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Release of dissolved organic nitrogen from *Scenedesmus quadricauda* (Chlorophyta) and *Microcystis novacekii* (Cyanobacteria)

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ABSTRACT: We measured the time-course of dissolved organic nitrogen (DON) release and inorganic nitrogen assimilation in the freshwater phytoplankton *Scenedesmus quadricauda* (Turp.) Brébission and *Microcystis novacekii* (Kom.) Comp. by the ^{15}N tracer method. The algae were cultivated under nitrogen-limited or -replete conditions. We estimated the total DON release, release of recently assimilated nitrogen (RANR) and net nitrogen assimilation (NNA), using ammonium chloride or sodium nitrate as the nitrogen source. In the ^{15}N tracer incubation of *S. quadricauda* prior to 1 h and in *M. novacekii* prior to 3–6 h, minimal release of total DON was observed under the nitrogen-limited conditions. This result suggests that nitrogen-limited cells efficiently use incorporated nitrogen. Appreciable release of total DON was observed in the ^{15}N incubation of nitrogen-replete *S. quadricauda* and *M. novacekii*. RANR in both nitrogen-limited and -replete algae was smaller than, and lagged behind, release of total DON, indicating that newly assimilated nitrogen was less easily released. NNA by nitrogen-limited *S. quadricauda* increased from the onset of the tracer incubation, showing that ambient nitrogen was needed for nitrogen-limited cells from the beginning of the incubation. Nitrogen-replete *S. quadricauda* showed no NNA during the first 1 h of the tracer incubation, suggesting that the alga did not require external nitrogen immediately after being transferred from nitrogen-replete conditions. This study shows that nitrogen release and assimilation are markedly affected by the nutritional states of the algae.

KEY WORDS: Ammonium · Nitrogen assimilation · Organic nitrogen release · *Microcystis novacekii* · Nitrate · *Scenedesmus quadricauda*

INTRODUCTION

Release of dissolved organic matter (DOM) by phytoplankton has been studied from various points of view: kinetics of DOM release (Fogg et al. 1965, Nalewajko et al. 1976, Lancelot 1979, Mague et al. 1980, Sellner 1981); relationships between DOM release and phytoplankton population density (Watt 1966, Anderson & Zeutschel 1970, Thomas 1971, Berman & Holm-Hansen 1974); and effects of ambient nutrient concentrations (Smith et al. 1977, Giordano et al. 1994) and temperature (Watanabe 1980) on DOM release.

Despite many studies on DOM release, slightly contradictory results have emerged. For example, the relationship between the total primary production and the ratio of DOM release to primary production has been variously reported (Baines & Pace 1991). Fogg et al. (1965), Anderson & Zeutschel (1970) and Berman (1976) showed that primary production was inversely related to the ratio, whereas Lancelot (1979), Sellner (1981), Brock & Clyne (1984) and Bell & Kuparinen (1984) did not detect an inverse relation. In addition, the composition of the released compounds has been

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variously reported in different DOM-release studies. Watt (1969) and Fogg (1983) suggested that the released substances consist mainly of low molecular weight compounds, such as amino acids and early photosynthates. However, Nalewajko & Schindler (1976), Chrost & Faust (1983) and Sundh (1989) reported that released DOM is often dominated by compounds with high molecular weights. These discrepancies seem related to the nutritional and physiological states of the algae tested.

Release of organic nitrogen by phytoplankton may be influenced by the nitrogen demand and physiology of the algal cells, as was suggested by the studies on DOM release. Nitrogen is an important factor for cellular nutrition and physiology. Nitrogen-limited cells will not readily release organic nitrogen, because nitrogen is required for growth and maintenance of optimal cell conditions. On the contrary, nitrogen-replete cells are likely to release more organic nitrogen. Nitrogen release is related to nitrogen uptake by the phytoplankton (Bronk & Glibert 1993, Flynn & Berry 1999). Studies on the relationship between DON (dissolved organic nitrogen) release and the nitrogen states of algae will contribute to the clarification of phytoplankton dynamics and nitrogen cycling in aquatic environments.

In this study, we determined DON release from the freshwater phytoplankton *Scenedesmus quadricauda* (Chlorophyta) and *Microcystis novacekii* (Cyanobacteria), using the method for measurement of total nitrogen release, recently assimilated nitrogen release (RANR) and net nitrogen assimilation (NNA) (Nagao & Miyazaki 1999). We used algae grown under different sets of nitrogen repletion, nitrogen limitation, nitrogen sources and light regimes, and we discuss the effects of nitrogen and light conditions on DON release.

MATERIALS AND METHODS

We used the green alga *Scenedesmus quadricauda* (Turp.) Brébission and the blue-green alga *Microcystis novacekii* (Kom.). *S. quadricauda* was isolated, maintained and is still available in our laboratory, and *M. novacekii* was kindly supplied by Dr M. Watanabe of Tsukuba Botanical Garden, National Science Museum, Japan (Tsukuba Algal Collection TAC 19).

