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Effect of nitrogen and phosphorus supply on growth, chlorophyll content and tissue composition of the macroalga *Chaetomorpha linum* (O.F. Müll.) Kütz in a Mediterranean coastal lagoon*

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SUMMARY: The effect of dissolved nutrients on growth, nutrient content and uptake rates of *Chaetomorpha linum* in a Mediterranean coastal lagoon (Tancada, Ebro delta, NE Spain) was studied in laboratory experiments. Water was enriched with distinct forms of nitrogen, such as nitrate or ammonium and phosphorus. Enrichment with N, P or with both nutrients resulted in a significant increase in the tissue content of these nutrients. N-enrichment was followed by an increase in chlorophyll content after 4 days of treatment, although the difference was only significant when nitrate was added without P. P-enrichment had no significant effect on chlorophyll content. In all the treatments an increase in biomass was obseved after 10 days. This increase was higher in the N+P treatments. In all the treatments the uptake rate was significantly higher when nutrients were added than in control jars. The uptake rate of N, as ammonium, and P were significantly higher when they were added alone while that of N as nitrate was higher in the N+P treatment. In the P-enriched cultures, the final P-content of macroalgal tissues was ten-fold that of the initial tissue concentrations, thereby indicating luxury P-uptake. Moreover, at the end of the incubation the N:P ratio increased to 80, showing that P rather than N was the limiting factor for *C. linum* in the Tancada lagoon. The relatively high availability of N is related to the N inputs from rice fields that surround the lagoon and to P binding in sediments.

Key words: macroalgae, coastal lagoons, growth, nitrogen, phosphorus.

RESUMEN: EFECTO DE LA ADICIÓN DE NITRÓGENO Y FÓSFORO EN EL CRECIMIENTO, CONCENTRACIÓN DE CLOROFILA Y NUTRIENTES EN LA MACROALGA *CHAETOMORPHA LINUM* (O.F. MÜLL) KÜTZ EN UNA LAGUNA COSTERA MEDITERRÁNEA. – A partir de experimentos en laboratorio se ha estudiado la influencia de la disponibilidad de nutrientes disueltos en el crecimiento, contenido en nutrientes y sus tasas de absorción en la especie de macroalga *Chaetomorpha linum* procedente de una laguna costera típicamente mediterránea (La Tancada, Delta del Ebro, NE España). Se fertilizó con diferentes formas de nitrógeno, nitrato o amonio y fósforo. Se ha observado un incremento significativo en el contenido de N y P en los tejidos vegetales tanto en las fertilizaciones con P o N como con N+P. La fertilización con N dio como resultado un aumento de la concentración de clorofila a los 4 días de iniciado el experimento aunque sólo se han observado diferencias significativas en el tratamiento con nitrato. La fertilización con P no produjo efecto significativo sobre la concentración en clorofila. En todos los tratamientos se ha observado un aumento de biomasa al cabo de 10 días y este incremento fue mayor cuando se fertilizaba con N+P. La tasa de absorción ha sido siempre más elevada en los microcosmos fertilizados que en los controles sin fertilizar. Las tasas de absorción de amonio y P fueron significativamente más elevadas en los tratamientos simples, con sólo amonio o P, sin embargo en el caso del nitrato la tasa de absorción fue mayor cuando se fertilizaba con N+P. Se ha observado un aumento en 10 veces el contenido inicial en los cultivos enriquecidos con P lo cual sería indicador de un consumo extra de fósforo, lo que unido al valor bajo inicial de N:P de 80 sería indicador de limitación del crecimiento por P en la laguna de la Tancada. La relativa disponibilidad alta de N con respecto al P podría estar relacionada con la entrada de N disuelto de los arrozales y la inmovilización de P en el sedimento.

Palabras clave: macroalgas, lagunas costeras, crecimiento, nitrógeno, fósforo.

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INTRODUCTION

Increased nutrient inputs from human activities have a relevant impact on coastal waters worldwide (Valiela *et al*., 1997). In temperate regions, the most throughly documented cases of heavily affected environments are the estuaries and coastal lagoons of the northeast USA (Valiela *et al*., 1992), Europe (Sfriso *et al.*, 1992, Viaroli *et al*., 1996), and Western Australia (Hodgkin and Birch, 1982; McComb and Humphries, 1992). Greater abundance of macroalgae is one of the direct effects of increased nutrient load (Valiela *et al*., 1992; Rivers and Peckol, 1995; Menéndez and Comin, 2000). Opportunistic macroalgae can uptake, assimilate and store a large amount of N, resulting in low concentrations of this nutrient in the water column, even in areas of high loading (Valiela *et al*., 1992; Peckol *et al.,* 1994).

In certain lagoons, the enrichment depends on high external inputs, mainly of dissolved inorganic N (DIN, Nixon *et al*., 1986). N is frequently accompanied by inputs of P. In Buttermilk Bay (Valiela *et al*., 1990) and Sacca di Goro (Viaroli *et al*., 1995) most DIN enters as nitrate. In others, such as Moriches Bay (Ryther, 1989), it enters mainly as ammonium or as an organic form. Decreased water quality, massive growth of macroalgae and severe distrophic crises are common in these N-enriched coastal environments (Amanieu *et al.,* 1975; Izzo and Hull, 1991, Sfriso *et al*., 1992; Viaroli *et al*.,1996).

