Simultaneous Short-term Uptake of Nitrate and Ammonium by *Oscillatoria agardhii* Grown in Nitrate- or Light-limited Continuous Culture

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The cyanobacterium *Oscillatoria agardhii* Gomont was grown in continuous culture under nitrate- or light-limited conditions. Short-term uptake rates of nitrate and ammonium were studied at various concentrations of nitrate and ammonium, both of which were present during the short-term assay. In nitrate-limited *O. agardhii*, the short-term ammonium uptake rate was not influenced by the presence of nitrate. Ammonium inhibited the rate of nitrate uptake in a non-competitive manner. An equation was derived for the short-term nitrate uptake rate which was a function of the ambient concentrations of both nitrate and ammonium. For a low ammonium concentration tested, the pattern of the nitrate–ammonium interaction in light-limited *O. agardhii* did not differ greatly from that found in nitrate-limited *O. agardhii*.

INTRODUCTION

Many phytoplankton species use ammonium in preference to nitrate when both are available simultaneously. For some species, nitrate uptake is completely inhibited in the presence of ammonium at concentrations in the range 0.5 to 1.0 μM (Morris & Syrett, 1963; Eppley *et al.*, 1969*b*; McCarthy & Eppley, 1972; McCarthy *et al.*, 1977). There are also reports of the simultaneous uptake of nitrate and ammonium by the marine diatom species *Monochrysis lutheri* (Caperon & Meyer, 1972) and *Dunaliella tertiolecta* (Bienfang, 1975) and by a marine red alga (Bird, 1976). There is even one report of a preferential use of nitrate over ammonium by a species of *Haematococcus* (Stross, 1963).

The rates of uptake of nitrate and ammonium and their interaction may be studied by two approaches. Firstly, the interaction may be examined over a long period of time, i.e. by studying the organism's response during growth in a medium containing both nitrogen sources. Secondly, the uptake rates can be studied by examining the short-term interaction, i.e. by supplying nitrate and ammonium simultaneously to organisms previously grown in the presence of only one nitrogen source and examining short-term uptake rates. This paper is concerned with the latter approach.

Short-term rates of uptake of nitrate or ammonium by *Oscillatoria agardhii* are a function of the respective external ion concentrations and follow Michaelis–Menten kinetics (Zevenboom & Mur, 1978*a, b*, 1981; Zevenboom, 1980; Zevenboom *et al.*, 1980). When nitrate and ammonium are available simultaneously, there may be a simultaneous, preferential or competitive use of one nutrient with respect to the other, and the uptake rates may not be the same as the rates for each ion alone.

In this paper, we report the results of short-term nitrate and ammonium uptake experiments with *O. agardhii* grown in continuous culture under nitrate- or light-limited conditions. In both cases, nitrate was the only nitrogen source present in the growth medium and, therefore, the organisms possessed the nitrate uptake system (Zevenboom, 1980; Zevenboom & Mur, 1978*a*).

Table 1. *Symbols, definitions and units*

Symbol	Definition	Unit
μ	specific growth rate	h^{-1}
E_0	incident irradiance	W m^{-2}
$\langle E \rangle$	average irradiance in culture vessel	W m^{-2}
$q_{\text{NO}_3^-}$	specific nitrate uptake rate during steady state growth	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
V	short-term (initial) nutrient uptake rate during linear decrease of S	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
$V_{\text{NO}_3^-}$	short-term (initial) specific nitrate uptake rate during linear decrease of $S_{\text{NO}_3^-}$	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
$V_{\text{NO}_3^-}^0$	$V_{\text{NO}_3^-}$ in the absence of ammonium	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
$V_{\text{NH}_4^+}$	short-term (initial) specific ammonium uptake rate during linear decrease of $S_{\text{NH}_4^+}$	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
V_{max}	potential maximum specific nutrient uptake rate; uptake capacity	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
$V_{\text{max}, \text{NO}_3^-}$	uptake capacity for nitrate	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
$V_{\text{max}, \text{NH}_4^+}$	uptake capacity for ammonium	$\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$
K_s^u	half-saturation constant for nutrient uptake; nutrient concentration, S , at which $V = \frac{1}{2}V_{\text{max}}$	μM
$K_{\text{NO}_3^-}^u$	half-saturation constant for nitrate uptake	μM
$K_{\text{NH}_4^+}^u$	half-saturation constant for ammonium uptake	μM
S	nutrient concentration used in the short-term experiments	μM
$S_{\text{NO}_3^-}$	nitrate concentration used in the short-term experiments	μM
$S_{\text{NH}_4^+}$	ammonium concentration used in the short-term experiments	μM