We pre-cultivated *Scenedesmus quadricauda* in chemostats or batch cultures and *Microcystis novacekii* in chemostats in the Modified WC medium (Guillard & Lorenzen 1972) containing NH_4Cl or $NaNO_3$ as the nitrogen source at 25°C. Nitrogen concentrations in the supplied medium were 50 µg-at N l^{-1} in batch cultures, and 40 µg-at N l^{-1} in chemostat cultures. Light was supplied at an irradiance of 150 mmol photons m^{-2} s^{-1} by cool-white fluorescent lamps (14:10 h light:dark cycle). The culture medium was continuously stirred by a magnetic bar and bubbled with air filtered through axenic membrane filters (0.2 µm). All chemostats were maintained at a constant dilution rate (0.3 to 0.4 d^{-1}) for at least 7 d until the turbidity at 665 nm became constant (Watanabe & Miyazaki 1996). N:C ratios (by atom) were ~0.07 for *S. quadricauda* and ~0.10 for *M. novacekii*, which were similar to the data used in Watanabe & Miyazaki (1996). Batch cultures of *S. quadricauda* were carried out for 4 d until the late exponential phase, during which the growth rate was 1.4 to 1.5 d^{-1}. N:C ratios of batch-cultured *S. quadricauda* (~0.183) were higher than those of chemostat-cultured *S. quadricauda* and the value (0.15) calculated from the Redfield ratio (Table 1). The cultivation conditions are listed in Table 1. Duplicate samples were removed at predetermined intervals for analysis.

Concentrations of ammonium and nitrate in the algal suspensions were determined according to the phenol-hypochlorite method detailed in Sagi (1966) and the hydrazinium reduction method (Mullin & Riley 1955), respectively. Concentrations of ammonium and nitrate under steady state in the chemostat cultures were below the detection limit, so algal cells were considered to be nitrogen-limited (Watanabe & Miyazaki 1996).

Aliquots (70 ml) of pre-cultivated algal suspension were dispensed into 100 ml glass bottles for ^{15}N incubation (Miyazaki et al. 1985). At the same time, we prepared samples incubated without added ^{15}N as controls in each treatment. The measured value of ^{15}N

Table 1. Summary of the precultivation and ^{15}N-tracer incubation of *Scenedesmus quadricauda* (Sq) and *Microcystis novacekii* (Mn). B: batch; C: chemostat; L: light; D: dark

Expt	Species	Preculture			^{15}N incubation	
		Culture	N-source	N:C (atomic ratio)	Irradiance	N-source
1A	Sq	B	NH_4^+	0.185 ± 0.002	L	NH_4^+
1B	Sq	B	NH_4^+	0.180 ± 0.002	L	NO_3^-
2A	Sq	C	NH_4^+	0.075 ± 0.001	L	NH_4^+
2B	Sq	C	NH_4^+	0.068 ± 0.001	L	NO_3^-
2C	Sq	C	NH_4^+	0.075 ± 0.001	D	NH_4^+
2D	Sq	C	NH_4^+	0.068 ± 0.001	D	NO_3^-
3A	Sq	C	NO_3^-	0.078 ± 0.003	L	NH_4^+
3B	Sq	C	NO_3^-	0.078 ± 0.003	L	NO_3^-
4A	Mn	C	NH_4^+	0.101 ± 0.003	L	NH_4^+
4B	Mn	C	NH_4^+	0.101 ± 0.000	L	NO_3^-
4C	Mn	C	NH_4^+	0.101 ± 0.003	D	NH_4^+
4D	Mn	C	NH_4^+	0.101 ± 0.003	D	NO_3^-

content was constant (0.365 %). $^{15}NH_4Cl$ (99.3 at %) or $Na^{15}NO_3$ (99.4 at %) (90 µg-at N · l^{-1}) was added to the bottles. Light-incubated bottles were incubated at 25°C at an irradiance of 150 mmol photons m^{-2} s^{-1}. Dark-incubated bottles were wrapped in aluminum foil. The incubation conditions are listed in Table 1. After incubation, samples were fixed with formalin (final conc. 0.2 %). In the present study, TDONR (total dissolved organic nitrogen release) was not observed or was minimal at the initial phase of the ^{15}N incubation using chemostat-precultivated algae, even though the samples were fixed with formalin, indicating minimal artificial release occurs. If artificial release was caused by the formalin treatment, more TDONR would have been observed during this initial phase. This indicates that artificial release due to fixation was minimal or negligible in the present study. We used formalin to fix samples to allow greater precision with the incubation time. If we had not used formalin, the incubation time would have become more inaccurate.

Fixed samples were filtered through Whatman GF/C filters (4 to 5 µg-at N). NaCl was added to filtrates to increase the salinity, and the filtrates were processed with the ion retardation column according to the method of Bronk & Gilbert (1991) with the modification described in Nagao & Miyazaki (1999). Nitrogen contents and ^{15}N at % in algal particles on filters and in filtrates were determined with a quadrupole mass spectrometer (Anelva TE-150) (Miyazaki et al. 1985).

We estimated TDONR (total DON released during the ^{15}N incubation), RANR (release of DON produced from DIN [dissolved inorganic nitrogen] assimilated into cells during the ^{15}N incubation) and NNA (gross nitrogen assimilation minus total released nitrogen during the ^{15}N incubation) by the calculation method of Nagao & Miyazaki (1999) (see Appendix 1 for summary of the estimation methods). Equations for the calculation of DON release and DIN assimilation are based on the mass balance of nitrogen among DIN, DON and PON (particulate organic nitrogen). Release of nitrogen assimilated before the ^{15}N incubation is discerned from the release of nitrogen assimilated during the ^{15}N incubation.