N has been described as the limiting nutrient for macroalgae in shallow coastal waters (Fong *et al*., 1994; Pedersen and Borum, 1996). However, macroalgae develop huge biomasses in shallow coastal waters which have distinct nutrient conditions. *Chaetomorpha linum*, a thin structured bloom-forming macroalga, is widely distributed in shallow eutrophic estuaries and coastal lagoons (Lavery and McComb, 1991; Lavery *et al.*, 1991; McGlathery *et al*., 1997; McComb *et al*., 1998). This macroalga is seldomly nutrient-limited, except after prolonged periods of low nutrient loadings (Lavery *et al*., 1991). In Tancada lagoon, Ebro Delta (NE Spain), enrichment is mainly due to DIN, indicating that P rather than N could be limiting for *C. linum* (Comin *et al*., 1990). At the beginning of spring, most of the DIN enters the lagoon as nitrate from fertilised ricefields and in autumn-winter as ammonium because of the mineralization of organic matter occurring during spring and summer and inputs from the adjacent Alfacs Bay (Comín *et al*., 1991; Menéndez and Comín, 2000; Vidal and Morguí, 2000; Menéndez unpublished data). In late spring and in summer the DIN concentration in the water column remains between 0-10 µmol DIN l^{-1} because of uptake mainly by floating macroalgae (*C. linum, Cladophora* sp.*, Gracilaria verrucosa, Ulva* sp.). The concentration of P in the lagoon is low mainly because of the strong capacity of the sediment to uptake and retain soluble reactive phosphorus (SRP) (Vidal, 1994).

Here we studied the effects of distinct forms of nutrients on the growth rates and nutrient and chlorophyll content of *C. linum* in Tancada lagoon. This macroalga was chosen because it is the dominant seaweed in the lagoon. Moreover, we also aimed to evaluate how the fluctuations and the potential input of N and P affect proliferations of this macroalga in Tancada lagoon.

MATERIALS AND METHODS

Site description

Chaetomorpha linum was collected from Tancada lagoon in June 1992. Tancada is a small (1.8 km^2), shallow (average depth of 37 cm) coastal lagoon located in the Ebro River delta, NE Spain (40°40'N, 0°36'E). The lagoon is formed by two almost equivalent sized basins. The West basin receives high freshwater inflows from irrigated ricefields in spring and summer, which leads to a relatively high concentration of nitrate (about 117 µmol l^{-1}) in April (Comin *et al*., 1995). In contrast, the East basin, because of its proximity to the sea, is less affected by freshwater inputs and more by seawater, mainly during easterly storms. In late spring and summer, DIN concentrations remained between 0 and 5 µmol l^{-1} but in autumn and winter the concentration of ammonium increased, mainly in the East basin (about 40 µmol l^{-1}). SRP ranged between 0.05 and 2.2 µmol l^{-1}, with minimum values in spring and summer and maximum values in autumn and winter. Water conductivity ranged from 17 to 45 mS cm^{-1} in the West basin and from 20 to 63 mS cm^{-1} in the East basin. Dissolved inorganic carbon concentration in the water column ranged between 3.0 and 3.9 mmol l^{-1}, and the pH remained fairly constant at about 8.2.

Experimental design

Approximately 10 ± 0.15 g (wet weight) of *C. linum* was placed in 2 l glass jars filled with filtered

(Whatman GF/F) water from the lagoon and left for three days prior to the start of the experiment. Background levels of nutrients in the incubation water were <2 μmol l^{-1} inorganic P, <20 μmol l^{-1} NH_4^+ and <6 μmol l^{-1} NO_3^-. Water was circulated in the recipient by bubbling with compressed air. The experiment was carried out in a temperature-controlled room at 20-22°C under a 15:9 h LD cycle. Light was provided by fluorescent (400W) lamps at 400 μmol photons m^{-2} s^{-1}, which saturates *C. linum* photosynthesis (Menéndez and Comin, 2000).

The experiment composed of six treatments with four replicates each: four jars received additions of PO_4^{3-} alone, four received additions of NO_3^- alone, four received NH_4^+ alone; four received NO_3^-+PO_4^{3-}; four received NH_4^++PO_4^{3-}, and four were maintained at the initial nutrient concentrations (Ci low). Additional recipients, in duplicate, that contained nutrients but not algae were used as controls. Nutrients were added from stock solutions of $NaNO_3$, NH_4Cl and KH_2PO_4. The final concentrations in each treatment were 10 times higher than those measured in the lagoon water during summer (NH_4^+: NO_3^-: PO_4^{3-} 6:4:1): 18 μmol l^{-1} PO_4^{3-} , 120 μmol l^{-1} NH_4^+ and 68 μmol l^{-1} NO_3^-. Water samples were collected at 0, 2, 17, 26, 41, 50, 65 and 74 hours of the experiment. Nitrate, nitrite and ammonium nitrogen concentrations and SRP were measured in filtered water samples (using Whatman GF/C filters pre-combusted at 500°C) in a Technicon autoanalyser following Grashoff *et al.* (1983). The net uptake rate of each nutrient was calculated as the difference between the initial and final amount of nutrients in the medium, related to time of incubation, water volume and plant biomass (Harlin and Wheeler, 1985):

$$\mu\text{mol N g}^{-1}\text{ DWh}^{-1} = \frac{(\text{Ci} - \text{Cf}) * \text{volume (l)}}{\text{length of incubation (h)}} \times \frac{1}{\text{g DW plant}}$$

where Ci is the initial concentration of the nutrient (μmol l^{-1}) and Cf is the final concentration (μmol l^{-1}), at each time interval (0-4 days and 4-10 days). Concentrations of each treatment (enriched and not enriched, Ci low) were corrected for those determined in the control jars (without macroalga).

