); (b) light and dark uptake, and effect of chloramphenicol inhibitor (CAP) (C-20 = 20 µmol l^{-1} addition, C-100 = 100 µmol l^{-1} addition)

DISCUSSION

Methods

Our study is one of the first to attempt to measure the uptake of DFAA by mixed communities of benthic microalgae. We tried to differentiate between prokaryotic and eukaryotic ^{3}H-AA uptake, and made a rough estimation of the contribution of DFAA to the N-demand of the MPB in semi-natural communities. We also tried to check and correct for non-biological adsorption, and to avoid pre-conditioning the cells to any particular type of N by using ASW with no nutrient additions. Nutrient pre-conditioning may influence uptake of one N substrate over another, affecting uptake rates (Dzurica et al. 1989, Mulholland et al. 2004). While our experimental approach was successful in providing us with measurable uptake rates, it is not without limitations. The algal communities used in our study were manipulated to some extent, and all experiments were done in the laboratory. Our

Fig. 3. DFAA uptake by isolated and semi-natural microphytobenthos communities without ($-NH_4$) and with addition of ammonium ($+NH_4$) for sediment collected from (a) 0.5 m, (b) 1 m and (c) 20 m depth of water, and differentiating between prokaryotic and eukaryotic uptake (+SE)

experiments were largely 'black box' experiments, where we simply measured the uptake of a mixture of ^{3}H-AA, without finding out the uptake mechanisms or in what form the N from the DFAA was used in the cells. We also did not measure the pool of available amino acids either. Despite all this, our experiments should serve as a starting point for further studies on the role of DON for natural MPB communities.

DFAA uptake

The MPB in all our experiments took up ^{3}H-AA when present at low concentrations. The amount of ^{3}H-AA added (0.13 to 0.5 µmol l^{-1}) falls within the lower range of naturally occurring DFAA concentrations in pore water, which usually varies from 0.3 to 30 µM in the sediment (Admiraal et al. 1987, Antia et al. 1991, Guldberg et al. 2002). However, the notion of low DFAA concentrations can be misleading given that there might be a tight coupling between production and uptake (i.e. a rapid turnover rate). Admiraal et al. (1984) concluded that isolated benthic diatom populations are able to take up DFAA even when concentrations are as low as 0.1 to 1 µmol l^{-1}. The results obtained in our study fall within this range. Despite the fact that we were able to measure uptake in all experiments, we do not know in what form the N entered the cell. Palenik & Morel (1990) first described a mechanism common to some marine phytoplankton species, where cell surface enzymes oxidize DFAA into NH_4 before entering the cell. Recent studies have confirmed the importance of this extracellular oxidation pathway (Mulholland et al. 2002, 2003).

