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Algal growth on organic compounds as nitrogen sources

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Abstract. Two experimental series were run to evaluate the potential of algal development on dissolved organic nitrogen (DON) compounds as the sole source of nitrogen (N) nutrition. Monocultures of several common Lake Kinneret algae (*Pediastrum duplex*, *Synechococcus* sp., *Microcystis aeruginosa*, *Aphanizomenon ovalisporum* and *Cyclotella* sp.) were incubated for 3 weeks in the laboratory with different inorganic (NH_4^+, NO_3^-) or organic (hypoxanthine, urea, guanine, ornithine, glucosamine, lysine) nitrogen sources. Even though the cultures were not axenic, marked differences were observed in algal growth response. *Pediastrum*, *Cyclotella* and *Aphanizomenon* grew well on most N sources, and cyanobacterial growth and yield were consistently greatest when urea was the only N source. We also followed algal growth and eventual species dominance in batch samples of GF/F-filtered lake water, supplemented with orthophosphate and different inorganic or organic N compounds and inoculated with concentrated lake phytoplankton. Although no clear impact on phytoplankton growth (as chlorophyll concentration) was observed, in seven out of 11 experiments we could discern changes in the algal species that became dominant in flasks with different organic and inorganic N sources. Our results are consistent with the proposition that components of the DON pool are not only an important potential, direct or indirect N source for phytoplankton, but also that different algal species can exploit these sources with varying capabilities so that different N substrates may selectively stimulate the development of dominant algal species.

Introduction

Although it has long been assumed that in most aquatic systems there is some flux of nitrogen (N) from the dissolved organic nitrogen (DON) pool to microorganisms (Strickland *et al.*, 1969; Jackson and Williams, 1985), there have been few studies to examine this process in any detail. Nevertheless, since the earlier investigations comprehensively reviewed by Antia *et al.* (1991), increasing research attention has been focused on the role of DON in various aquatic environments. Lately, several studies have emphasized the importance of DON as a potential source of available N for bacteria and algae in estuarine and coastal waters (Carlsson *et al.*, 1993; Seitzinger and Sanders, 1997; John and Flynn, 1999), and there have been suggestions that increased levels of DON may be responsible for the rising incidence of toxic phytoplankton in many locations (Graneli *et al.*, 1985; Carlsson *et al.*, 1995). Bacterial degradation of organic nitrogen compounds and the ability of some algal species to utilize these compounds directly have been demonstrated (North and Stephens, 1967; Flynn and Butler, 1986; Berman *et al.*, 1991; Antia *et al.*, 1991; Ietswart *et al.*, 1994; Lisa *et al.*, 1995; Palenik and Hensen, 1997). There is also evidence for photochemically mediated release of biologically available N from DON in surface and near-surface DON in natural waters (Bushaw *et al.*, 1996). Recently, it was shown that ammonia and/or urea could be released from the DON pool in lake, estuarine and coastal sea water that had been pre-filtered through 1 μm filters (Berman *et al.*, 1999). These experiments confirmed the potential for the release of available N from DON by indigenous bacteria or dissolved enzymes. Another study (Berman,

1997) indicated that components of the DON pool in the epilimnion of Lake Kinneret, either indirectly or directly, were the main source of N for the development of a bloom of the cyanobacterium *Aphanizomenon ovalisporum.*

In this paper, we describe experiments that were designed (i) to examine the ability of monocultures of several common Lake Kinneret algal species to grow on a variety of organic N substrates and (ii) to determine how batch samples of an initial inoculum of indigenous phytoplankton developed in lake water when dependent on various organic and inorganic sources of N. These studies were intended to clarify whether the available fraction of DON may be important in determining not only the amounts, but also the species composition, of the developing phytoplankton assemblage.

Method

Algal monocultures

Non-axenic monocultures of algae isolated from Lake Kinneret, *Pediastrum duplex*, Meyer (Chlorophyta), *Synechococcus* sp., *Microcystis aeruginosa*, *A.ovalisporum* (Cyanobacteria) and *Cyclotella* sp. (Diatomaea), were maintained on Lindstrom medium (LM; Lindstrom, 1985) or, in the latter case, on diatom medium (DM; modified Woods Hole, MBL medium; Nichols, 1973). Cultures in exponential phase were washed three times and concentrated by centrifugation in the same growth medium, but without an N source. (We modified the above media so that they contained only inorganic salts without either NH_4^+ or NO_3^- ions.) Aliquots (0.1–0.5 ml) of the concentrated algae were then added to a series of 125 ml clear polycarbonate bottles (Nalgene) containing 100 ml of the same growth medium (LM or DM minus N source), supplemented with 100 μM of N of one of the following: urea, hypoxanthine, guanine, ornithine, glucosamine, lysine, NH_4^+ or NO_3^-. The organic N supplements were chosen as representative of some of the more labile compounds derived from major biological cell constituents that might be expected to be present in the natural DON pool (Antia *et al.*, 1991). Contamination of NH_4^+ or urea in these compounds was never >0.2%. Control bottles were incubated with the same algal inoculum in unsupplemented (–N) medium.

All treatments and controls were run in triplicate flasks and incubated for ~3 weeks at 22°C at a photon flux of ~80 μM m^{-2} s^{-1} with a 12 h photoperiod. Subsamples (3 ml) were taken at 1–3 day intervals during the incubation period and growth was followed by changes in *in vivo* chlorophyll *a* concentrations determined with a Turner Designs Model 10 fluorometer.

In some of these growth experiments, subsamples were taken concurrently with chlorophyll determinations in order to measure changes in NH_4^+ concentrations with time in these bottles.