Macroalgae were extracted twice during the experiment, on days 4 and 10. After 4 days, two jars from each treatment were discarded and at the end of the experiment the two jars remaining for each treatment were analysed. Dry weight at 60°C, tissue carbon, N and P were analysed. Total C and N were determined in the finely ground biomass samples using a Carlo-Erba CHN elemental analyser. After acid digestion, total P content was measured with a spectrophotometric technique (Jackson, 1970). Total chlorophyll concentration was measured spectrophotometrically on 90% acetone extracts. Extractions were carried out following the method described by Sestak (1971). Calculations were based on MacKinney (1941) equations. The net growth rate (μ) of *C. linum* was calculated from changes in the dry biomass for each experimental period:

$$\mu = (\ln B_t - \ln B_0)\, t^{-1}$$

Statistical analysis

The effects of enrichment on the nutrient and chlorophyll content and on the growth rate of *C. linum* were analysed after 4 and 10 days of fertilisation using one-way ANOVA with a level of significance of 5% (Legendre and Legendre, 1998). One-way ANOVA was used to compare the effects of the addition of P, nitrate and ammonium on uptake rates in the fertilised and non-fertilised treatments, and two-way ANOVA to test differences among N uptake rates as nitrate or ammonium alone or in combination with P. Tukey's test was used to make multiple comparisons between treatment means from significant ANOVA tests. Homogeneity of variance was determined using the F_{max} test. The CSS-Statistica computer program was used for statistical analysis.

RESULTS

Tissue concentrations of N, P and C

N-enrichment resulted in a significant increase ($F=57.17$, $p<10^{-6}$) in the concentration of N in tissues, while P-enrichment had no effect ($p>0.05$) on this (Fig. 1). P-enrichment resulted in a significant ($F=90.22$, $p<10^{-6}$) increase in the tissue concentration of P. This increase was higher when macroalga was fertilised with P alone than when fertilised in combination with N (both nitrate or ammonium, Tukey's, $p< 0.0005$). N-enrichment had no effect ($p>0.05$) on the concentration of P in tissues.

The atomic N:P ratio in tissue in the P-treatment at the end of the experiment was very low compared with the initial N:P ratio (6 vs. 80), indicating a deficiency of N relative to P. N-enrichment diminished this ratio to 21 in N+P treatments, and enhanced it to