METHODS

Organism. *Oscillatoria agardhii* Gomont was isolated from the Veluwemeer, a Dutch shallow eutrophic lake, and supplied by the Rijksinstituut voor Drinkwatervoorziening, in The Hague. The cultures were free of other algae and cyanobacteria, but were not free of bacteria. In culture, the bacterial biomass was less than 0.5% (w/w) of the total biomass, an amount that was judged not to influence significantly the results of the experiments.

Culture conditions and measurements. *Oscillatoria agardhii* was grown in a chemostat system, described by Van Liere (1979), using a nitrate-limited growth medium (0.2 mM-nitrate in inflow) as described previously (Zevenboom & Mur, 1978a; Zevenboom, 1980). Light-limited organisms (5.9 mM-nitrate in inflow), kindly supplied by Dr L. van Liere, were grown in complete medium in the presence of nitrate as the sole nitrogen source. The nitrate- and light-limited cultures were aerated at flow rates of 100–120 l h⁻¹ and were grown under standard conditions of temperature (20 ± 1 °C) and pH (8.0 ± 0.1). [In previous studies it was shown that temperature and pH had a significant influence on the unrestricted growth rate of *O. agardhii* (Van Liere, 1979; Zevenboom, 1980). An optimum growth temperature of 32 °C and an optimum pH value of 9.0 were found. Since these conditions bear little resemblance to natural situations (Zevenboom, 1980) the standard conditions described above were chosen.] The cultures were continuously irradiated with light from circular white fluorescent lamps (Philips TLE 22 W/33). The incident irradiance (E_0) and average irradiance in the culture vessel ($\langle E \rangle$) were determined by the methods described by Van Liere *et al.* (1978) and Loogman & Van Liere (1978).

Nitrate-limited *O. agardhii* was studied at a fixed dilution rate of 0.007 h⁻¹ and the value of $\langle E \rangle$ was 7 W m⁻². [Later experiments showed that this irradiance value was non-saturating (Van Liere, 1979; Zevenboom *et al.*, 1980), but it should be noted that the growth rate was limited by the availability of nitrate, as emphasized in a previous paper (Zevenboom *et al.*, 1980).] Light-limited organisms were studied at a fixed dilution rate of 0.030 h⁻¹ and an E_0 of 13 W m⁻². Under these conditions, the growth rate was light-limited and $\langle E \rangle$ was approximately 6 W m⁻² (Van Liere, 1979; Van Liere & Mur, 1979).

A steady state was assumed to have been established when the absorbance, biomass concentration and pigment content of the culture remained constant for at least 1 week.

Short-term uptake of nitrate and ammonium. In the short-term uptake experiments, standard conditions were used with respect to saturating light intensity ($\langle E \rangle = 15 \text{ W m}^{-2}$), temperature (20 °C) and pH (8.0). [Temperature and pH have an influence on the uptake rates of both nitrate and ammonium (Zevenboom, 1980; Zevenboom & Mur, 1981); the V_{max} value decreases with decreasing incubation temperature or increasing pH, while the K_s^u value is not affected.] Nitrate and ammonium were simultaneously supplied to washed samples of *O. agardhii* from continuous cultures.

For this organism, the short-term rates of uptake of nitrate and ammonium are a function of the respective external concentrations, according to the Michaelis–Menten equation (Zevenboom & Mur, 1978a, 1981; Zevenboom *et al.*, 1980) (see Table 1 for definitions of symbols and units):

$$V = V_{\text{max}} [S / (K_s^u + S)] \quad (1)$$

The values of $V_{\max}$ and K_s^u were calculated using a linear transformation of equation 1, the Woolf plot (as used in a nutrient uptake study by Eppley *et al.*, 1969 *a*):

$$S/V = (1/V_{\max}) \cdot S + (K_s^u/V_{\max}) \quad (2)$$

with S/V as the ordinate and S as the abscissa. For calculation of the 95% confidence intervals of $V_{\max}$ and K_s^u , the Eadie–Hofstee plot was used:

$$V = -K_s^u \cdot V/S + V_{\max} \quad (3)$$

with V as the ordinate and V/S as the abscissa. This transformation is inferior to the Woolf transformation (Dowd & Riggs, 1965) but can be used to calculate the 95% confidence intervals in order to show significant differences in the values of the parameters.