Phytoplankton growth on organic compounds as nitrogen sources in lake water

Lake water, collected at 1 m depth from a pelagic lake station, was passed through a GF/F (~0.8 μm) glass fiber filter to remove almost all biota except bacteria and

viruses, and distributed in 500 ml portions into a series of 1 l Erlenmeyer flasks. These flasks were supplemented with 10 μM phosphorus (P) [as $Na_2(PO_4)_3$] and with 100 μM (as N) of one of the following organic or inorganic N compounds: hypoxanthine, lysine, guanine, urea, NH_4^+ or NO_3^-. Control flasks had no N source additions. A concentrated sample of the phytoplankton in lake near-surface water, taken at the same time, was obtained by filtration on a 1 μm Nitex net. The algal slurry was resuspended in 10 ml of GF/F-filtered lake water; 0.5 ml portions were then inoculated into duplicate Erlenmeyer flasks with or without supplements that were incubated at ambient lake temperatures and at ~80 μM m^{-2} s^{-1} photon flux (12 h light period) for ~3 weeks.

Growth of phytoplankton in lake water was monitored by measurements of *in vivo* fluorescence and acetone extracts of chlorophyll *a* (Holm-Hansen *et al.*, 1965). The dominant algal species in the initial lake water samples and in the flasks after 21 days were determined visually on Lugol-fixed samples by microscopy.

The analytical methods used to determine N concentrations were as follows: DON (Nydahl, 1978), ammonium (Solarzano, 1969), nitrate (American Public Health Association, 1992) and urea (McCarthy, 1970). The latter method was modified by using a dialysed preparation of jack bean urease Type IV (Sigma). The results of all chemical analyses were based on averages of 3–5 replicate samples. Note that all concentrations of the N compounds are given as μM N. The addition of various N sources did not change the initial pH of the lake water or of the culture media.

Results

Algal monocultures

Of the algal monocultures tested, *Pediastrum* (Chlorophyta), *Cyclotella* (diatom) and *A.ovalisporum* (cyanobacteria) grew as well on some of the organic nitrogen (ON) substrates as on NH_4^+ and NO_3^- (Figure 1). *Pediastrum* growth was rapid and continuous throughout the incubation period on all N sources. After an initial 4–5 day lag, *Cyclotella* grew rapidly until day 14 and reached even higher maximum yields with the ON substrates than with NH_4^+ or NO_3^-. The filamentous diazotroph *A.ovalisporum* consistently developed most rapidly and achieved the highest yield on urea, but also grew on other ON substrates and on NH_4^+ and NO_3^-. When *A.ovalisporum* was transferred to unsupplemented (–N) medium, there was a considerable lag before growth commenced, presumably due to the time required for induction and activation of nitrogenase. Under our conditions, *Microcystis* and *Synechococcus*, the other cyanobacteria tested, also developed best on urea; these organisms grew more slowly on inorganic N and least well on other DON sources (Figure 1).

In a further set of experiments with *Synechococcus*, *Microcystis* and *A.ovalisporum*, in which only urea, NH_4^+ or NO_3^- were added as N sources, we again observed enhanced growth with urea. Similar results were observed when the experiments were carried out either in LM or in filtered (0.2 μm) lake water supplemented with 10 μM P (data not shown).

We followed the time course of changes in NH_4^+ concentrations in the growth

media in some of these experiments. Surprisingly, different patterns of NH_4^+ uptake and appearance were discerned for different algal species (Figure 2). The same general patterns of NH_4^+ concentration change were obtained at least twice in separate experiments and appeared to be consistent. With the exception of *Cyclotella* (see below), monocultures to which NH_4^+ was added showed a rapid (3–4 day) depletion of this compound, which then remained close to or below detection levels throughout the incubations. In the case of *A.ovalisporum* and *Synechococcus*, almost no NH_4^+ appeared in any of the media to which ON compounds had been added. *Pediastrum* monocultures with guanine or ornithine showed a brief release of NH_4^+ in the medium; with glucosamine addition, a longer period of NH_4^+ accumulation was observed. In two separate experiments with *Microcystis* cultures, we noted a rise and accumulation of high levels of NH_4^+

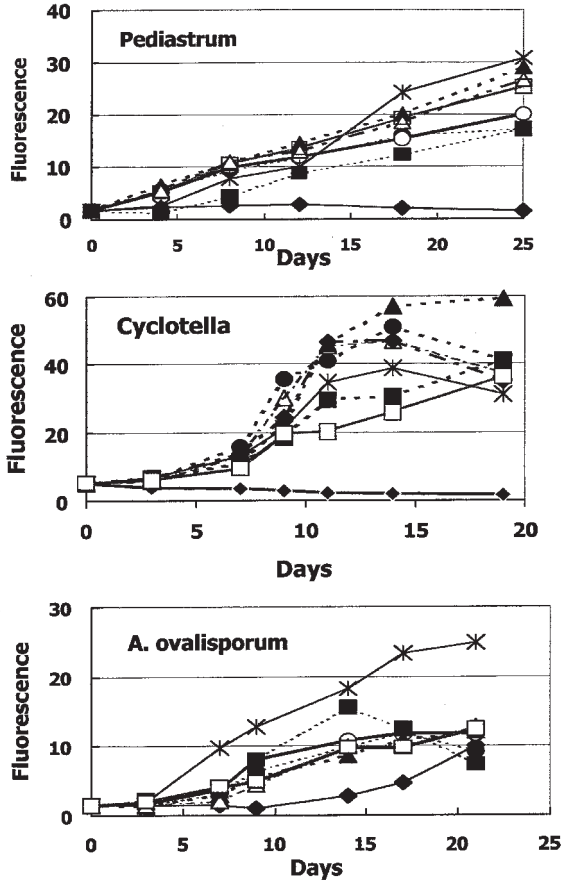

Fig. 1. Growth of monoalgal cultures on inorganic and organic nitrogen sources (see the text for details). ◆, medium – N; ■, NH_4^+; □, NO_3^-; ✳, urea; ▲, ornithine or lysine; △, glucosamine; ●, hypoxanthine; ○, guanine. Note that the data points represent the average of triplicate samples; for clarity, error bars are not shown.