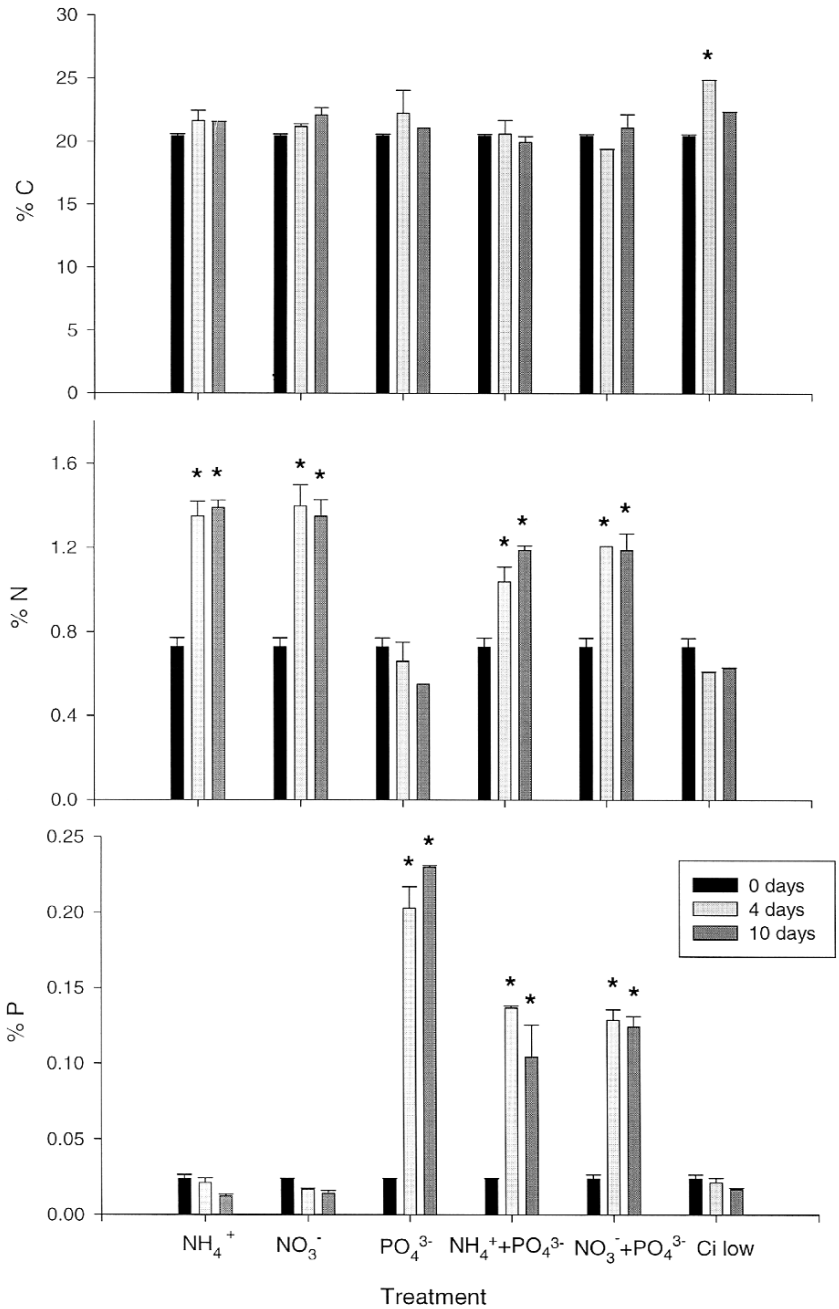

FIG. 1. – Carbon, nitrogen and phosphorus contents as percentage of dry weight in *C. linum* at 0, 4 and 10 days of incubation. Vertical bars are standard errors. * : significant differences versus the control at $p<0.05$.

almost 200 in the jars fertilised with nitrate and ammonium alone (Table 1).

Significant differences were observed in the C content of *C. linum* only after 4 days of fertilisation when nitrate plus P were added in relation to unenriched treatment (Ci low) ($F=4.0$, $p<0.05$) (Fig. 1).

Chlorophyll content

N-enrichment was followed by an increase in the chlorophyll content 4 days after treatment (initial

Table 1.- Atomic N:P ratios in *C. linum* tissues at the beginning and end of the incubation period. Mean ± standard errors of four replicates are reported.

	N:P ratio
Initial	80 ± 0.2
NO_3^-	194 ± 11.4
NH_4^+	192 ± 12.5
PO_4^{3-}	6 ± 0.9
$NH_4^+ + PO_4^{3-}$	21 ± 4.2
$NO_3^- + PO_4^{3-}$	21 ± 0.2
Ci low	82 ± 0.1

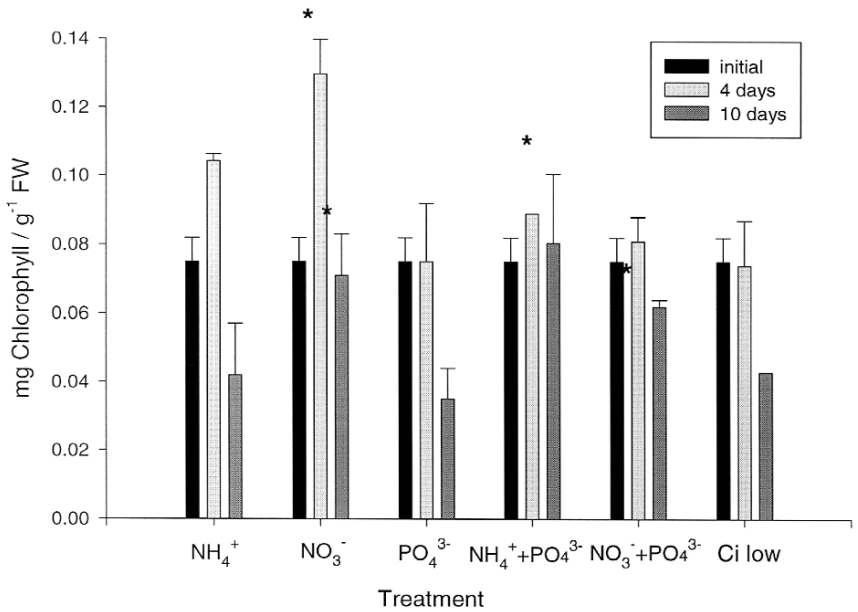

FIG. 2. – Variation in the concentration of chlorophyll in *C. linum* during the laboratory experiments. Vertical bars are standard errors. *: significant differences versus the control at p<0.05.

Table 2.- *C. linum* growth rates (μ) in each treatment, during the two extractions, between 0 and 4 days, between 4 and 10 days and throughout the experimental period, between 0 and 10 days. Values are means ± standard errors.