RESULTS AND DISCUSSION

Influence of ammonium on nitrate uptake by nitrate-limited O. agardhii

Data concerning the short-term uptake of nitrate at various ammonium concentrations ($S_{\text{NH}_4^+}$) by nitrate-limited *O. agardhii* are given in Table 2. Simultaneous uptake of nitrate and ammonium occurred at ammonium concentrations lower than 90 μM . With 90 μM ammonium, the uptake of nitrate present at a concentration of about 50 μM was completely inhibited (Table 2).

These findings are in agreement with the uptake of nitrate and ammonium by a marine red alga (Bird, 1976) and by two marine diatoms, *Monochrysis lutheri* (Caperon & Meyer, 1972) and *Dunaliella tertiolecta* (Bienfang, 1975). They are in contrast to the short-term studies with some other marine phytoplankton species and *Chlorella vulgaris* (Morris & Syrett, 1963; Eppley *et al.*, 1969 *b*; McCarthy & Eppley, 1972; McCarthy *et al.*, 1977), in which nitrate uptake occurred only when the ammonium concentration was below 0.5 to 1.0 μM .

In the absence of ammonium or in the presence of a fixed ammonium concentration ($S_{\text{NH}_4^+}$ = about 10 μM and 20 μM) the short-term nitrate uptake rate ($V_{\text{NO}_3^-}$) was a function of the ambient nitrate concentration ($S_{\text{NO}_3^-}$) (Fig. 1). These data could be described by Michaelis–Menten kinetics (Table 3). In the presence of ammonium, $V_{\max, \text{NO}_3^-}$ was markedly decreased while $K_{\text{NO}_3^-}^u$ was not significantly affected (Table 3, note confidence intervals).

The $V - S$ curves in Fig. 1 were extrapolated to lower nitrate concentrations as shown by the dashed lines. The nitrate uptake rate during steady-state growth ($q_{\text{NO}_3^-}$) at the low steady-state nitrate concentration extant in the culture (0.3 μM) was calculated to be 0.042 $\mu\text{mol (mg dry wt)}^{-1} \text{ h}^{-1}$. This value is higher than the one which can be calculated, according to equation 1, using the values of $V_{\max, \text{NO}_3^-}$ and $K_{\text{NO}_3^-}^u$ in the absence of ammonium (Table 3) and the low steady-state nitrate concentration [$V_{\text{NO}_3^-} = 0.021 \mu\text{mol (mg dry wt)}^{-1} \text{ h}^{-1}$, at $S_{\text{NO}_3^-} = 0.3 \mu\text{M}$ and $S_{\text{NH}_4^+} = 0 \mu\text{M}$]. With nitrate-limited *O. agardhii* grown under light-saturating conditions, we previously found that for low nitrate concentrations ($S_{\text{NO}_3^-} < 20 \mu\text{M}$) the short-term uptake data could be described by another $V - S$ (Michaelis–Menten) curve which was independent of μ (Zevenboom & Mur, 1979). The $q_{\text{NO}_3^-}$ values coincided with this curve. These findings indicate that for nitrate-limited *O. agardhii* the $V - S$ curves for nitrate are biphasic. Thus it is not valid to calculate $V_{\text{NO}_3^-}$ for low $S_{\text{NO}_3^-}$ values using the values for $V_{\max, \text{NO}_3^-}$ and $K_{\text{NO}_3^-}^u$ listed in Table 3. These data should be obtained experimentally by examining the short-term rates of uptake of nitrate at very low nitrate concentrations both in the absence and the presence of ammonium.