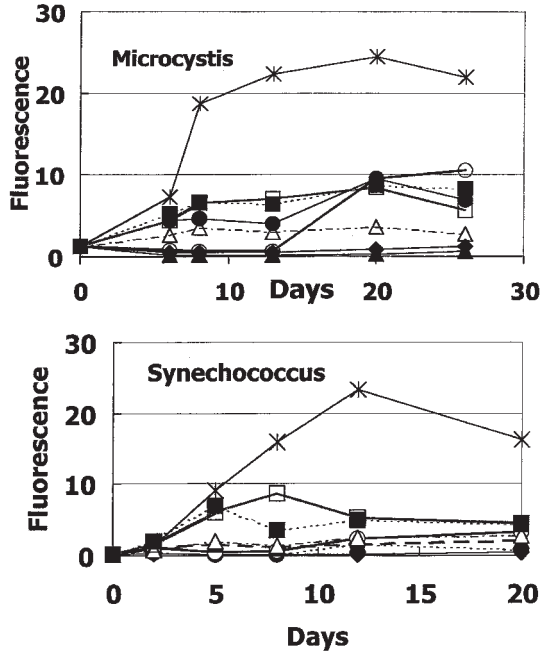

Fig. 1. continued.

in media that had been supplemented with ornithine, but not with any other ON addition.

A very different time course of NH_4^+ concentration change was observed with *Cyclotella*. In flasks supplemented with NH_4^+, there was a much slower and less complete fall in NH_4^+ concentrations than with other cultures. With all ON supplements, a significant rise in NH_4^+ concentrations was observed by day 3; these NH_4^+ levels only declined after day 7. An exception to this was observed in the case of urea supplements, where NH_4^+ increased steadily until the end of the experiment.

Phytoplankton growth and species dominance patterns in amended lake water

In Tables I–VII, we show some results from the experimental series in which we examined the changes in algal biomass (as chlorophyll) and the predominant algal species that developed after 3 weeks incubation of lake water supplemented with various organic or inorganic N sources. Duplicate flasks showed very similar chlorophyll concentrations ($\pm$ <5%) and similar phytoplankton population composition. Algal standing stock change was expressed as the increase in chlorophyll after 21 days relative to the initial chlorophyll concentration as indicated in the tables. In addition, the initial concentrations of NH_4^+, NO_3^- and DON measured in the lake water at the time of sampling are given. Note that supplements of P (10 μM) were added to all the samples in this series.

In late June–July 1996 (Table I), 14- to 20-fold growth increases (measured as chlorophyll) were recorded in all supplemented flasks, while a 7-fold increase occurred with only P addition in the control flasks. *Microcystis*, initially the dominant species, remained the principal component of the assemblage in all treatments and was accompanied by small chlorophytes. With added hypoxanthine or lysine, *Oocystis* and *Synedra*, respectively, also developed. Similar results (not shown) were obtained in an experiment in August 1996. This was not the case in August 1997 (Table II) when *Microcystis* was again initially dominant

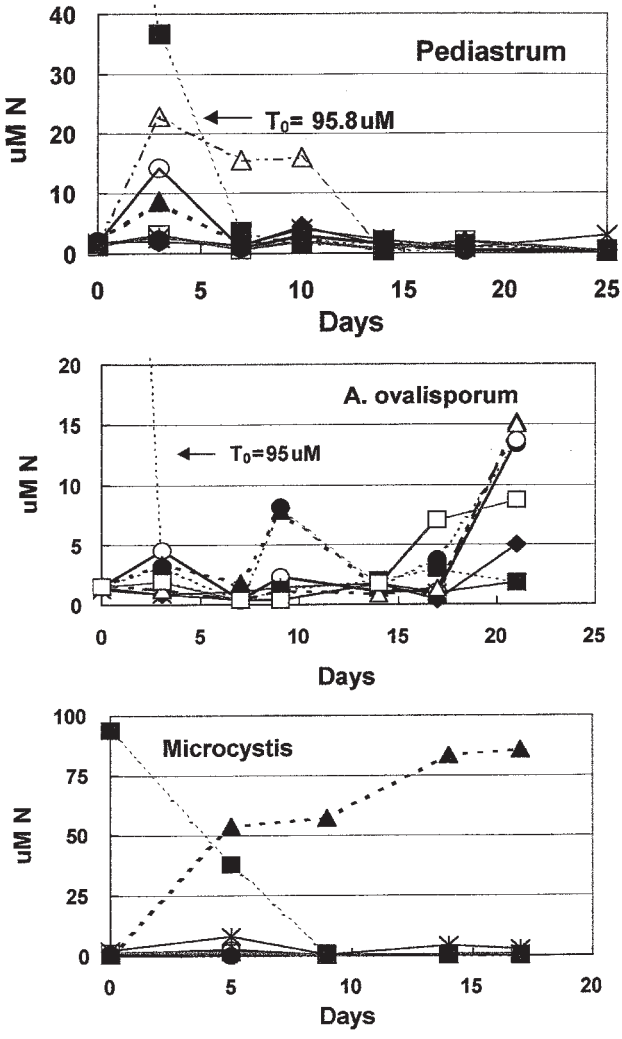

Fig. 2. Changes in NH_4^+ concentration (μM N) in monoalgal cultures growing on inorganic and organic nitrogen sources (see the text for details). ◆, medium – N; ■, NH_4^+; □, NO_3^-; ✳, urea; ▲, ornithine or lysine; △, glucosamine; ●, hypoxanthine; ○, guanine. Note that the data points represent the average of triplicate samples; for clarity, error bars are not shown.