Since no previous uptake data for mixed MPB communities are available, we will instead compare our results with those reported for plankton communities. Although potential differences in uptake efficiency may exist, such comparison allows us to discuss our findings on a community level. Mulholland et al. (2003) found that natural plankton communities along an estuarine nutrient gradient derived up to 11% of the total N uptake in the form of DFAA. According to our estimations, the SNC from 1 m depth in Expt 8 could have gained roughly 6 to 12% of their hypothetical N demand from DFAA, which agrees well with the data reported by Mulholland et al. (2003). In a subsequent study, Mulholland et al. (2004) found that DFAA also played an important role in the C and N nutrition of the brown tide pelagophyte *Aureococcus anophagefferens*, where DFAA was a predominant nutrient source (ca. 18%). Deep-dwelling, shade-adapted communities (20 m depth) in our study could have gained, according to our estimations, as much as 55 to 100% of their N-demand from
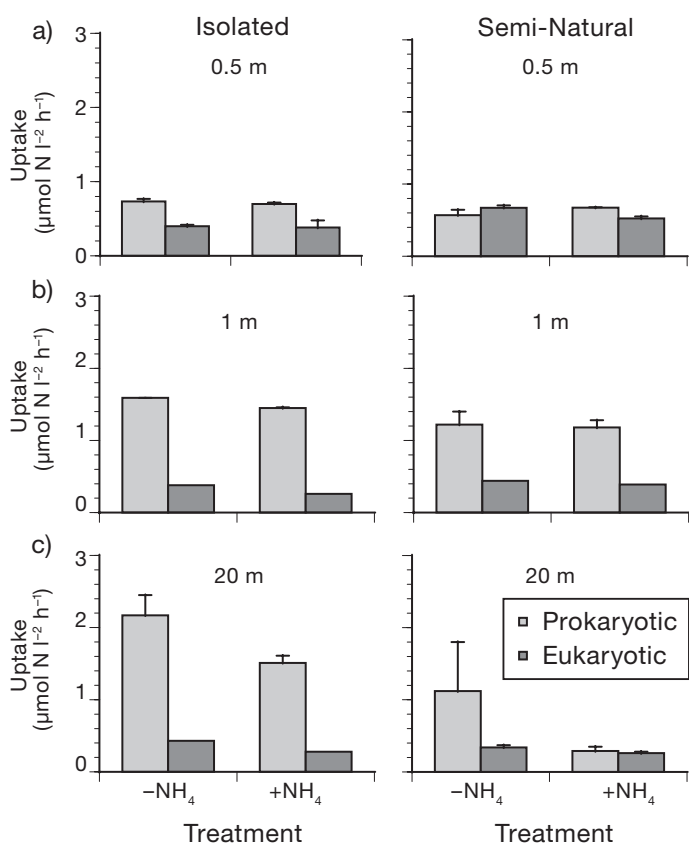
DFAA. This finding supports the idea that DON may become more important as both a N and C source as light decreases with depth (Sundbäck et al. 2004). Veuger et al. (2004) found that DFAA could contribute up to 80% of the total microbial N uptake, implying that DON may be an important N substrate for microbial communities. Middelburg & Nieuwenhuize (2000) found similar results near the mouth of the Thames estuary.

The proportion of ^{3}H-AA taken up by the eukaryotic (assumed microalgal) fraction was occasionally as high as, or even higher (55%) than the proportion taken up by prokaryotes (bacteria) (Table 3). While CAP is primarily used as a bacteriostatic inhibitor (Shaw 1992, Steppe & Paerl 2005), a few studies have used it to inhibit macro- and microalgae (Hader et al. 2002, Segovia & Berges 2005). As such, the eukaryotic rates reported in our study might be underestimates, espe-

cially if cyanobacteria are present (cf. Rondell et al. 2000). The observed high proportion of eukaryotic ^{3}H-AA uptake is intriguing, since bacteria are generally considered to be the major transformers of organic matter, dominating DFAA uptake in the sediment (Blackburn & Blackburn 1993, Herbert 1999), where they rapidly degrade amino acids (Jørgensen et al. 1981, Therkildsen et al. 1996, Lomstein et al. 1998). However, microalgae are known to effectively compete for these organic resources (Flynn & Butler 1986), and DON uptake can be assumed to be particularly important when inorganic nitrogen concentrations are low (Lomstein et al. 1998). In fact, the importance of DON as a N source for plants, on a wider global scale, in nutrient-poor ecosystems has lately been emphasized by van Breemen (2002).