RESULTS

DON release from *Scenedesmus quadricauda*

Release of DON and NNA were examined in the light in nitrogen-replete *Scenedesmus quadricauda*. We prepared *S. quadricauda* by batch pre-cultivation with ammonium. We considered the batch-cultured *S. quadricauda* as nitrogen-replete, because the *S. quadricauda* cells had cellular N:C ratios (0.18 to 0.185), 2.4 to 2.8 times higher than nitrogen-limited cells cultured in chemostats (0.068 to 0.075) (Table 1). In the ammonium incubation (Expt 1A) of nitrogen-replete *S. quadricauda*, a TDONR of 0.38 µg-at N µg-at N^{-1} was observed at 1 h (Fig. 1A). In the nitrate incubation (Expt 1B), TDONR observed was 0.28 µg-at N µg-at N^{-1} at 1 h (Fig. 1B). For both ammonium and nitrate incubations (Fig. 1A,B) TDONR was observed to increase slightly from 1 until 12 h. RANR in the ammonium incubation was nearly zero during the incubation (Expt 1A; Fig. 1A). RANR in the nitrate incubation was almost zero until 3 h and then increased to 0.2 µg-at N µg-at N^{-1} at 12 h (Expt 1B; Fig. 1B). The percentages of RANR to TDONR at 12 h were 2.5 % in the ammonium incubation and 51 % in the nitrate-incubation (Table 2). NNA at 1 h was zero in both the ammonium and nitrate incubations. After 1 h, NNA increased. NNA was higher than TDONR after 4 h in the ammonium and nitrate incubations.

TDONR, RANR and NNA were measured in the nitrogen-limited *Scenedesmus quadricauda* precultivated with ammonium in chemostats in the light (Expts 2A and 2B; Fig. 2A,B). TDONR was lower at 1 to 3 h (Fig. 2A,B) compared with the nitrogen-replete *S. quadricauda* (Fig. 1). RANR was nearly zero in the ammonium incubation, while it was zero until 3 h and increased to 0.24 µg-at N µg-at N^{-1} in the nitrate incu-

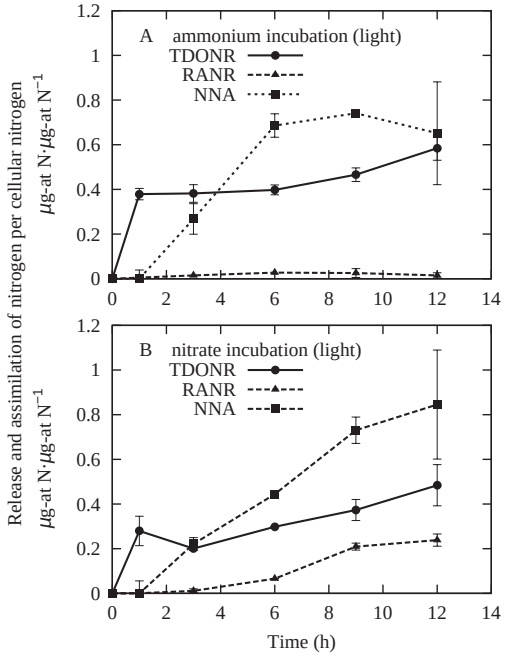

Fig. 1. Cellular N specific DON release and DIN assimilation by nitrogen-replete *Scenedesmus quadricauda* in the light during ^{15}N-tracer incubation ($^{15}NH_4Cl$ or $Na^{15}NO_3$: 90 µg-at N·l^{-1}). The alga was prepared by cultivation under an excess supply of ammonium (50 µg-at N·l^{-1}) in batch. (A) Incubation in ammonium (Expt 1A). (B) Incubation in nitrate (Expt 1B)