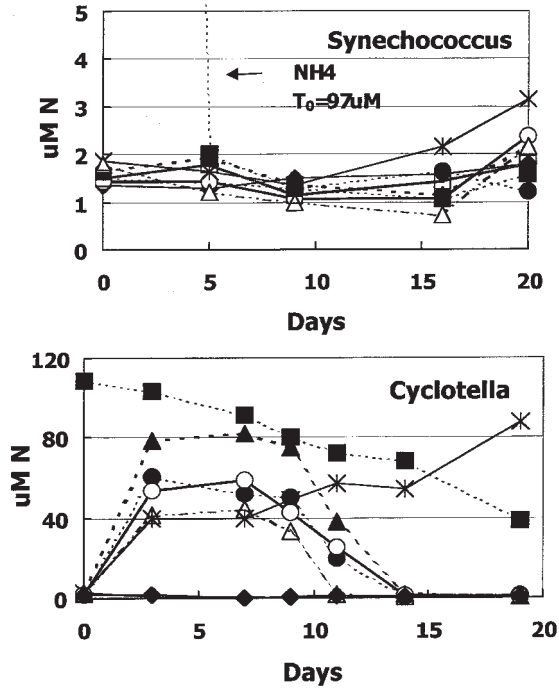

Fig. 2. continued.

Table I. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
10 June 1996	–	1.0	*Microcystis*, chlorophytes (ciliates)
3 July 1996	KW	6.9	*Microcystis*, chlorophytes (ciliates)
	NH_4^+	21.4	*Microcystis*, chlorophytes
	NO_3^-	16.3	*Microcystis*, chlorophytes
	Hypoxanthine	14.3	*Microcystis*, chlorophytes, *Oocystis*
	Lysine	16.0	*Microcystis*, chlorophytes, *Synedra*
	Guanine	14.4	*Microcystis*, chlorophytes
	Urea	16.0	*Microcystis*, chlorophytes

KW, Lake Kinneret surface water with no added N source. All samples received 10 μM P, as $Na_3(PO_4)_3$. Dominant species are underlined.
[a]Ambient concentrations in lake water at time zero: NH_4^+ 1.4 μM, NO_2^- + NO_3^- 2.2 μM and DON 14.1 μM.
[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (2.0 μg chlorophyll l^{-1}) taken as 1.0.

in a phytoplankton assemblage similar to that of the previous August. However, in this experiment, *Microcystis* did not persist and was replaced by small unidentified round flagellates in the flasks with hypoxanthine, lysine and urea, by *Synedra* in unsupplemented lake water or with NO_3^- and by *A.ovalisporum* in the case of guanine addition. Only moderate growth occurred in the control

flask, but very large increases in chlorophyll (40- to 140-fold) were observed with N additions.

During the winter season (December 1996; Table III), considerable increases in chlorophyll were observed in all supplemented flasks, while unsupplemented lake water showed only a 3-fold increase in growth. Surprisingly, NH_4^+ supported less growth than other N supplements in this case. (Note the initial, high level of ambient NH_4^+ in the lake water.) The benthic diatom *Synedra* became dominant in all treatments and was accompanied by *Microcystis*. *Selenastrum* appeared with NO_3^-, *Scenedesmus* with urea and lysine. Although some *Peridinium* cells were present initially, none survived in any of the incubated samples. Large numbers of ciliates also appeared in all supplemented treatments, but not in the untreated water.

In samples taken on 16 January 1997 (Table IV), all N supplements except NO_3^- promoted rapid growth and high yields. (In this case, initial, ambient levels of NO_3^- were high.) The diatom *Synedra* became prominent with the addition of hypoxanthine, lysine or urea. Large numbers of the green flagellate *Carteria* appeared with inorganic, but not with organic, N additions. The large diatom *Aulacoseira* that was initially present also grew out in some, but not all, of the supplemented samples. Similar results were also obtained in the experiment of February 1997 (not shown) where initially dominant *Aulacoseira* was only maintained in NH_4^+ and NO_3^-, whereas with organic N sources, species such as *Monoraphidium*, *Actinastrum* and *Synedra* developed.

Again in April 1997 (Table V), a very marked increase in chlorophyll concentrations occurred in all except the control flasks, especially with urea and NO_3^- supplements. This increase was mainly due to the proliferation of a small unidentified chlorophyte. Normally in Lake Kinneret during this season, *Peridinium* is the major dominant of the phytoplankton assemblage (Berman *et al.*, 1995), but

Table II. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
10 August 1997	–	1.0	*Microcystis*, chlorophytes (ciliates)
4 September 1997	KW		*Synedra*, round chlorophytes (ciliates)
	NH_4^+	75.6	Round chlorophytes, *Synedra* (ciliates)
	NO_3^-	116.3	*Synedra*, round chlorophytes, *Tetraedron*, *Collodictium*
	Hypoxanthine	87.2	Round chlorophytes, *Synedra*, *Aphanizomenon*
	Lysine	139.5	Round chlorophytes, *Synedra*, *Collodictium*
	Guanine	69.8	*Aphanizomenon*, round chlorophytes, *Synedra*, *Cyclotella*, *Tetraedron* (ciliates)
	Urea	40.7	Round flagellates, chlorophytes, *Synedra*, *Aphanizomenon*, *Tetraedron*

KW, Lake Kinneret surface water with no added N source. All samples received 10 µM P, as $Na_3(PO_4)_3$. Dominant species are underlined.

[a]Ambient concentrations in lake water at time zero: NH_4^+ 0.6 µM, NO_2^- + NO_3^- 0.2 µM and DON 17.5 µM.

[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (0.9 µg chlorophyll l^{-1}) taken as 1.0.