μ (gDW day^{-1})	0-4 days	4-10 days	0-10 days
NO_3^-	0.0524 ± 0.008	0.1121 ± 0.0022	0.0673 ± 0.001
NH_4^+	0.0192 ± 0.008	0.0945 ± 0.0152	0.0567 ± 0.009
PO_4^{3-}	-0.0098 ± 0.0004	0.0565 ± 0.0272	0.0339 ± 0.016
NH_4^++PO_4^{3-}	0.1005 ± 0.0235	0.1054 ± 0.0261	0.0633 ± 0.015
NO_3^-+PO_4^{3-}	0.0368 ± 0.0287	0.1402 ± 0.0043	0.0841 ± 0.002
Ci low	-	0.0842 ± 0.007	0.0505 ± 0.004

concentration of chlorophyll 0.075 mg g^{-1} fresh weight (FW)), being highest when nitrate was added without P (F=4.54, p<0.05; Tukey's, p< 0.05). After 10 days, although the macroalga chlorophyll content decreased in all the treatments compared with the concentrations measured after 4 days (Fig. 2), significant differences were observed when cultures were enriched with N as nitrate, and with N plus P (F=4.35, p<0.05; Tukey's, p<0.05) in comparison with the unenriched treatment (Ci low). P-enrichment had no significant (p>0.05) effect on the chlorophyll content 4 or 10 days after fertilisation.

Growth rates

In general, all the treatments showed an increase in biomass 10 days after treatment, being highest (F=11.09, p<0.005) in the N+P treatments.

The growth rate was significantly higher between days 4 and 10 than between days 0 and 10 (F= 11.34, p<0.001) (Table 2) except in the ammonium plus P treatment.

N-enrichment only increased the growth rate of *C. linum* significantly (F= 3.77, p<0.05) after 4 days in the nitrate plus P treatment, and at the end of the experiment in the N+P treatment (Table 2).

Nutrient uptake rates

In all the treatments the uptake rate was significantly higher (ANOVA, p<10^{-6}) when nutrient was added (high level of nutrient) than in Ci low jars (not

Table 3.- Net nutrient uptake rates by *C. linum,* after the first 74 hours of incubation in control jars (Ci low), in those enriched with NO_3^- or NH_4^+ or PO_4^{3-} (Ci high), and in those enriched with NO_3^- + PO_4^{3-} and NH_4^+ + PO_4^{3-}. Values are means ± standard errors.

μmol g^{-1} DW h^{-1}	Ci low	Ci high	NO_3^-+PO_4^{3-}	NH_4^++PO_4^{3-}
NO_3^-	0.0642 ± 0.006	0.9525 ± 0.015	1.2941 ± 0.036	-
NH_4^+	0.3946 ± 0.005	2.0625 ± 0.016	-	1.8190 ± 0.055
PO_4^{3-}	0.0254 ± 0.002	0.4003 ± 0.006	0.3595 ± 0.015	0.2544 ± 0.007

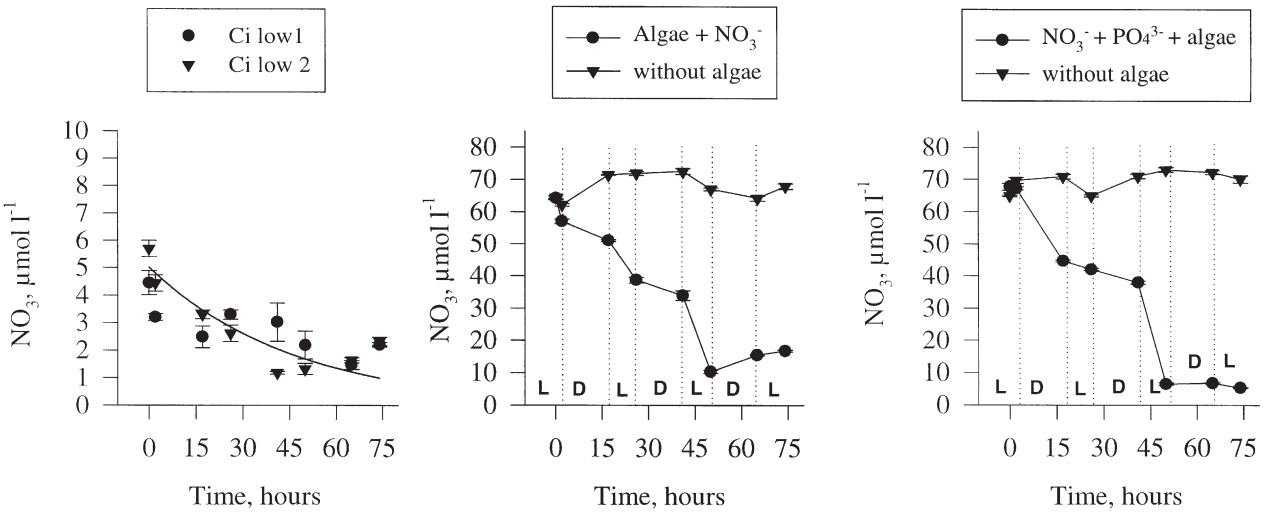

FIG. 3. – Nitrate uptake of *C. linum* during the first 75 hours after fertilisation, under a range of concentrations: low concentration (Ci low), high concentration (algae plus nitrate) and in combination with phosphate (algae plus nitrate and phosphate). **D**: dark, **L**: light. Vertical bars are standard errors.