Light effects

No effect from light on ^{3}H-AA uptake by MPB could be found during the incubations. Studies have suggested that light can affect the uptake of DFAA by microalgal communities (Admiraal & Peletier 1979, Admiraal et al. 1984, Flynn & Butler 1986). However, light effects reported in the literature vary. For example, the incorporation rate of DFAA into proteins has been found to be much higher in the light than in the dark (Flynn & Butler 1986). In contrast, maximum rates of DFAA uptake have been suggested to occur in dark waters depleted of DIN (Flynn & Butler 1986). Facultatively heterotrophic diatoms are capable of growth under low light conditions using DON as a nutrient and carbon source (Rivkin & Putt 1987); moreover, DON does not necessarily enhance their growth rate when they are subjected to high irradiance (Admiraal & Peletier 1979). One possible explanation for the lack of light effect in our study could be the incubation time, where the change in light conditions lasted only for a few hours. Shifts in DON uptake and growth of MPB using DON as a nutrient source can take days to develop (Rondell et al. 2000). When IC and SNC are grown under different light conditions for a period of 3 to 4 wk, DFAA stimulates the biomass and production of the MPB to a greater degree under high irradi-
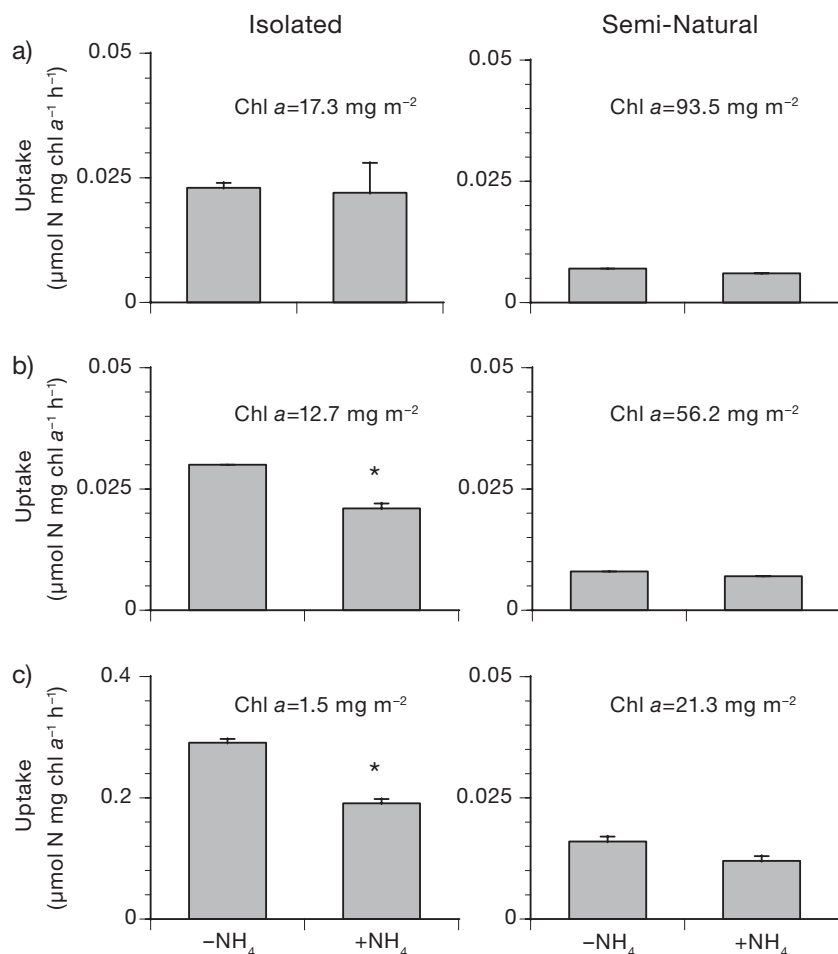

Fig. 4. DFAA uptake normalized to chlorophyll *a* by isolated and semi-natural microphytobenthos communities (+SE), without (–NH$_4$) and with addition of ammonium (+NH$_4$), for sediment collected from (a) 0.5 m, (b) 1 m and (c) 20 m depth of water. Note the difference in scales. *Statistically significant (p < 0.05) difference between treatments

ance (Linares in press). Thus, it seems that short-term changes in the light climate do not affect the DFAA uptake mechanism.

Differences in DFAA uptake efficiency between communities adapted to light conditions at different depths suggested long-term effects of light. Shade-adapted eukaryotic cells in the IC were more efficient in taking up ^{3}H-AA (avg. 0.13 µmol mg chl a^{-1} h^{-1}) than light-adapted communities (avg. 0.02 µmol N mg chl a^{-1} h^{-1}). The fact that these observations were made in the IC in particular could be because, at least initially, the IC were probably more nutrient limited than the SNC. Darkness and N-deprivation has been found to favor the development of DFAA-uptake systems (Antia et al. 1991). Our results agree with findings that darkness favors, and N-deprivation further accelerates, the development of DFAA-uptake systems (Flynn & Syrett 1985, Flynn & Butler 1986). A recent study along a depth gradient in the same study area where our light and shade-adapted

sediment was collected from, found that MPB were still active at depths >5 m (Sundbäck et al. 2004). Thus, it seems that these communities are well adapted to survive at these depths, which may be attributed to their effective use of organic compounds such as DFAA. Benthic microalgae are very efficient at adapting their metabolism to limiting light conditions (Admiraal & Peletier 1979, Glud et al. 2002). Our findings also agree with those reported by Rivkin & Putt (1987), who found that DFAA were important for periphyton growth in Antarctica under light-limiting conditions. Thus, it may be that in shallow-water sediments DFAA is mainly a N-source when DIN is in short supply, but deeper down (or under the shade of green-algal mats for example, cf. Sundbäck et al. 1996), where light limits photosynthesis, the microalgae use DFAA both as a N and C source.