Table III. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
2 December 1996	–	1.0	<u>*Peridinium*</u>, *Microcystis*, *Scenedesmus*, chlorophytes
25 December 1996	KW	3.5	<u>*Synedra*</u>, *Microcystis*, chlorophytes, *Oocystis*
	NH_4^+	16.0	<u>*Synedra*</u>, *Microcystis*, chlorophytes (ciliates)
	NO_3^+	30.0	<u>*Synedra*</u>, *Selenastrum*, *Microcystis*, chlorophytes (ciliates)
	Hypoxanthine	24.0	<u>*Synedra*</u>, *Microcystis*, chlorophytes (ciliates)
	Lysine	34.0	<u>*Synedra*</u>, *Microcystis*, *Scenedesmus*, chlorophytes (ciliates)
	Guanine	30.0	<u>*Synedra*</u>, *Microcystis*, chlorophytes (ciliates)
	Urea	48.0	<u>*Synedra*</u>, *Microcystis*, *Scenedesmus*, chlorophytes (ciliates)

KW, Lake Kinneret surface water with no added N source. All samples received 10 μM P, as $Na_3(PO_4)_3$. Dominant species are underlined.
[a]Ambient concentrations in lake water at time zero: NH_4^+ 10.7 μM, $NO_2^- + NO_3^-$ 1.4 μM and DON 18.1 μM.
[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (2.5 μg chlorophyll l^{-1}) taken as 1.0.

Table IV. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
16 January 1997	–	1.0	<u>*Microcystis*</u>, *Aulacoseira*, *Synedra*, chlorophytes
6 February 1997	KW	3.3	<u>*Synedra*</u>, *Microcystis Aulacoseira*, chlorophytes
	NH_4^+	38.5	<u>*Microcystis*</u>, *Carteria*, *Synedra*, chlorophytes (ciliates)
	NO_3^-	11.1	<u>*Carteria*</u>, *Synedra*, *Aulacoseira*, *Microcystis*, *Actinastrum*
	Hypoxanthine	50.0	<u>*Synedra*</u>, chlorophytes, *Microcystis*, *Aulacoseira* (ciliates)
	Lysine	23.1	<u>*Synedra*</u>, *Microcystis*, chlorophytes, *Aulacoseira* (ciliates)
	Guanine	40.4	<u>Chlorophytes</u>, *Synedra*, *Aulacoseira*, *Microcystis*
	Urea	51.9	<u>*Synedra*</u>, *Actinastrum*, *Microcystis*

KW, Lake Kinneret surface water with no added N source. All samples received 10 μM P, as $Na_3(PO_4)_3$. Dominant species are underlined.
[a]Ambient concentrations in lake water at time zero: NH_4^+ 2.3 μM, $NO_2^- + NO_3^-$ 14.3 μM and DON 22.3 μM.
[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (2.6 μg chlorophyll l^{-1}) taken as 1.0.

in 1997 only a few dinoflagellate cells were present initially. *Peridinium* grew only in flasks supplemented with hypoxanthine and guanine, but disappeared from all other treatments. *Microcystis* persisted with all N additions except lysine.

In May 1997 (Table VI), the initial assemblage mainly consisted of *Microcystis*, *Chodatella*, *Peridinium*, *Staurastrum* and small chlorophytes. Incubation with different N sources led to the development of phytoplankton assemblages with differing dominant species. In June 1997 (not shown), most of the increases in chlorophyll observed in supplemented flasks during the incubation were due to

Table V. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
8 April 1997	–	1.0	*Microcystis*, chlorophytes, *Peridinium*
4 May 1997	KW	2.8	Chlorophytes, *Microcystis* (ciliates)
	NH_4^+	2.5	Chlorophytes, small flagellates, *Microcystis*
	NO_3^-	130.0	Chlorophytes, *Microcystis*, dinoflagellates
	Hypoxanthine	45.0	Chlorophytes, diatoms, *Microcystis*, *Peridinium* (ciliates)
	Lysine	45.0	Chlorophytes, small flagellates (ciliates)
	Guanine	87.5	Chlorophytes, *Peridinium*, *Microcystis*
	Urea	162.5	Chlorophytes, *Microcystis*, *Scenedesmus*, *Synedra*

KW, Lake Kinneret surface water with no added N source. All samples received 10 μM P, as $Na_3(PO_4)_3$. Dominant species are underlined.
[a]Ambient concentrations in lake water at time zero: NH_4^+ 1.2 μM, $NO_2^- + NO_3^-$ 16.2 μM and DON 17.4 μM.
[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (2.0 μg chlorophyll l^{-1}) taken as 1.0.

Table VI. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
26 May 1997	–	1	*Microcystis*, *Chodatella*, *Peridinium*, *Staurastrum*, chlorophytes
16 June 1997	KW	3	*Synedra*, *Microcystis*, chlorophytes, (ciliates)
	NH_4^+	53	Chlorophytes, *Synedra*, (ciliates)
	NO_3^-	47	*Synedra*, *Microcystis*, chlorophytes, (ciliates)
	Hypoxanthine	36	Chlorophytes, *Microcystis*, *Synedra*, (ciliates)
	Lysine	33	Chlorophytes, *Synedra*, *Actinastrum*, (ciliates)
	Guanine	13	Chlorophytes, *Microcystis*
	Urea	36	*Microcystis*, *Selenastrum*, chlorophytes

KW, Lake Kinneret surface water with no added N source. All samples received 10 μM P, as $Na_3(PO_4)_3$. Dominant species are underlined.
[a]Ambient concentrations in lake water at time zero: NH_4^+ 0.4 μM, $NO_2^- + NO_3^-$ 1.0 μM and DON 15.6 μM.
[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (1.8 μg chlorophyll l^{-1}) taken as 1.0.

the multiplication of the small chlorophytes accompanied by *Microcystis* and *Synedra. Peridinium* cells, although initially present in May and June, did not persist in any treatment.