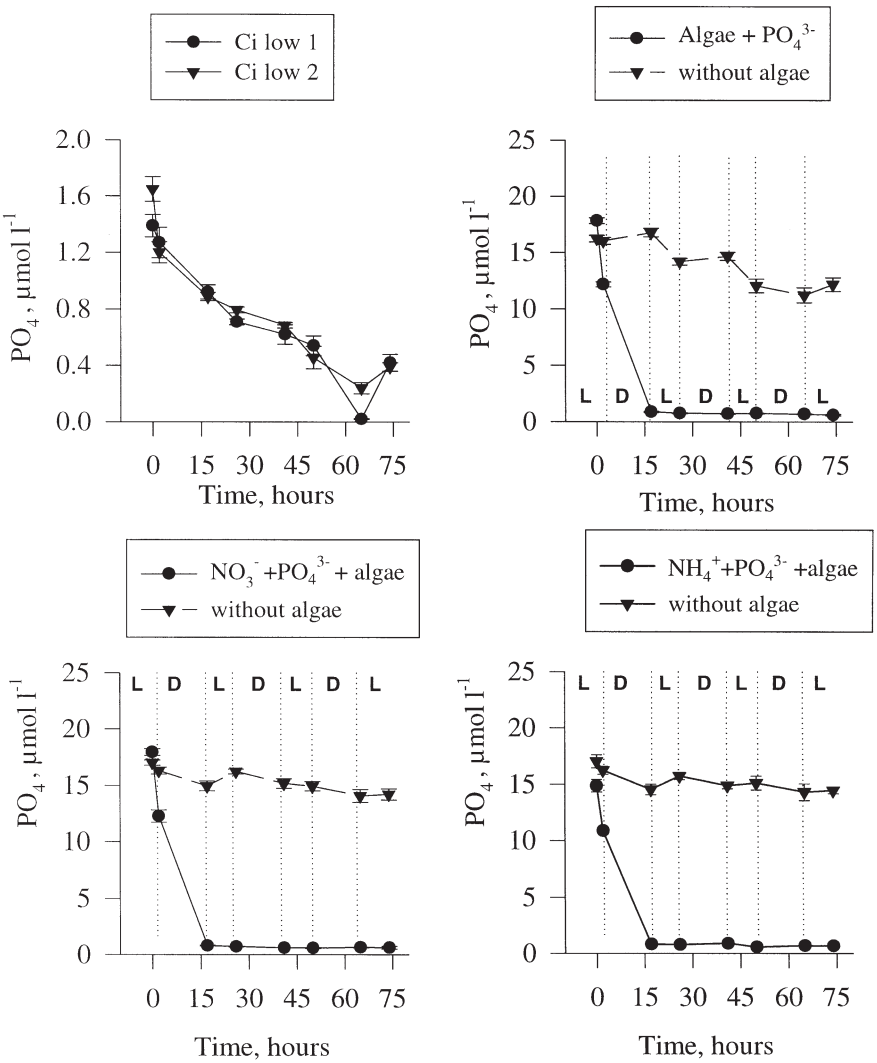

FIG. 4. – Phosphate uptake of *C. linum* during the first 75 hours after fertilisation, under a range of concentrations: low concentration (Ci low), high concentration (algae plus phosphate) and in combination with nitrogen as ammonium or nitrate (algae plus nitrogen as nitrate or ammonium, and phosphate). **D**: dark, **L**: light. Vertical bars are standard errors.

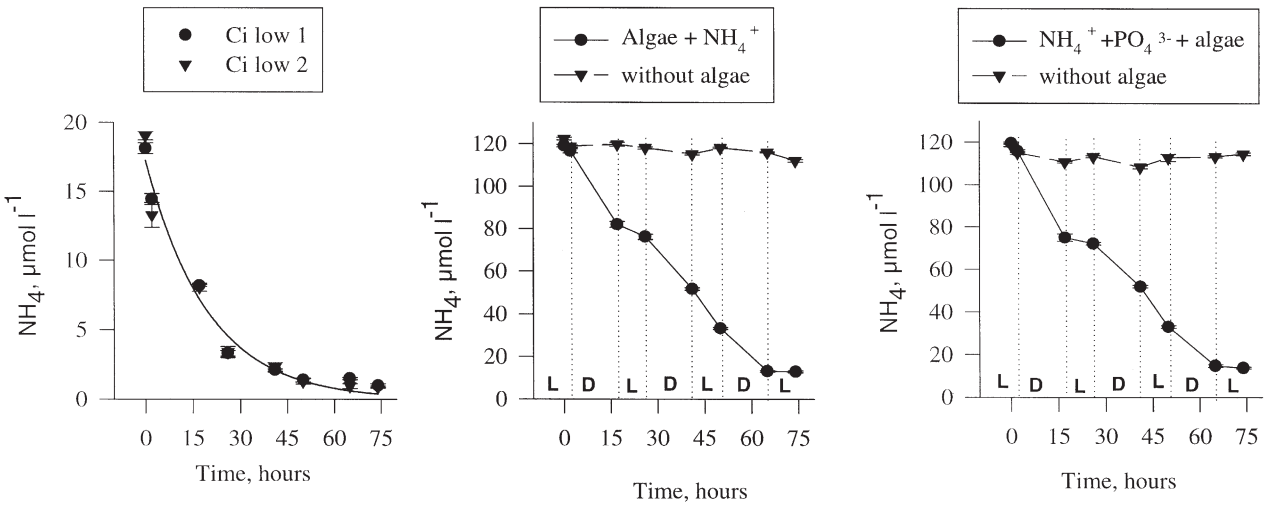

FIG. 5. – Ammonium uptake of *C. linum* during the first 75 hours after fertilisation, under a range of concentrations: low concentration (Ci low), high concentration (algae plus ammonium) and in combination with phosphate (algae plus ammonium and phosphate). **D**: dark, **L**: light. Vertical bars are standard errors.