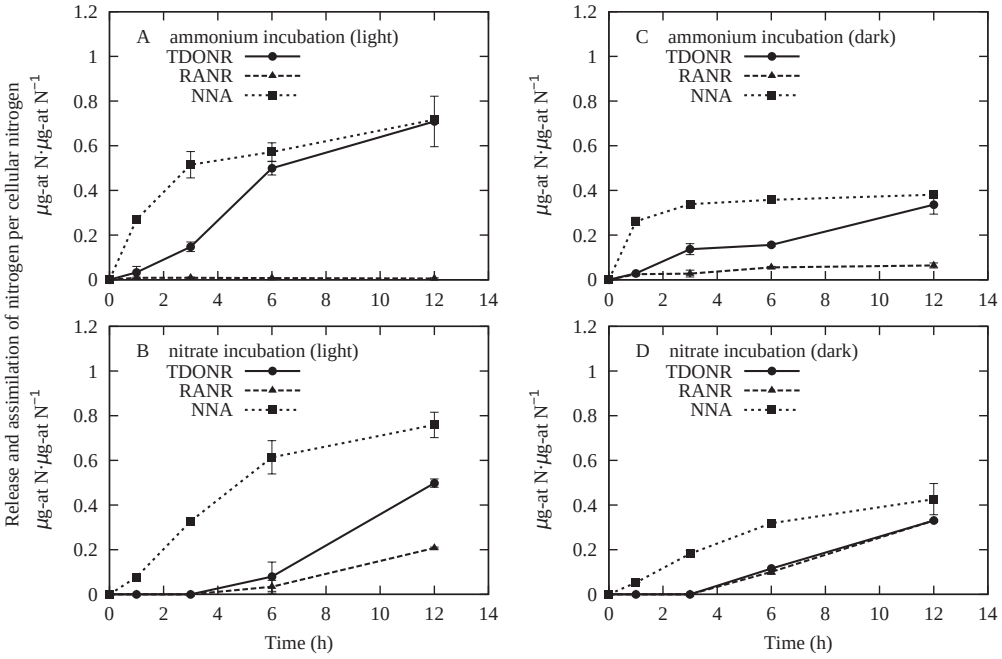

Fig. 2. Cellular N specific DON release and DIN assimilation by nitrogen-limited *Scenedesmus quadricauda* during ^{15}N-tracer incubation ($^{15}NH_4Cl$ or $Na^{15}NO_3$: 90 μg-at N·l^{-1}). The alga was prepared by cultivation under a supply of ammonium (40 μg-at N·l^{-1}) in chemostats. (A) Incubation in ammonium and in the light (Expt 2A). (B) Incubation in nitrate and in the light (Expt 2B). (C) Incubation in ammonium and in the dark (Expt 2C). (D) Incubation in nitrate and in the dark (Expt 2D)

bation. The percentage of RANR to TDONR at 12 h was 0.8% when ammonium was supplied as the nitrogen source in the ^{15}N incubation (Expt 2A; Fig. 2A) and 42% when nitrate was supplied (Expt 2B; Fig. 2B) (Table 2). NNA increased with time in the ammonium (Expt 2A; Fig. 2A) and the nitrate incubations (Expt 2B; Fig. 2B) (Fig. 2). This NNA increase was similar to that in Expt 1 using nitrogen-replete *S. quadricauda*, except that NNA in nitrogen-replete *S. quadricauda* was zero at 1 h. NNA at 12 h reached 0.71–0.72 μg-at N μg-at N^{-1}.

In the dark incubations for nitrogen-limited *Scenedesmus quadricauda*, TDONR showed a gradual increase (Expts 2C and 2D; Fig. 2C,D). TDONR at 12 h was 0.34 μg-at N μg-at N^{-1} in the ammonium incubation and 0.33 μg-at N μg-at N^{-1} in the nitrate incubation. RANR was nearly constant (less than 0.64 μg-at N μg-at N^{-1}) in the ammonium incubation. RANR in

Table 2. Percentages of the recently assimilated nitrogen release to total DON release (RANR/TDONR × 100) and TDONR to gross nitrogen assimilation (TDONR/GNA × 100) in ^{15}N-tracer incubation of *Scenedesmus quadricauda* (Sq) and *Microcystis novacekii* (Mn). Data values were calculated from duplicate samples. B: batch; C: chemostat; L: light; D: dark

Expt	Species	Preculture		^{15}N incubation		RANR/TDONR (%)	TDONR/GNA (%)	
		Culture	N-source	Irradiance	N-source	12 h	3 h	12 h
1A	Sq	B	NH_4^+	L	NH_4^+	2.5 ± 2.7	59.3 ± 7.8	48.9 ± 10.8
1B	Sq	B	NH_4^+	L	NO_3^-	51.1 ± 13.2	47.3 ± 3.4	37.4 ± 9.4
2A	Sq	C	NH_4^+	L	NH_4^+	0.8 ± 0.6	22.3 ± 3.7	49.4 ± 4.6
2B	Sq	C	NH_4^+	L	NO_3^-	41.7 ± 2.1	0.0 ± 0.0	41.6 ± 2.5
2C	Sq	C	NH_4^+	D	NH_4^+	19.4 ± 4.9	28.6 ± 4.3	46.7 ± 3.7
2D	Sq	C	NH_4^+	D	NO_3^-	98.7 ± 2.5	0.0 ± 0.0	44.2 ± 4.7
3A	Sq	C	NO_3^-	L	NH_4^+	33.4 ± 20.7	14.0 ± 7.8	48.6 ± 8.9
3B	Sq	C	NO_3^-	L	NO_3^-	42.3 ± 2.4	11.6 ± 2.2	42.4 ± 1.6
4A	Mn	C	NH_4^+	L	NH_4^+	99.5 ± 15.2	4.7 ± 1.3	25.9 ± 1.9
4B	Mn	C	NH_4^+	L	NO_3^-	–	5.1 ± 5.8	25.5 ± 18.3
4C	Mn	C	NH_4^+	D	NH_4^+	100.5 ± 23.4	4.5 ± 4.7	33.4 ± 4.6
4D	Mn	C	NH_4^+	D	NO_3^-	–	0.1 ± 0.1	2.6 ± 2.4

the nitrate incubation showed almost the same trend as the TDONR. The ratios of RANR to TDONR at 12 h were 19% in the ammonium incubation and 98.0% in the nitrate incubation (Table 2). NNA in the dark increased with time. NNA at 12 h was 0.38 and 0.43 µg-at N µg-at N^{-1} in the ammonium and nitrate incubations, respectively.

TDONR, RANR and NNA in the light incubations for *Scenedesmus quadricauda*, which was chemostat-precultivated with nitrate as the nitrogen source (Expt 3; Fig. 3), were similar to those in Expt 2 using ammonium-precultivated, nitrogen-limited cells (Fig. 2).