Peridinium, *Staurastrum*, *Microcystis*, *Synedra* and small chlorophytes were prominent phytoplankton in December 1997 (not shown). Upon incubation, *Synedra* predominated in all treatments and the dinoflagellates disappeared. Although there was no outgrowth in the control flasks, 18- to 43-fold increases in chlorophyll occurred with N additions, notably with hypoxanthine and lysine.

In February 1998 (Table VII), the lake phytoplankton was dominated by *Aulacoseira* and *Peridinium.* With incubation, the dinoflagellates disappeared, but *Aulacoseira* persisted in supplemented flasks. Small round chlorophytes

Table VII. Phytoplankton growth and development of dominant algae in Lake Kinneret water with added N substrates

Date	Substrates[a]	Growth[b]	Dominant phytoplankton
12 February 1998	–	1.0	*Aulacoseira*, *Peridinium*, chlorophytes
5 March 1998	KW	1.0	*Pediastrum*, *Oocystis*, *Actinastrum*, *Scenedesmus*
	NH_4^+	31.9	*Actinastrum*, *Scenedesmus*, *Synedra*, *Aulacoseira*
	NO_3^-	25.0	Chlorophytes, *Synedra*, *Aulacoseira*, *Actinastrum*
	Hypoxanthine	15.6	Chlorophytes, *Actinastrum*, *Aulacoseira*, *Scenedesmus*
	Lysine	10.9	Chlorophytes, *Synedra*, *Aulacoseira* small flagellates, (ciliates)
	Urea	28.9	Chlorophytes, small flagellates, *Synedra*, *Aulacoseira*, *Scenedesmus* (ciliates)

KW, Lake Kinneret surface water with no added N source. All samples received 10 μM P, as $Na_3(PO_4)_3$. Dominant species are underlined.
[a]Ambient concentrations in lake water at time zero: NH_4^+ 3.1 μM, $NO_2^- + NO_3^-$ 11.8 μM and DON 14.8 μM.
[b]Growth measured as chlorophyll concentrations after 21 days incubation relative to initial concentrations (2.4 μg chlorophyll l^{-1}) taken as 1.0.

developed with all N supplements except NH_4^+, but the other predominant algal species varied with treatment. *Actinastrum* grew out with hypoxanthine and inorganic N supplements.

In order to assess the relative growth stimulus (as yield of chlorophyll after 21 days incubation) of the various N compounds added, we averaged the observed increases in chlorophyll relative to concentrations measured at the start of each incubation for all 11 experiments in this series (Figure 3). The variability of the growth response between different experiments was very high and a pairwise *t*-test comparison showed that there was no significant difference between any of the N-addition treatments (organic and inorganic N). All N supplements resulted in significantly ($P < 0.001$) increased yields in comparison to the control flasks which only received added P.

Discussion

The ability of some algal species in axenic cultures to exploit ON sources has been previously demonstrated (North and Stephens, 1967; Antia *et al*., 1975; Berman *et al*., 1991; Palenik and Henson, 1997; John and Flynn, 1999) and it seems obvious that some components of the DON pool in natural waters can serve as either direct or indirect sources of N nutrition for phytoplankton and bacteria. Previous work (Berman *et al*., 1998) has indicated that both NH_4^+ and urea can be generated by indigenous bacteria from DON in freshwater and coastal marine waters.

One of the aims of this study was to examine the extent to which monoalgal cultures (i.e. algae in the presence of bacteria) developed when N was available only from an organic N source. In this case, it was probable that in all the cultures bacterial degradation of the ON source would lead to the release of NH_4^+ and/or urea, and perhaps even NO_3^- which would be subsequently exploited by algae.

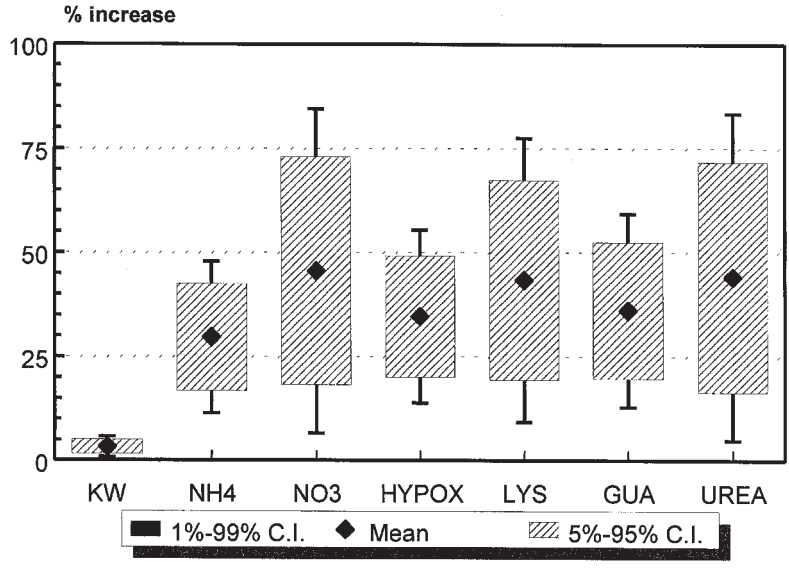

Fig. 3. Summary of 11 growth experiments in Lake Kinneret water with different sources of nitrogen: averages, 5–95% and 1–99% confidence intervals of the increase in chlorophyll concentrations (relative to initial concentrations taken as 1.0). KW, lake water with no added N; treatments with N supplements as indicated: NH_4^+; NO_3^-; HYPOX, hypoxanthine; LYS, lysine; GUA, guanine; urea.