fertilised) (Figs. 3-5, Table 3). The uptake rate of ammonium was significantly higher ($F=1551$, $p<10^{-6}$; Tukey's, $p< 0.0005$) (Fig. 5) when it was added alone than in combination with P. In contrast, the nitrate uptake rate was higher ($F=1536$, $p<10^{-6}$; Tukey's, $p< 0.0005$) in the N+P-treatments than in the N-treatments (Table 3, Fig. 3). The uptake rate of N in the ammonium treatments was two-fold greater than that with nitrate enrichment ($F=2917$, $p<10^{-6}$; Tukey's, $p<0.0005$).

The P uptake rate was higher when this nutrient was added alone. In N+P-treatments, the P uptake rate was faster when N was added as nitrate than when it was added as ammonium ($F=716$, $p<10^{-6}$; Tukey's, $p< 0.0005$) (Table 3).

If we calculate the net N and P accumulation in the macroalgal biomass ($(Nutrient_{initial} * Weight_{initial}) - (Nutrient_{final} * Weight_{final})$), significant differences were observed between fertilised and unfertilised treatments ($F=45.54$ and $F=39.66$, $p<0.0005$ in N and P fertilisation respectively (Table 4). However, no significant differences were detected in either forms of N fertilisation (ammonium vs. nitrate) (Tukey's, $p>0.05$).

Table 4.- Net macroalgae accumulation of nitrogen ($\Delta N=(B_{10}*N_{10})-(B_0*N_0)$; B_{10}: dry weight at day 10, N_{10}: nitrogen content at day 10, B_0: initial dry weight and N_0: initial nitrogen content) and phosphorus ($\Delta P=(B_{10}*P_{10})-(B_0*P_0)$; P_{10}: phosphorus content after 10 days, P_0: initial phosphorus content) by *C. linum* calculated after the 10 days of incubation in the jars enriched with NO_3^-, NH_4^+, PO_4^{3-}, $NO_3^- + PO_4^{3-}$, $NH_4^+ + PO_4^{3-}$, and without enrichment. Values are means ± standard errors.

	ΔN (mg)	ΔP (mg)
NO_3^-	11.575 ± 0.216	0.027 ± 0.002
NH_4^+	10.456 ± 1.355	-0.010 ± 0.013
PO_4^{3-}	0.318 ± 0.767	1.831 ± 0.320
$NO_3^- + PO_4^{3-}$	12.899 ± 1.065	1.666 ± 0.111
$NH_4^+ + PO_4^{3-}$	12.066 ± 0.636	1.302 ± 0.056
Ci low	1.906 ± 0.280	0.024 ± 0.007

DISCUSSION

Effect of fertilisation on nutrient content and chlorophyll

For *C. linum* in Tancada lagoon the availability of P is as relevant as that of N for growth, as demostrated by the high initial value of the N:P ratio in macroalgae, the fast uptake of P during the first 15 hours of incubation (Fig. 4), the increase in P content in the tissues, and the significant increase in biomass observed 10 days after fertilisation in N + P-treatment.

An N:P ratio greater than 11-24 is indicative of P limitation for macroalgae, whereas ratios lower than 8-16 indicate N limitation (Wheeler and Björnsäter, 1992). Our results show that *C. linum* was limited by N and P, except in the N+P-treatment. The initial N:P ratio of *C. linum* used in this experiment was 80, thereby indicating P limitation. Presumably, this explains the rapid uptake of P observed and the 10-fold increase in the concentration of this nutrient in the tissues of *C. linum* 4 days after treatment with P. The high P content in the algae subjected to P-enrichment indicates that this species may be capa-

ble of luxury uptake for this nutrient, as occurs in several tropical and temperate macroalgae (Fujita *et al*., 1989). However, no significant increase in biomass was observed in comparison with the Ci low treatment, indicating a secondary N limitation according to the N:P ratio of 6 observed in *C. linum* tissues of the P treatment.

N deficiency typically decreases photosynthetic pigments (Falkowski and Owens, 1980). The chlorophyll concentration increased during the first 4 days of incubation in N-treatments. After 10 days, this concentration decreased, but N content in the tissues remained stable. These results indicate that *C. linum* used chlorophyll to store N when surplus N was available, and that N was lost from the chlorophyll pool immediately after the removal of the external N supply. In laboratory experiments in which *C. linum* was subjected to dfferent periods of N availability and depletion, McGlathery and Pedersen (1999) observed a continuous flux into the protein pool. *C. linum* grown at low irradiance (100 μmol m^{-2} s^{-1}) lost N from the chlorophyll pool immediately after removing the external N supply. In contrast, at high irradiance (300 μmol m^{-2} s^{-1}), chlorophyll synthesis continued until day 6 after N-depletion, indicating an efflux from the chlorophyll to the protein pool. These results coincided with the decrease in chlorophyll concentration observed in our study during the N starvation period, detected after 8 days of N-depletion under the irradiance of 400 μmol m^{-2} s^{-1}, suggesting a potential translocation of N from the chlorophyll to the protein pool when N availability in the environment falls.