It should be noted here that nitrate-limited *O. agardhii* grown under light-non-saturating conditions (see Methods) had a higher $V_{\max, \text{NO}_3^-}$ value at $S_{\text{NH}_4^+} = 0$ (Table 3) than was found with nitrate-limited *O. agardhii* grown under light-saturating conditions (Zevenboom & Mur, 1978 *a*, 1979). In previous papers the effects of light on nitrate-limited *O. agardhii*, grown in chemostat cultures, were reported and discussed (Van Liee *et al.*, 1979; Zevenboom, 1980; Zevenboom *et al.*, 1980). It was shown that under nitrate-limitation and light-non-saturating conditions the growth rate was limited throughout by only one factor, namely, nitrate

Table 2. Short-term nitrate and ammonium uptake data for nitrate-limited *O. agardhii* ($\mu = 0.007 \text{ h}^{-1}$, $\langle E \rangle = 7 \text{ W m}^{-2}$) at various concentrations of nitrate and ammonium

Expt	$S_{\text{NO}_3^-}$ (μM)	$S_{\text{NH}_4^+}$ (μM)	$V_{\text{NO}_3^-}$ [μmol (mg dry wt) $^{-1} \text{ h}^{-1}$]	$V_{\text{NO}_3^-}/V_{\text{NO}_3^-}^0$ * (%)	$V_{\text{NH}_4^+}$ [μmol (mg dry wt) $^{-1} \text{ h}^{-1}$]	$V_{\text{NH}_4^+}/V_{\text{NH}_4^+}^0$ † (%)
1	19	1	0.55	86	0.08	91
2	19	9	0.22	34	0.43	96
3	25	21	0.18	25	0.45	89
4	53	8	0.29	32	0.33	90
5	55	9	0.31	34	0.38	86
6	55	20	0.23	25	0.42	76
7	50	36	0.14	16	0.55	79
8	50	51	0.10	11	0.68	93
9	50	74	0.01	1	0.72	96
10	52	90	0.00	0	0.77	99
11	91	9	0.36	36	0.40	90
12	103	21	0.23	23	0.48	80

* Nitrate uptake rate as a percentage of the nitrate uptake rate at zero ammonium concentration ($V_{\text{NO}_3^-}^0$). Since $V_{\text{NO}_3^-}^0$ values (at $S_{\text{NH}_4^+} = 0 \mu\text{M}$) were experimentally determined at six different $S_{\text{NO}_3^-}$ values (Fig. 1) which were not identical to those reported in this table, $V_{\text{NO}_3^-}^0$ for the given $S_{\text{NO}_3^-}$ value had to be calculated using the equation: $V_{\text{NO}_3^-}^0 = 1.17[S_{\text{NO}_3^-}/(16 + S_{\text{NO}_3^-})]$ (see Table 3).

† Ammonium uptake rate as a percentage of the ammonium uptake rate at zero nitrate concentration ($V_{\text{NH}_4^+}^0$) (see also Fig. 4).

Fig. 1

Fig. 2

Fig. 1. Short-term uptake of nitrate by nitrate-limited *O. agardhii* ($\mu = 0.007 \text{ h}^{-1}$, $\langle E \rangle = 7 \text{ W m}^{-2}$) in the presence of ammonium at concentrations of about $10 \mu\text{M}$ (●; Table 2, expts 2, 4, 5, 11) and about $20 \mu\text{M}$ (▲; Table 2, expts 3, 6, 12), and in the absence of ammonium (○). The horizontal and vertical bars indicate $V_{\text{max}, \text{NO}_3^-}$ and $K_{\text{NO}_3^-}$ values, respectively, calculated according to the Woolf plot (see also Tables 2 and 3).

Fig. 2. (a) $V_{\text{NO}_3^-}/V_{\text{NO}_3^-}^0$ (expressed as a percentage of $V_{\text{NO}_3^-}^0$) as a function of $S_{\text{NH}_4^+}$ at nitrate concentrations of about $20 \mu\text{M}$ (●; Table 2, expts 1–3), about $50 \mu\text{M}$ (○; Table 2, expts 4–10) and about $100 \mu\text{M}$ (▲; Table 2, expts 11, 12).

(b) $V_{\text{NO}_3^-}/V_{\text{NO}_3^-}^0$ ratio as a function of $S_{\text{NH}_4^+}$. The line is fitted to equation 4. Symbols as in (a).