Thus, we expected to observe more or less similar growth patterns in all samples. Nevertheless, marked differences were noted in the growth response of these monoalgal cultures (Figure 1). *Pediastrum*, *A.ovalisporum* and *Cyclotella* developed as well on some ON sources as on inorganic N. We observed in repeated experiments that all three cyanobacteria, *A.ovalisporum*, *Microcystis* and *Synechococcus*, grew well on urea, markedly better than on either NO_3^- or NH_4^+. The latter two species developed poorly on other ON sources. This may have been due, however, to the fact that the cultures were growing on LM which was almost certainly not optimal for cyanobacteria.

In addition to functioning as an N source, urea may also act as a readily available source of CO_2 carbon for photosynthetic organisms. Nevertheless, it is unlikely that carbon availability would have been a limiting factor for growth in these experiments using media or Lake Kinneret water with initial high levels of dissolved inorganic carbon. (Note, the pH in these media was unaffected by the addition of 100 μM urea.) Although our study does not provide conclusive data to support the suggestion that urea may act as a preferred source of N for some cyanobacteria in natural aquatic systems, it would be of considerable interest to ascertain whether this also holds for cyanobacterial species other than those observed in this study. It would be particularly intriguing if this preference could be shown for the ubiquitous strains of *Synechococcus* found in both freshwater and marine environments where the importance of urea as an N source for phytoplankton is well documented (McCarthy, 1972; McCarthy *et al.*, 1982).

Furthermore, it might be important to clarify whether urea supply plays any role in the reported increasing incidence of toxic cyanobacterial blooms (Carmichael, 1994).

We did not follow the N transformations and cycling in any detail in the experimental flasks, although these processes would be expected to be occurring extensively. One indication of this was the extremely rapid fall in the concentrations of NH_4^+ that had been added to the culture flasks (Figure 2). This decrease can be mostly attributed to intense uptake and recycling by the bacterial populations present in these algal monocultures. Only a relatively small amount of the initially added 100 μM NH_4^+ would be required for actual algal growth observed in these experiments. Uptake of inorganic N by phytoplankton with subsequent recycling of DON has been shown to occur in nature (Bronk *et al.*, 1994).

A priori, we assumed that similar changes in the patterns of NH_4^+ concentrations during the incubations would occur from the same ON compounds in all the culture experiments. The fact that this was not observed may have been due either to the presence of different bacterial assemblages associated with the different algal cultures and/or with modes of ON break down and uptake mediated differently by consortia of various algal and bacterial species. Whatever the exact reason(s) for the results shown in Figures 1 and 2, the inference is that utilization of ON by different assemblages of algae and bacteria can vary considerably. Therefore, it is not unreasonable to suggest that differential exploitation of constituents of the DON pool can act as a selective factor in the development of phytoplankton populations in nature.

In the series of experiments with lake water, the growth response (as measured by chlorophyll increase) with various inorganic or organic sources was extremely variable (Tables I–VII). This was not surprising considering that the water samples were taken at different seasons and that different initial phytoplankton inocula were used. The chlorophyll concentrations in these treatments usually increased considerably during the 3 week incubation, but, overall, there were no significant differences between the growth yields with any of the N sources added (Figure 3). Controls without N supplements had significantly lower growth increments despite a substantial addition (10 μM) of P, irrespective of season. Previous work (Berman, 1970; Pollingher *et al.*, 1988) has indicated that during the summer and fall, phytoplankton in Lake Kinneret are mainly limited by P, but also occasionally by N, whereas P limitation might occur towards the end of the late winter–spring bloom of *Peridinium*.

Although the overall growth response to N compounds added to lake water was not specific (Figure 3), it was notable that we could often discern changes in the development of dominant algal species in treatments with different organic and inorganic N sources. Sometimes, the same dominant algal species grew out with all N sources (e.g. Tables I, III and V), but frequently this was not the case (Tables II, IV, VI and VII). Out of a total of 11 experiments, seven showed apparent selective effects of the different supplements. These observations further support the idea that different N substrates can selectively stimulate the development of dominant algal species. (Large numbers of ciliates also appeared in some of these samples, but with no clear relationship to N source.)

We note that *Peridinium*, the dominant lake dinoflagellate, never developed to any extent in the lake water incubations, but we have repeatedly observed that these algae do not grow or even survive for long under similar experimental conditions. Therefore, we suggest that no definite conclusions can be reached at present regarding the capabilities of *Peridinium* to exploit ON compounds.

To conclude, we submit that despite the limited number of algal species and ON compounds which were tested, the results of this experimental study are consistent with the idea that some components of the natural DON pool may not only be important as potential sources of phytoplankton N nutrition, but also that different algal species may be able to exploit these sources with varying capabilities. Thus, the kind of ON compounds available in any given aquatic environment may affect the species composition of the phytoplankton. Clearly, results obtained from experiments in small containers may not have direct relevance to the 'real world'. Nevertheless, we suggest that ideas and insights leading to reasonable hypotheses can be generated by this approach. An important challenge for future research is to develop methods to characterize and to quantify the actual fluxes of N from DON pools to phytoplankton and bacteria in lakes and oceans.

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References

American Public Health Association (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. APHA, Washington, DC.

Antia,N.J., Berland,B.R., Bonin,D.J. and Maestrini,S.Y. (1975) Comparative evaluation of certain organic and inorganic sources of nitrogen for phototrophic growth of marine microalage. *J. Mar. Biol. Assoc. UK*, **55Z**, 519–539.

Antia,N.J., Harrison,P.J. and Oliveira,L. (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia*, **30**, 1–89.

Berman,T. (1970) Alkaline phosphatases and phosphorus availability in Lake Kinneret. *Limnol. Oceanogr.*, **17**, 663–667.