The differences in C content between control and P-enrichments, and N- and N+P-enrichments, may be related to the accumulation of carbohydrates in N-limited algae, which may facilitate N uptake and amino acid synthesis when N availability returns. Similar results were observed by McGlatery *et al.* (1996). When the internal N store of *C. linum* was depleted and the concentration of simple organic compounds (mainly amino acids) reached their minimum pool size, a significant increase in the starch pool in the high-light algae was detected.

Variation in ammonium and nitrate uptake

Altough the initial amount of ammonium added was higher than that of nitrate, both in low and high fertilisation treatment, and uptake rates were higher when ammonium rather than nitrate was added as a source of N, no differences in the N content of the *C. linum* tissues were observed between treatments. On the other hand, growth rates were high when nitrate was added, mainly in combination with P and during the starvation period. This observation indicates that *C. linum* has a high nitrate storage capacity, as reported for other macroalgae (Rosenberg and Ramus, 1982, Hwang *et al*., 1987, Lopes *et al*., 1997). The differences observed in N uptake rates in the two treatments (nitrate or ammonium added) may be related to loss of ammonium by volatilisation caused by the increase of pH inside the photosynthetic algal mat (from 8.9 to 9.5 after 1.5 hours of exposure to light) during light periods. Alternatively, ammonium may be converted to nitrate by nitrification in water with high concentrations of oxygen owing to oxygen bubbling. These hypotheses are supported by the observation of a lack of significant differences in the net N accumulation in macroalgae biomass between nitrate and ammonium treatments.

Nitrate is the most thermodynamically stable form of DIN in oxidised aquatic environments, and hence it is the predominant form of fixed N in most aquatic systems, but not necessarily the most readily available form (Falkowski and Raven, 1997). Following translocation to the plasmalema, which is an energy-dependent process, the assimilation of nitrate requires chemical reduction to ammonium. One of the universal responses in fungi and plants following exposure to nitrate is the induction of nitrate reductase (NR) activity (Crawford, 1995). This activity is enhanced when nitrate is added to algae maintained in nitrate-limited conditions and nitrate uptake occurs mainly during the night, whereas NR activity is greatest during the day (Lopes *et al*., 1997). In our study this diurnal cycle of uptake was observed only when nitrate alone was added to water. When nitrate is added in combination with P this diurnal cycle was observed 9 hours after the depletion of PO_4^{3-} in water. This observation might be related to rapid uptake of PO_4^{3-} during the first 15 hours of incubation owing to a low content of P in the cells and the ATP needed to take up nitrate. In experiments with plants grown without N, when nitrate was added to the environment a lag was observed, and uptake rates were low at the beginning and then increased progressively (Barceló *et al*., 1995). In our experiment, the uptake rate of P was higher when it was added with nitrate than with ammonium, and the nitrate uptake rate was faster when it was added in combination with P than alone. This indicates a coupling mechanism for P and

nitrate uptake. On the other hand, it may indicate ammonium inhibition of the formation of ATP by photophosphorylation (Barceló *et al.,* 1995).

According to McGlathery *et al*. (1997), diurnal variation in ammonium uptake is dependent on algal N status. In N-saturated macroalgae, ammonium uptake during the light period exceeds that which takes place in the dark (McGlathery *et al.,* 1997, D'Elia and DeBoer, 1978). These diurnal cycles of ammonium uptake appear to be related to the C requirements for N assimilation. N-saturated algae lack organic C reserves and thus to build amino acids they are dependent on the provision of C skeletons from recent photosynthesis (Turpin, 1991). On the other hand, N-limited algae accumulate carbohydrates during photosynthesis which can facilitate N uptake and amino acid synthesis during the dark (Turpin, 1991). This change in the diurnal pattern of ammonium uptake was observed in our experiments, with uptake of ammonium during the night when nitrogen content in *C. linum* was low at the beginning of the experiment, and uptake of ammonium both during the night and the day following nitrogen increase in the tissues. Also, this result may be related to C limitation of photosynthesis in relation to an observed decrease in alkalinity from 1.91 meq l^{-1} to 1.53 meq l^{-1} and pH values of around 9 after 2 days of incubation, which decrease the availability of C in the water.

CONCLUSIONS

Our results show the physiological response of *C. linum* to a nutrient pulse enrichment over 1-2 weeks, and do not necessarily reflect a longer term ecosystem-level response. *C. linum* in Tancada lagoon have distinct responses depending on the source, availability and periodicity of inputs of dissolved nutrients. Seasonality, which affects irradiance and water temperature and thereby influences the life cycles of other primary producers such as other species of macroalgae, phytoplankton and rooted macrophytes, can alter the physiological response of this macroalga.

P rather than N is the limiting factor for *C. linum* growth in Tancada lagoon in our experimental conditions. The relatively high availability of N may be related to the N inputs from rice fields and to P binding in the sediments. Therefore nutrient limitation of macroalgae may be related to the magnitude and frequency of external inputs.

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