DON release from *Microcystis novacekii*

Minimal TDONR appeared at 1 to 3 h in the light incubation of nitrogen-limited *Microcystis novacekii* precultivated with ammonium in chemostats (Expts 4A and 4B, Fig. 4A,B). TDONR increased after 3 h. This TDONR change until 3 h was similar to the trends observed during the initial phase of Expts 2 to 4 using nitrogen-limited *Scenedesmus quadricauda*. In Expts 4A and 4B, RANR was almost the same as TDONR. This result contrasted with those of Expts 2A and 2B, where RANR by ammonium-precultivated, nitrogen-limited *S. quadricauda* was almost zero. NNA by am-

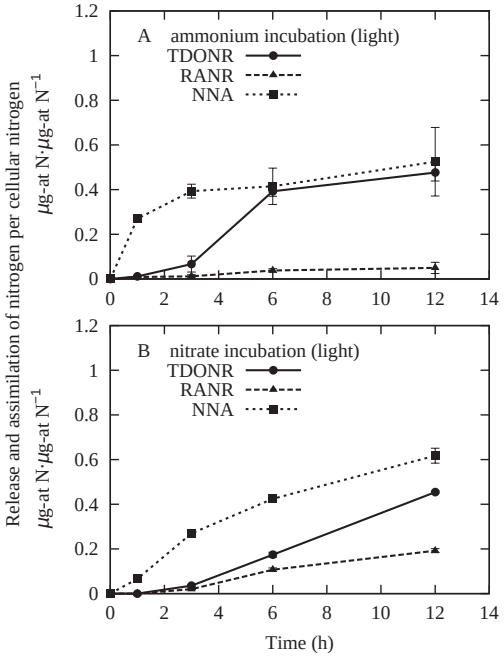

Fig. 3. Cellular N specific DON release and DIN assimilation by nitrogen-limited *Scenedesmus quadricauda* in the light during ^{15}N-tracer incubation ($^{15}NH_4Cl$ or $Na^{15}NO_3$: 90 µg-at N l^{-1}). The alga was prepared by cultivation under a supply of nitrate (40 µg-at N l^{-1}) in chemostats. (A) Incubation in ammonium (Expt 3A). (B) Incubation in nitrate (Expt 3B)

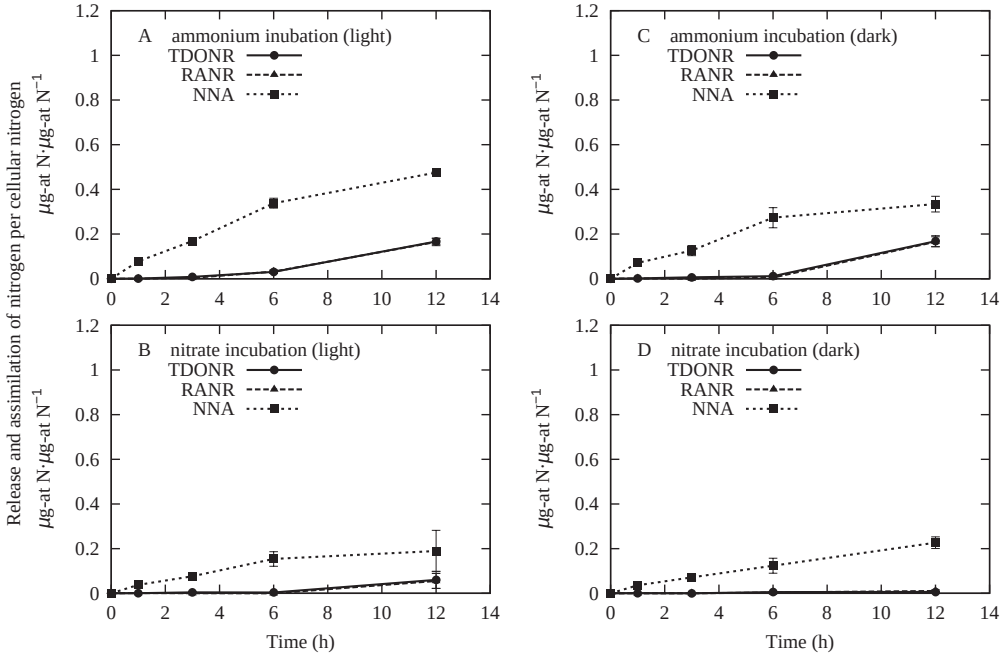

Fig. 4. Cellular N specific DON release and DIN assimilation by nitrogen-limited *Microcystis novacekii* during ^{15}N-tracer incubation ($^{15}NH_4Cl$ or $Na^{15}NO_3$: 90 µg-at N l^{-1}). The alga was prepared by cultivation under a supply of ammonium (40 µg-at N l^{-1}) in chemostats. (A) Incubation in ammonium and in the light (Expt 4A). (B) Incubation in nitrate and in the light (Expt 4B). (A) Incubation in ammonium and in the dark (Expt 4C). (B) Incubation in nitrate and in the dark (Expt 4D)

monium-precultivated, nitrogen-limited *M. novacekii* was lower than that by ammonium-limited *S. quadricauda* (Fig. 2).