Table 3. Short-term nitrate uptake data for nitrate-limited *O. agardhii* ($\mu = 0.007 \text{ h}^{-1}$, $\langle E \rangle = 7 \text{ W m}^{-2}$) in the absence and presence of ammonium

Data are for ammonium concentrations of about 10 μM and about 20 μM (see Fig. 1 and Table 2, expts 2, 4, 5, 11 and expts 3, 6, 12). Values of $V_{\text{max}, \text{NO}_3^-}$ and $K_{\text{NO}_3^-}^u$ were calculated using the Woolf plot (equation 2) and the data points (n) in Fig. 1. The values and 95% confidence intervals calculated using the Eadie–Hofstee plot (equation 3) are given in parentheses.

$S_{\text{NH}_4^+}$ (μM)	$V_{\text{max}, \text{NO}_3^-}$ [μmol (mg dry wt) $^{-1} \text{ h}^{-1}$]	$K_{\text{NO}_3^-}^u$ (μM)	n	r	$V_{\text{max}, \text{NO}_3^-}/V_{\text{max}, \text{NO}_3^-}^0$ (%)
0	1.17 (1.15 $\pm$ 0.08)	16 (15 $\pm$ 2)	6	0.994	100
10	0.44 (0.40 $\pm$ 0.06)	21 (16 $\pm$ 6)	4	0.992	38
20	0.25 (0.26 $\pm$ 0.07)	12 (11 $\pm$ 8)	3	0.997	21

(Zevenboom, 1980; Zevenboom *et al.*, 1980). Thus, light-non-saturating conditions did not affect the nitrate-limited growth rate. However, effects were found on pigment content (Van Liere *et al.*, 1979), cell nitrogen content and $V_{\text{max}, \text{NO}_3^-}$ value (Zevenboom, 1980; Zevenboom *et al.*, 1980). The values of these parameters were found to be higher in nitrate-limited *O. agardhii* grown at a fixed dilution rate (i.e. growth rate) and under light-non-saturating conditions compared with the values found in nitrate-limited cells grown at the same μ value and under light-saturating conditions. Of the increase in cell nitrogen, 30 to 50% was associated with the increase in the nitrogen-containing pigments. The increased pigment content was apparently necessary to maintain the rate of photosynthesis constant when less light was available (i.e. under light-non-saturating conditions). It is reasonable to suppose that part of the remaining additional cell nitrogen was used for the 'nitrate-uptake machinery' as reflected by the increased $V_{\text{max}, \text{NO}_3^-}$ value found under nitrate-limiting, light-non-saturating conditions.

Figure 2(a) shows the influence of $S_{\text{NH}_4^+}$ on the value of $V_{\text{NO}_3^-}/V_{\text{NO}_3^-}^0$, i.e. the nitrate uptake rate in the presence of ammonium ($V_{\text{NO}_3^-}$) as a percentage of the nitrate uptake rate in the absence of ammonium ($V_{\text{NO}_3^-}^0$) at a given nitrate concentration. This value decreased with increasing ammonium concentration and was found to be zero at $S_{\text{NH}_4^+} = 90 \mu\text{M}$ (Fig. 2a, Table 2). The data shown in Fig. 2(a) appeared to be well described by one hyperbolic curve, which would suggest that the relationship between the ratio $V_{\text{NO}_3^-}/V_{\text{NO}_3^-}^0$ and $S_{\text{NH}_4^+}$ was independent of the nitrate concentration. To examine the effect of $S_{\text{NH}_4^+}$ more closely it was useful to plot the ratio $V_{\text{NO}_3^-}^0/V_{\text{NO}_3^-}$ against $S_{\text{NH}_4^+}$ (Fig. 2b). From linear regression analysis of the data ($n = 10$; r , correlation coefficient = 0.988), the ratio was found to be linearly related to $S_{\text{NH}_4^+}$ and could be described by the equation:

$$V_{\text{NO}_3^-}^0/V_{\text{NO}_3^-} = 1.30 + 0.148 S_{\text{NH}_4^+} \quad (4)$$

The line shown in Fig. 2(b) deviates slightly from that which was expected: in the absence of ammonium ($S_{\text{NH}_4^+} = 0$), the value of the ordinate should be 1.0.