Berman,T. (1997) Dissolved organic nitrogen utilization by an *Aphanizomenon* bloom in Lake Kinneret. *J. Plankton Res.*, **19**, 577–586.

Berman,T., Chava,S., Kaplan,B. and Wynne,D. (1991) Dissolved organic substrates as phosphorus and nitrogen sources for axenic batch cultures of freshwater green algae. *Phycologia*, **30**, 339–345.

Berman,T., Stone,L., Yacobi,Y.Z., Kaplan,B., Schlichter,M., Nishri,A. and Pollingher,U. (1995) Primary production and phytoplankton in Lake Kinneret. A long-term record. *Limnol. Oceanogr.*, **40**, 1064–1076.

Berman,T., Bechemin,C. and Maestrini,S.Y. (1999) Release of ammonium and urea from dissolved organic nitrogen in aquatic ecosystems. *Aquat. Microb. Ecol.*, **16**, 295–302.

Bronk,D.A., Glibert,P.M. and Ward,B.B. (1994) Nitrogen uptake, dissolved organic nitrogen release and new production. *Science*, **265**, 1843–1846.

Bushaw,K.L., Zepp,R.C., Tarr,M.A., Schulz-Jander,D., Boubonniere,R.A., Hodson,R.E., Miller,W.L., Bronk,D.A. and Moran,M.A. (1996) Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. *Nature*, **381**, 401–404.

Carlsson,P., Zuleika Segatto,A. and Graneli,E. (1993) Nitrogen bound to humic matter of terrestrial origin—a nitrogen pool for coastal phytoplankton? *Mar. Ecol. Prog. Ser.*, **97**, 105–116.

Carlsson,P., Graneli,E., Tester,P. and Boni,L. (1995) Influences of riverine humic substances on bacteria, protozoa, phytoplankton and copepods in a coastal plankton community. *Mar. Ecol. Prog. Ser.*, **127**, 213–221.

Carmichael,W.W. (1994) The toxins of cyanobacteria. *Sci. Am.*, **270**, 64–72.

Flynn,K.J. and Butler,I. (1986) Nitrogen sources for the growth of marine microalgae—role of dissolved free amino acids. *Mar. Ecol. Prog. Ser.*, **34**, 281–304.

Graneli,E., Edler,L., Gedziorowska,D. and Nyman,U. (1985) Influence of humic and fulvic acids on *Prorocentrum minimum* (Pav.) J. Schiller. In Anderson,D.M., White,A.W. and Baden D.G. (eds), *Proceedings of the Third International Conference on Toxic Dinoflagellates*, *St. Andrews*, *N.B. Canada. June 8–12*, *1985*. Elsevier, New York, 201 pp.

Holm-Hansen,O., Lorenzen,C.J., Holmes,R.W. and Strickland,J.D.H. (1965) Fluorometric determination of chlorophyll. *J. Cons. Cons. Int. Explor. Mer*, **30**, 3–15.

Ietswaart,T.P., Schneider,J. and Prins,R.A. (1994) Utilization of organic nitrogen sources by two phytoplankton species and a bacterial isolate in pure and mixed cultures. *Appl. Environ. Microbiol.*, **60**, 1554–1560.

Jackson,G.A. and Williams,P.M. (1985) Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. *Deep-Sea Res.*, **32**, 223–235.

John,E.H. and Flynn,K.J. (1999) Amino acid uptake by the toxic dinoflagellate *Alexandrium fundyense. Mar. Biol.*, **133**, 11–19.

Lindstrom,K. (1985) Selenium requirement of the dinoflagellate *Peridiniopsis borgei* (Lemma). *Int. Rev. Ges. Hydrobiol.*, **70**, 77–85.

Lisa,T., Piedras,P., Ca'rdenas,J. and Pineda,M. (1995) Utilization of adenine and guanine as nitrogen source by *Chlamydomonas reinhardtii* cells. *Plant Cell Environ.*, **118**, 583–588.

McCarthy,J.J. (1970) A urease method for urea in seawater. *Limnol. Oceanogr.*, **15**, 303–313.

McCarthy,J.J. (1972) The uptake of urea by natural populations of marine phytoplankton. *Limnol. Oceanogr.*, **17**, 738–748.

McCarthy,J.J., Wynne,D. and Berman,T. (1982) The uptake of dissolved nitrogenous nutrients by Lake Kinneret (Israel) microplankton. *Limnol. Oceanogr.*, **27**, 673–680.

Nichols,H.W. (1973) Growth media, freshwater. In Stein,J.R. (ed.), *Phycological Methods*. Cambridge University Press, Cambridge, pp. 8–23.

North,B.B. and Stephens,G.C. (1967) Uptake and assimilation of amino acids by *Platymonas. Biol. Bull. Mar. Biol. Lab. Woods Hole*, **133**, 391–400.

Nydahl,F. (1978) On the peroxodisulphate oxidation of total nitrogen in waters to nitrate. *Water Res*., **12**, 1123–1130.

Palenik,B. and Henson,S.E. (1997) The use of amides and other organic nitrogen sources by the phytoplankton *Emiliania huxleyi*. *Limnol. Oceanogr.*, **42**, 1544–1551.

Pollingher,U., Berman,T., Kaplan,B. and Scharf,D. (1988) Lake Kinneret phytoplankton: response to N and P enrichments in experiments and in nature. *Hydrobiologia*, **166**, 65–75.

Seitzinger,S.P. and Sanders.R.W. (1997) Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Mar. Ecol. Prog. Ser*., **159**, 1–12.

Solarzano,L. (1969) Determination of ammonia in natural waters by the phenol-hypochlorite method. *Limnol. Oceanogr*., **14**, 799–801.

Strickland,J.D.H., Holm-Hansen,O., Eppley,R.W. and Linn,R.J. (1969) The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. *Limnol. Oceanogr*., **14**, 23–34.

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