In the dark, TDONR by ammonium-precultivated, nitrogen-limited *Microcystis novacekii* (Expts 4C and 4D; Fig. 4C,D) did not appear until 6 h during the ^{15}N-ammonium incubation (Expt 4C; Fig. 4C) and not until 12 h in the ^{15}N-nitrate incubation (Expt 4D; Fig. 4D). TDONR in the dark was lower than results for *Scenedesmus quadricauda* and *M. novacekii* incubated under light conditions. NNA in the dark by *M. novacekii* linearly increased during the tracer incubations and was lower than values in the light for *S. quadricauda* and *M. novacekii*.

DISCUSSION

The possibility of an artificial DON release

Several factors such as the containment of samples in bottles, fixation (Shimura et al. 1978) and filtration (Vogel et al. 1978) may result in the artificial releases of DON (Bronk & Glibert 1994). Assimilation of DIN and/or DON by bacteria present in samples might make it difficult to estimate exactly the DON release from algae (Li 1990, Stockner et al. 1990, Hoch & Kirchman 1995). Re-assimilation of released DON by the algal cells themselves may lead to an underestimation of DON release. Therefore, it is necessary to assess the effects of these factors on the DON release measurements.

We incubated the sample algae in glass incubation bottles. After incubation, we fixed the samples with formalin (0.2%) and filtered them gravitationally with glass-fiber filters for DON-release measurements. Previous studies have reported that fixation with formalin and rough filtration caused an anomalous release of DOM (Shimura et al. 1978, Vogel et al. 1978). Kirchman et al. (1989) found that even gentle gravity filtration increased the release of dissolved free amines. In the present study, TDONR was not observed or was minimal at the initial phase of the ^{15}N incubation (Figs. 2 to 4) using chemostat-precultivated algae, even though the samples were fixed and filtered. This indicates that any artificial release by containment, fixation and filtration was negligible, though further studies will be needed to confirm this negligible artificial release.

Uptake of DIN and/or DON by bacteria might alter the estimation of DON release. When ammonium is used as a nitrogen source, bacteria sometimes take up significant amounts of ammonium from the sample water when both algae and bacteria are present (Li 1990, Hoch & Kirchman 1995). This uptake might change the ambient nitrogen concentrations and would affect the estimation of DON release. In this study, we used axenic samples, and thus, excluding contamination, there was no or minimal uptake by bacteria. Even if bacteria were present in the samples, most bacteria would pass into filtrates, since we used Whatman GF/C filters to collect particles larger than 1.2 mm in diameter. If so, nitrogen incorporated by bacteria would be estimated as DON release. Ammonium uptake by bacteria might lead to an overestimation of DON release. In the present study, TDONR was nearly zero even at the initial phase of the ^{15}N incubation using chemostat-precultivated algae when the ammonium concentration was higher (Figs. 2 & 4). Therefore, we consider that nitrogen uptake by bacteria had minimal influence on the estimation of DON release. However, it is known that phytoplankton can take up DON (Collos et al. 1992, Bronk & Glibert 1993, Flynn & Berry 1999), and as we did not measure the reassimilation of DON by the algae, the release of DON may be underestimated.

Flynn & Berry (1999) explained the difference in DON release between low and high nitrogen systems, considering the equilibrium of organic nitrogen between release leakage and transport back into algae. In the present tracer incubation, total system nitrogen was about 100 µg-at N l^{-1}. According to Flynn & Berry (1999), the DON released would be less than 5% of the total system nitrogen at 100 µg-at N l^{-1}. The percentage in the present study was 0 to 20% before 3 h. At later times, a higher percentage was observed. These values were not necessarily comparable with the data of Flynn & Berry (1999). This is in part because carbon would become limiting at later times, and the system did not necessarily attain the equilibrium of organic nitrogen as mentioned in Flynn & Berry (1999). Though the present incubation systems were not necessarily equilibrated in the same way as in Flynn & Berry (1999), we can discuss the relative difference in nitrogen release among algae with different nutrient status.

DON release and nitrogen assimilation

Scenedesmus quadricauda precultivated in nitrogen-replete conditions excreted DON (TDONR) from the beginning of the tracer incubation (Fig. 1). On the contrary, *S. quadricauda* precultivated in nitrogen-limited conditions released minimal DON (TDONR) at 1 h during the tracer incubation (Figs. 2 & 3). TDONR increased after 1 to 3 h. Similarly, *Microcystis novacekii* grown in nitrogen-limited conditions released minimal DON (TDONR) at 1 h during the tracer incubation (Fig. 4). This result is contrary to the observa-

tions by Marlow et al. (1989) that phytoplankton under stress such as nutrient limitation increase DOM release. The smaller TDONR by the nitrogen-limited algae suggests that the algae suppress TDONR to fulfill the cellular nitrogen demand.

In the present study, RANR by *Microcystis novacekii* precultivated in nitrogen-limited conditions was larger at 6 h or later under the supply of ammonium than that of nitrate in the tracer incubations. On the contrary, RANR by *Scenedesmus quadricauda* precultivated in nitrogen-limited conditions was smaller at 6 h or later under ammonium supply than under nitrate supply in the tracer incubations. This result shows that planktonic algae do not necessarily release recently assimilated nitrogen more readily under ammonium supply than nitrate supply, and it suggests that RANR depends on the species of nitrogen source and algae.