The dependence of $V_{\text{NO}_3^-}^0$ on $S_{\text{NO}_3^-}$ is given by the equation:

$$V_{\text{NO}_3^-}^0 = V_{\text{max}, \text{NO}_3^-}^0 [S_{\text{NO}_3^-}/(K_{\text{NO}_3^-}^u + S_{\text{NO}_3^-})] \quad (5)$$

Fig. 3. Dependence of short-term nitrate uptake rate ($V_{\text{NO}_3^-}$) on the concentrations of nitrate and ammonium simultaneously present in the uptake experiments: calculated according to equation 8. The arrow indicates the $K_{\text{NO}_3^-}^u$ value.

Combining equations 4 and 5 and rearranging, the dependence of $V_{\text{NO}_3^-}$ on $S_{\text{NO}_3^-}$ and $S_{\text{NH}_4^+}$ is given by:

$$V_{\text{NO}_3^-} = V_{\text{max}, \text{NO}_3^-}^0 [S_{\text{NO}_3^-} / (K_{\text{NO}_3^-}^u + S_{\text{NO}_3^-}) (1.30 + 0.148 S_{\text{NH}_4^+})] \quad (6)$$

Equation 6 resembles the non-competitive type of enzyme inhibition, which can be expressed by the general equation:

$$V = V_{\text{max}} [S / (K_s^u + S) (1 + i / K_i)] \quad (7)$$

in which i is the concentration of the inhibitor ($S_{\text{NH}_4^+}$) and K_i is an inhibition constant. The value of K_i , represented by the reciprocal of the slope of the line in Fig. 2(b), was calculated to be 6.8 μM . From equation 7 it is clear that the inhibitor affects V_{max} , while K_s^u is unaffected. The experimental data fit this pattern (Table 3).

Under the conditions used, $V_{\text{max}, \text{NO}_3^-}^0$ is 1.17 $\mu\text{mol (mg dry wt)}^{-1} \text{h}^{-1}$ and $K_{\text{NO}_3^-}^u$ is 16 μM (Table 3). Substitution of these data into equation 6 gives, after rearranging:

$$V_{\text{NO}_3^-} = 7.9 S_{\text{NO}_3^-} / (16 + S_{\text{NO}_3^-}) (8.8 + S_{\text{NH}_4^+}) \quad (8)$$

The measured $V_{\text{NO}_3^-}$ data, listed in Table 2, fitted well with equation 8, except at the lowest and highest $S_{\text{NH}_4^+}$ values (Table 2, expts 1, 9 and 10) where errors larger than 10% were found. A three-dimensional graph of equation 8 is given in Fig. 3, in which $V_{\text{NO}_3^-}$ is described as a function of $S_{\text{NO}_3^-}$ and $S_{\text{NH}_4^+}$.

For several reasons, it is difficult to make a clear comparison between our results and those reported in the literature. Firstly, the preconditioning of the organisms frequently is not comparable (continuous culture versus batch culture). Secondly, a comparison between 'short-term' and 'long-term' studies is difficult to make, since they deal with two different aspects. The answers which are obtained from the two different studies are not necessarily the same.

For several marine phytoplankton species it has been suggested that the inhibition of nitrate uptake by ammonium takes place at the membrane, i.e. the primary transport step (Eppley & Rogers, 1970; Falkowsky, 1975a, b). In these species a membrane-associated enzyme, (nitrate-chloride)-activated ATPase, seems primarily to be involved in the active transport of nitrate across the plasmalemma (Falkowsky, 1975a, b). In *O. agardhii*, the mechanism(s) of the non-competitive type of inhibition of nitrate uptake by ammonium

Table 4. Short-term nitrate uptake data for light-limited *O. agardhii* ($\mu = 0.030 \text{ h}^{-1}$, $E_0 = 13 \text{ W m}^{-2}$) in the absence and presence of ammoniumValues of $V_{\max, \text{NO}_3^-}$ and $K_{\text{NO}_3}^u$ were calculated as indicated in the legend to Table 3.