Another possible explanation for the difference in uptake between light- and shade-adapted MPB communities could be that of species composition. Our sediment communities were composed mainly of pennate diatoms (i.e. *Navicula* spp., *Nitzchia* spp.). However, Wulff et al. (2005), who studied the depth distribution of benthic diatoms along the same depth gradient, found a difference in species composition between shallow and deep sediment communities. Sundbäck et al. (2004), also working on the same depth gradient, found that deeper sediments (>10 m) consisted mainly of large-sized diatoms (*Pleurosigma* cf. *clevei*, *Polytrichum formosum*), while epipelic-epipsammic taxa (i.e. *Navicula* spp.) were dominant in the shallow sediments. Such a difference in species composition might also help explain the difference found between the shallow and deep sediments.

Effect of NH$_4$

The addition of NH$_4$ during the incubation had no effect on DFAA uptake, except in Expt 8, where eukaryotic DFAA uptake decreased, although only in the IC. Few studies exist in which the uptake of both organic and inorganic N sources by benthic microalgae have been studied simultaneously. Admiraal et al. (1987) studied uptake (by measuring concentrations in the media) of several amino acids, as well as DIN by 2 benthic diatom species isolated from tidal
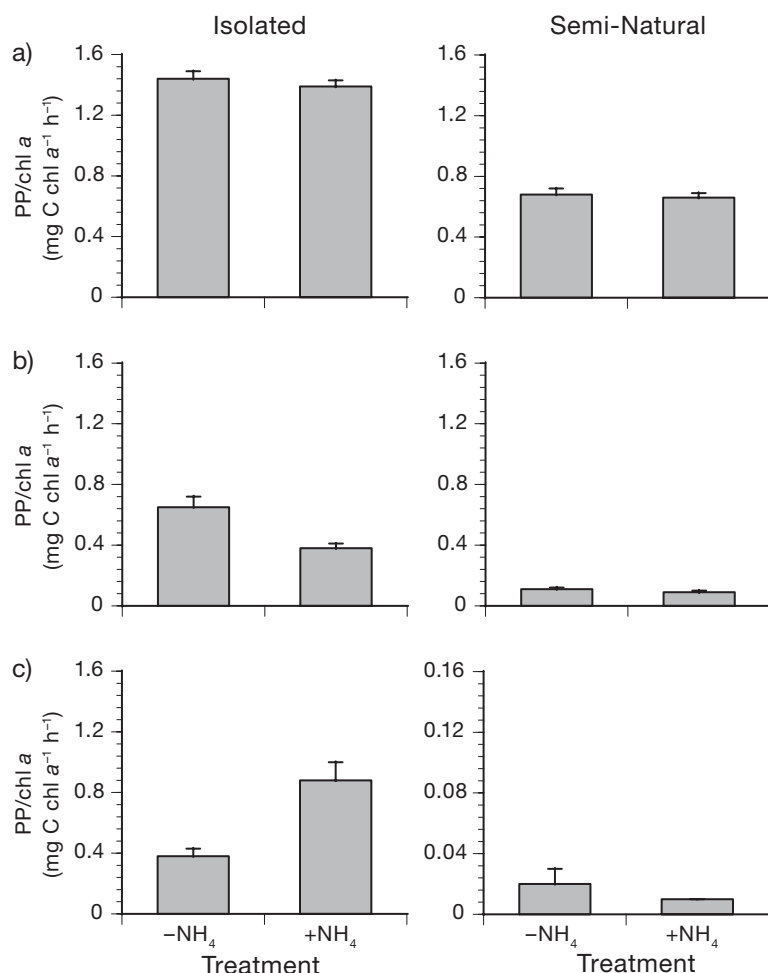

Fig. 5. Chlorophyll *a* normalized productivity (PP/chl *a*) by isolated and semi-natural MPB communities (+SE), without (−NH$_4$) and with addition of ammonium (+NH$_4$), for sediment collected from: (a) 0.5 m, (b) 1 m and (c) 20 m depth of water. Note differences in scale

flats. They found that NH_4 did not suppress the uptake of DFAA, whereas uptake of NO_3 was suppressed. They also found that amino acids were used in parallel with ammonium, even though the concentration of DFAA (1 μM) was an order of magnitude lower than that of NH_4. Our experiments, using mixed MPB communities and a low concentration of DFAA (0.5 μM), partly support these findings.