The ratios of RANR to TDONR at 12 h were mostly smaller in *Scenedesmus quadricauda* than in *Microcystis novacekii* when the algae were precultivated in nitrogen-limited conditions. This suggests that *S. quadricauda* releases more DON stored before the tracer incubations compared with *M. novacekii*. Mague et al. (1980) proposed the hypothesis that DOC (dissolved organic carbon) is released when pools of DOC are saturated. Flynn & Butler (1986) suggested that the release of DOM was due to overflow from DOM pools. If the nitrogen was released in a similar way, the difference in the ratio of RANR to TDONR between *S. quadricauda* and *M. novacekii* may be due to the difference in the cellular DON pool size.

We can only speculate on the components of previously and recently assimilated nitrogen, as we did not directly measure them. Mague et al. (1980) suggested that exudates originating from carbon recently taken up into cells were rich in low-molecular compounds. Wiebe & Smith (1986) stated that actively growing phytoplankton excreted mostly low-molecular compounds. Compounds released from recently assimilated nitrogen in the present study might thus be mainly composed of low-molecular compounds such as amino acids. In contrast, Bronk & Glibert (1991) speculated that cell components discharged by cell lysis had higher molecular weights. DON released from previously assimilated nitrogen might be of higher molecular weight compounds compared with that from recently assimilated compounds, as some of the previously assimilated nitrogen might have been synthesized into components with higher molecular weights.

NNA after 1 h in tracer incubations reflected the difference between nitrogen sources. In *Scenedesmus quadricauda*, NNA in the ^{15}N-ammonium incubation was higher than that in the ^{15}N-nitrate incubation (Figs. 2 & 3). In *Microcystis novacekii*, NNA after 1 h of tracer incubations was higher in the ^{15}N-ammonium incubation than in the ^{15}N-nitrate incubation. One of the possible reasons for these results may be that ammonium is more reduced than nitrate. Energy cost for amino-acid synthesis from inorganic nitrogen compounds seems lower for ammonium than for nitrate (Thompson et al. 1989, Levasseur et al. 1993), so ammonium may be taken up more readily; however, Larsson et al. (1985) showed that there was no difference in the levels of reduced pyridine nucleotides after the addition of ammonium or nitrate in the presence of carbon dioxide.

The difference in nitrogen source did not lead to a difference in NNA after 12 h tracer incubation in *Scenedesmus quadricauda* (0.72 µg-at N µg-at N^{-1} for ammonium; 0.72 for µg-at N µg-at N^{-1} for nitrate). Total inorganic carbon measured with the near-infrared method in the incubation medium became zero after 6 h. Carbon would be limiting after 6 h. After 6 h, nitrogen assimilation would be limited by carbon, and thus the difference in NNA during the initial phase of the incubation would disappear.

Chan & Campbell (1978) found that the release of nitrogen increased in the light in a Canadian Shield lake. In the present study, light increased the TDONR in both *Scenedesmus quadricauda* and *Microcystis novacekii*. This increase was observed in both the ^{15}N-ammonium and ^{15}N-nitrate tracer incubations (Figs. 2 to 4). In the dark, algae cannot synthesize organic carbon through photosynthesis. Since algae cannot newly produce organic carbon which is required for the synthesis of organic nitrogen compounds, a lower release of DON would be expected in dark incubations.

TDONR by nitrogen-limited *Microcystis novacekii* was lower than that by nitrogen-limited *Scenedesmus quadricauda* (Figs. 2 to 4). The ratio of TDONR to gross nitrogen assimilation (GNA = TDONR + NNA) was lower in *M. novacekii* than in *S. quadricauda* after 3 and 12 h incubations in the presence of tracer ammonium (Table 2). This suggests that *M. novacekii* utilizes the assimilated ammonium-nitrogen more effectively. This more effective assimilation by *M. novacekii* could be related to the lower half-saturation constant of ammonium uptake (K_m) compared with *S. quadricauda* (Watanabe & Miyazaki 1996). NNA in the nitrogen-limited conditions was lower in *M. novacekii* than in *S. quadricauda*. This lower NNA may lead to a lower maximum uptake (V_{max}) of ammonium in *M. novacekii*. Thus, the results of nitrogen release and assimilation can be used to partly explain the V_{max} and K_m values, which in turn can be used to assess the competitive interaction between *M. novacekii* and *S. quadricauda* (Watanabe & Miyazaki 1996), though some changes in the kinetic parameters may occur because of the change in the rate of transport due to feedback processes (Flynn 1998).

Bronk & Ward (2000) and Slawyk et al. (2000) discussed the sources of DON release. The present calculation assumes that DIN is assimilated into organic nitrogen compounds of algal cells whether the compounds are dissolved or components of cell structure and that DON released originates from the organic nitrogen compounds in the cells. We did not use TCA to separate DON into component DONs. We considered the mass balance of tracers among DIN, DON and PON. The present NNA was assumed to correspond to the net DIN uptake measured in Dugdale & Goering (1967). The summed values of TDONR and NNA will be considered to correspond to the gross DIN uptake (Slawyk et al. 1998).