$S_{\text{NH}_4^+}$ (μM)	$V_{\max, \text{NO}_3^-}$ [μmol (mg dry wt) $^{-1} \text{ h}^{-1}$]	$K_{\text{NO}_3}^u$ (μM)	n	r	$V_{\max, \text{NO}_3^-}/V_{\max, \text{NO}_3^-}^0$ (%)
0	0.37 (0.37 ± 0.04)	10 (11 ± 4)	4	0.999	100
7	0.16 (0.16 ± 0.02)	16 (16 ± 3)	4	0.999	43

remain(s) obscure, because of a lack of information regarding the enzyme kinetics and cellular pools of assimilation products.

Influence of ammonium on nitrate uptake by light-limited O. agardhii

The short-term kinetics of nitrate uptake by light-limited *O. agardhii* are shown in Table 4. Only one ammonium concentration was tested. As was found with nitrate-limited *O. agardhii*, there was no delay in nitrate uptake, but instead an immediate, simultaneous uptake of both nitrate and ammonium.

It should be noted here that light-limited cells had a much lower $V_{\max, \text{NO}_3^-}$ value (at $S_{\text{NH}_4^+} = 0$) (Table 4) compared with the values found for nitrate-limited cells (at $S_{\text{NH}_4^+} = 0$) grown under light-non-saturating conditions (Table 3) or light-saturating conditions [$V_{\max, \text{NO}_3^-} = 0.8 \mu\text{mol (mg dry wt)}^{-1} \text{ h}^{-1}$; Zevenboom *et al.*, 1980]. The low $V_{\max, \text{NO}_3^-}$ value of light-limited cells is not surprising, since these organisms were grown in the presence of excess nitrate (see Methods) and, therefore, the $V_{\max, \text{NO}_3^-}$ value equals the nitrate uptake rate measured during steady-state growth ($q_{\text{NO}_3^-}$) (Zevenboom & Mur, 1978*b*; Zevenboom, 1980). This is not the case for nitrate-limited organisms, which have a much higher, growth rate-independent, $V_{\max, \text{NO}_3^-}$ value compared with the value of $q_{\text{NO}_3^-}$ (Zevenboom & Mur, 1979; Zevenboom *et al.*, 1980).

In the presence of ammonium ($S_{\text{NH}_4^+} = 7 \mu\text{M}$), the $V_{\max, \text{NO}_3^-}$ value of light-limited *O. agardhii* was markedly decreased ($V_{\max, \text{NO}_3^-}$ was 43 % of $V_{\max, \text{NO}_3^-}^0$; Table 4), while $K_{\text{NO}_3}^u$ was not significantly affected (Table 4, note confidence intervals). Using equation 4 and $S_{\text{NH}_4^+} = 7 \mu\text{M}$, the $V_{\max, \text{NO}_3^-}$ of nitrate-limited cells was calculated to be 50 % of $V_{\max, \text{NO}_3^-}^0$. The data suggest that the short-term nitrate–ammonium interaction shown by light-limited cells and nitrate-limited cells are rather similar. However, this must await a more thorough investigation of the effects of higher ammonium concentrations on the short-term nitrate uptake rates of light-limited organisms.

In this study, organisms were grown at two different growth rates (nitrate-limited cells, $\mu = 0.007 \text{ h}^{-1}$; light-limited cells, $\mu = 0.030 \text{ h}^{-1}$). In previous studies with *O. agardhii* it was concluded that growth rate has an influence on the nitrogen (nitrate or ammonium) uptake kinetics of this species. Under light-limitation, μ influenced the uptake kinetics for nitrate and ammonium in a similar way ($V_{\max}$ increased with increasing μ , while K_s^u remained constant) (Zevenboom, 1980). Under nitrate-limitation, the $V_{\max}$ values for nitrate and ammonium were identical, high and independent of μ , while the K_s^u values for nitrate and ammonium were differently affected by μ (the former increased with increasing μ , while the latter was hardly influenced by μ) (Zevenboom & Mur, 1979, 1981; Zevenboom *et al.*, 1980). From the above, it seems important to examine the nitrate–ammonium interaction by nitrate-limited cells more closely by growing the species at other, higher, growth rates than were applied in this study.