However, we also found that the presence of NH_4 decreased ^{3}H-AA uptake (Expt 8). This effect was clearest in the IC when viewed as ^{3}H-AA$^{chl\,a}$ uptake. One drawback of our study is that we did not measure uptake of DIN (cf. Mulholland et al. 2002) and we can only speculate about the reason for the suppression of DFAA uptake. The IC represented a more N-starved community than the SNC, where an indigenous sediment pool of both DIN and DON was available. It could be that the N-starved algae in the IC preferred to take up NH_4 when made available in a concentration 1 order of magnitude higher (7.5 μM) than DFAA (0.5 μM). We assume that no N source was available in the chemically clean sand. Both ^{14}C and ^{3}H-AA uptake per unit chl a were significantly higher in the IC than in the SNC, suggesting that the algal cells in the IC were more active than those in the SNC. Admiraal et al. (1984) observed a variation between species regarding the effect of DIN on the DFAA uptake rate. Thus, as pointed out by Admiraal et al. (1984), incorporation of DFAA by natural microalgal communities is an extremely variable process, dependent on species composition and nutritional state.

The generally accepted idea that NH_4 is the preferred N-source of microalgae (Antia et al. 1991, Herbert 1999) comes mainly from studies on phytoplankton species. Studies using diatoms from the sediment suggest that benthic microalgae might regulate the uptake of inorganic and organic sources in a different way than phytoplankton. The varying availability of DIN and DON may in fact determine the dominant metabolism (e.g. autotrophy vs. heterotrophy) of the community (Mulholland et al. 2004), and in some cases they may actually prefer DFAA to NH_4 and NO_3 (Admiraal et al. 1987). This might explain why in a previous study the addition of DFAA stimulated the growth and production of IC and SNC as much as, or even more, than the addition of NO_3 (Linares in press).

Estimated N-demand

DFAA concentration in the sediment pore water was not measured. However, we estimated this concentration by assuming that 10 to 20% of DON is in the form of DFAA (Guldberg et al. 2002). One of our initial aims

was to test whether shade-adapted MPB communities were more efficient at taking up DFAA than light-adapted communities. This assumption is based on the idea that facultative heterotrophy (including uptake of dissolved organic carbon [DOC] and DON) of microalgae becomes more important as light becomes scarce (Admiraal & Peletier 1979). The estimated contribution from DFAA to the microalgal N-demand was much higher in the 20 m sediment (55 to 100%) than in the 1 m sediment (6 to 12%). A recent study conducted along a depth gradient in the Gullmar fjord found occasional DON uptake by the sediment, as well as light effects on DON flux, particularly at depths greater than 5 m. These findings suggest that DON uptake by microalgae may be important at sublittoral depths (Sundbäck et al. 2004). The relatively high ^{3}H-AA uptake rates measured in the 20 m sediment, and the high percentage of DFAA estimated to contribute to the total N-demand, support these findings. Moreover, it is possible to theorize from the estimated percentage that the microalgae use DFAA as both a N and C source (cf. Mulholland et al. 2002).

CONCLUSIONS

Our results showed that sediments with mixed MPB communities can take up DFAA when occurring in naturally low concentrations (<1 μM). Short-term changes in light conditions do not appear to affect the uptake rate of DFAA and rates are only marginally lowered by ammonium availability. Although prokaryotic uptake of DFAA often dominates, eukaryotic (algal) uptake can occasionally be as high as the prokaryotic uptake. Tentative estimations suggest that DFAA can provide MPB communities with a considerable portion of their total N-demand (~10 to 100%). An efficient uptake of DFAA may be an important adaptation mechanism particularly under light-limited conditions, where DFAA can be assumed to also provide an alternative C source when photosynthesis is strongly light-limited. It is suggested that labile fractions of DON, such as DFAA, may be an important alternative N source for natural MPB communities, in particular when ambient DIN concentrations are low.

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