The present results show that nitrogen release was influenced by the nitrogen status of the algae. DON was released more readily by nitrogen-replete algae than by nitrogen-limited algae. The patterns of release were related to the assimilation of the nitrogen. Differences between *Microcystis novacekii* and *Scenedesmus quadricauda* were observed. The differences would affect the competitive advantage of the algae under differing environmental nitrogen sources. It is necessary to study the relationship between DON release and ecological interactions of algae to obtain clearer insights into phytoplankton competitive interactions.

Appendix 1. Calculation of DON release and DIN assimilation (Nagao & Miyazaki 1999)

Equations for the calculation of DON release and DIN assimilation are based on the mass balance of nitrogen among DIN, DON and PON.

When DON release and assimilation occur simultaneously, during δt (time difference between t and t_1: $t_1 = t + \delta t$), the mass balance of total nitrogen ($^{14}N+^{15}N$) is described as:

$$\mathrm{TDONR} + \mathrm{PON}_{t_1} = \mathrm{PON}_t + \mathrm{GNA} \quad \text{(A1)}$$

where TDONR is the total DON released during δt. PON_{t_1} and PON_t are PON at t_1 and t, respectively. GNA (gross nitrogen assimilation) is the gross (released + unreleased) nitrogen assimilated during δt.
The mass balance of ^{15}N is described by:

$$A_{\mathrm{PON}_t} \cdot \mathrm{PON}_t + A_{\mathrm{DIN}} \cdot \mathrm{GNA} = A_{\mathrm{PON}_{t_1}} \cdot \mathrm{PON}_{t_1} + A_{\mathrm{DON}_{t_1}} \cdot \mathrm{DON}_{t_1} - A_{\mathrm{DON}_t} \cdot \mathrm{DON}_t \quad \text{(A2)}$$

where A_{PON_t} and $A_{\mathrm{PON}_{t_1}}$ are the ^{15}N at % of PON at t and t_1, respectively. A_{DIN} is the ^{15}N at % of DIN. DON_{t_1} and DON_t are DON at t_1 and t, respectively. $A_{\mathrm{DON}_{t_1}}$ and A_{DON_t} are the ^{15}N at % of DON at t_1 and t, respectively. We assume that nitrogen taken up is converted to PON and DON.
TDONR is expressed as:

$$\mathrm{TDONR} = \mathrm{DON}_{t_1} - \mathrm{DON}_t \quad \text{(A3)}$$

From Eqs. (A1), (A2) and (A3), GNA and TDONR are calculated as follows:

$$\mathrm{GNA} = \frac{A_{\mathrm{DON}_t} - A_{\mathrm{PON}_{t_1}}}{A_{\mathrm{DON}_t} - A_{\mathrm{DIN}}} \times \mathrm{PON}_{t_1} - \frac{A_{\mathrm{DON}_{t_1}} - A_{\mathrm{DON}_t}}{A_{\mathrm{DON}_t} - A_{\mathrm{DIN}}} \times \mathrm{DON}_{t_1} + \frac{A_{\mathrm{PON}_t} - A_{\mathrm{DON}_t}}{A_{\mathrm{DON}_t} - A_{\mathrm{DIN}}} \times \mathrm{PON}_t \quad \text{(A4)}$$

and

$$\mathrm{TDONR} = \frac{A_{\mathrm{DIN}} - A_{\mathrm{PON}_{t_1}}}{A_{\mathrm{DON}_t} - A_{\mathrm{DIN}}} \cdot \mathrm{PON}_{t_1} - \frac{A_{\mathrm{DON}_{t_1}} - A_{\mathrm{DON}_t}}{A_{\mathrm{DON}_t} - A_{\mathrm{DIN}}} \cdot \mathrm{DON}_{t_1} + \frac{A_{\mathrm{PON}_t} - A_{\mathrm{DIN}}}{A_{\mathrm{DON}_t} - A_{\mathrm{DIN}}} \cdot \mathrm{PON}_t \quad \text{(A5)}$$

We define NNA (net nitrogen assimilation) as:

$$\mathrm{NNA} = \mathrm{GNA} - \mathrm{TDONR} \quad \text{(A6)}$$

TDONR is composed of RANR (recently assimilated nitrogen release: release of DON produced from DIN assimilated during δt) and PANR (previously assimilated nitrogen release: nitrogen assimilated before t and released during δt). Thus, we have:

$$\mathrm{TDONR} = \mathrm{RANR} + \mathrm{PANR} \quad \text{(A7)}$$

The mass balance of ^{15}N present in TDONR is described by:

$$A_{\mathrm{DIN}} \cdot \mathrm{RANR} + A_{\mathrm{PON}_t} \cdot \mathrm{PANR} = A_{\mathrm{DON}_{t_1}} \cdot \mathrm{DON}_{t_1} - A_{\mathrm{DON}_t} \cdot (\mathrm{DON}_{t_1} - \mathrm{TDONR}) \quad \text{(A8)}$$

From Eqs. (A7) and (A8), RANR may be calculated as follows:

$$\mathrm{RANR} = \frac{A_{\mathrm{DON}_t} - A_{\mathrm{PON}_t}}{A_{\mathrm{DIN}} - A_{\mathrm{PON}_t}} \cdot \mathrm{TDONR} + \frac{A_{\mathrm{DON}_{t_1}} - A_{\mathrm{DON}_t}}{A_{\mathrm{DIN}} - A_{\mathrm{PON}_t}} \cdot \mathrm{DON}_{t_1} \quad \text{(A9)}$$

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