Bates (1976) studied the nitrate–ammonium interaction by *Skeletonema costatum* grown under different light conditions. From his work it can be calculated that $V_{\max, \text{NO}_3^-}$ (as a percentage of $V_{\max, \text{NO}_3^-}^0$) was 90 % in 'sun-adapted' cells and 38 % in 'shade-adapted' cells.

Fig. 4. Short-term uptake of ammonium by nitrate-limited *O. agardhii* ($\mu = 0.007 \text{ h}^{-1}$, $\langle E \rangle = 7 \text{ W m}^{-2}$) in the presence of nitrate at concentrations of about $20 \text{ } \mu\text{M}$ (●), about $50 \text{ } \mu\text{M}$ (○) and about $100 \text{ } \mu\text{M}$ (▲) (see Table 2), and in the absence of nitrate (---; Zevenboom, 1980). The horizontal and vertical bars indicate $V_{\max, \text{NH}_4}$ and $K_{\text{NH}_4}^u$ values, respectively, calculated according to the Woolf plot. Curves shown were fitted to the following equations.

Curve —: $V_{\text{NH}_4} = 0.86 [S_{\text{NH}_4} / (14 + S_{\text{NH}_4})]$; $n = 12$, $r = 0.988$

Curve ----: $V_{\text{NH}_4} = 0.85 [S_{\text{NH}_4} / (8 + S_{\text{NH}_4})]$; $n = 8$, $r = 0.998$

Values and 95 % confidence intervals (calculated according to the Eadie-Hofstee plot): for curve —, $V_{\max, \text{NH}_4} = 0.72 \pm 0.17$, $K_{\text{NH}_4}^u = 8 \pm 3$; for curve ----, $V_{\max, \text{NH}_4} = 0.85 \pm 0.10$, $K_{\text{NH}_4}^u = 8 \pm 4$.

From these results it can be concluded that the pre-conditioning with respect to light had a marked influence on the nitrate–ammonium interaction in *S. costatum*. In this paper results are presented of a study of nitrate-limited *O. agardhii* grown under light-non-saturating conditions. As was mentioned previously, light affected the $V_{\max, \text{NO}_3^-}$ of nitrate-limited cells. Experiments with nitrate-limited cells grown under light-saturating conditions are required in order to examine more thoroughly the effect of light on the nitrate–ammonium interaction in nitrate-limited *O. agardhii*.

Influence of nitrate on ammonium uptake by O. agardhii

Short-term rates of uptake of ammonium, at various nitrate concentrations, by nitrate-limited *O. agardhii* are shown in Table 2 and Fig. 4. All data could be described by one $V-S$ curve. Also shown is the $V-S$ curve for ammonium uptake by nitrate-limited cells in the absence of nitrate (Fig. 4; dashed line). The V_{NH_4} data, expressed as a percentage of $V_{\text{NH}_4}^0$, ranged from 76 to 99 % (Table 2, see also Fig. 4). If there was no influence of nitrate on the ammonium uptake rate, a value of 100 % should have been found. However, from the 95 % confidence intervals of $V_{\max, \text{NH}_4}$ and $K_{\text{NH}_4}^u$ (listed in the legend of Fig. 4), it can be concluded that the short-term ammonium uptake curve obtained in the presence of nitrate was not significantly different from that obtained in the absence of nitrate. The short-term ammonium uptake rate of light-limited cells determined at $S_{\text{NH}_4} = 7 \text{ } \mu\text{M}$ and at different nitrate concentrations ($S_{\text{NO}_3^-} = 7, 30, 58$ and $84 \text{ } \mu\text{M}$) was $0.20 \pm 0.01 \text{ } \mu\text{mol (mg dry wt)}^{-1} \text{ h}^{-1}$. In the absence of nitrate and at $S_{\text{NH}_4} = 7 \text{ } \mu\text{M}$, V_{NH_4} was $0.19 \text{ } \mu\text{mol (mg dry wt)}^{-1} \text{ h}^{-1}$ (Zevenboom, 1980). These data, as well as those shown in Fig. 4, suggest that ammonium uptake by *O. agardhii* is not influenced by nitrate, as tested in the short-term assay. This pattern seems to be very common and is to be found in many other reports on phytoplankton species and bacteria (Brown *et al.*, 1974).

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