THE EFFECT OF GROWTH IRRADIANCE ON THE COUPLING OF CARBON AND NITROGEN METABOLISM IN *CHAETOMORPHA LINUM* (CHLOROPHYTA)\(^1\)

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The influence of growth irradiance on the non-steady-state relationship between photosynthesis and tissue carbon (C) and nitrogen (N) pools in *Chaetomorpha linum* (Muller) Kutzing in response to abrupt changes in external nitrogen (N) availability was determined in laboratory experiments. For a given thallus N content, algae acclimated to low irradiance consistently had a higher rate of light-saturated photosynthesis (\(P_{\text{max}}\) normalized to dry weight) than algae acclimated to saturating irradiance; for both treatments, \(P_{\text{max}}\) was correlated to thallus N. Both \(P_{\text{max}}\) and the photosynthetic efficiency (\(a_{\text{dw}}\)) were correlated in *C. linum* grown at either saturating or limiting irradiance over the range of experimental conditions, indicating that variations in electron transport were coupled to variations in C-fixation capacity despite the large range of tissue N content from 1.1% to 4.8%. Optimizing both \(a\) and \(P_{\text{max}}\) and thereby acclimating to an intermediate light level may be a general characteristic of thin-structured opportunistic algae that confers a competitive advantage in estuarine environments in which both light and nutrient conditions are highly variable. Nitrogen-saturated algae had the same photosynthesis-irradiance relationship regardless of light level. When deprived of an external N supply, photosynthetic rates did not change in *C. linum* acclimated to low irradiance despite a two-fold decrease in tissue N content, suggesting that the active pools of chlorophyll and Rubisco remained constant. Both \(a\) and \(P_{\text{max}}\) decreased immediately and continuously in algae acclimated to high irradiance on removal of the N supply even though tissue N content was relatively high during most of the N-starvation period, indicating a diversion of energy and reductant away from C fixation to support high growth rates. Carbon and nitrogen assimilation were equally balanced in algae in both light treatments throughout the N-saturation and -depletion phases, except when protein synthesis was limited by the depletion of internal N reserves in severely N-starved high-light algae and excess C accumulated as starch stores. This suggests that the ability for short-term adjustment of internal allocation to acquire N and C in almost constant proportions may be especially beneficial to macroalgae living in environments characterized by high variability in light levels and nutrient supply.

**Key index words:** carbon; *Chaetomorpha linum*; chlorophylls; growth; irradiance; macroalgae; nitrogen; photosynthesis; storage

Light and nitrogen availability are the primary factors controlling macroalgal productivity in temperate coastal waters. Because both resources vary considerably in natural environments (e.g., Hanisak 1983, Fujita 1985, McGlathery 1992, Valiela et al. 1992), macroalgae would be expected to have mechanisms that ensure a balance between resource variability and growth. Changes in carbon (C) and nitrogen (N) allocation to optimize growth do occur in some macroalgae in response to seasonal variation in light and N supply (e.g. Chapman and Craigie 1977, Rosenberg and Ramus 1982, Pedersen and Borum 1996) and to long-term steady-state laboratory conditions (Lapointe and Duke 1984). In estuarine environments, nutrient availability may vary as much over the shorter times scales of hours to days as on a seasonal cycle (e.g. Fujita 1985, Ramus and Venable 1987, McGlathery 1992). Thus, ephemeral, opportunistic macroalgae that rely on rapid growth to escape high tissue-loss rates in estuarine environments must be able to adjust their C and N metabolism over these short time scales to optimize growth. Evidence exists that such rapid responses do occur in some algae from studies on (1) non-steady-state relationships between internal N pools, uptake, assimilation, and growth (Ramus and Venable 1987, Fujita et al. 1988, Pedersen 1994, McGlathery et al. 1996) and (2) transient depressions in photosynthesis of N-limited algae and mobilization of carbohydrate stores in response to ammonium resupply (Williams and Herbert 1989, Vergara et al. 1995). However, we lack a complete understanding of the direct mechanisms coupling C and N metabolism in marine macroalgae and the time scales on which these operate.

The mechanisms that optimize growth in response to changing light or nutrient conditions in-
volve the reallocation of N and C cellular components to optimize photosynthetic yield. Tissue N levels and photosynthetic C fixation are strongly coupled in all plants because the energy and C skeletons required for N uptake and assimilation are provided by photosynthesis and because over 50% of tissue N is allocated to the chloroplast (Chapin et al. 1987, Turpin 1991, Huppe and Turpin 1994). Photosynthetic efficiency at low irradiances (α) is regulated mainly by the effect of pigment level and configuration on light absorption and electron transport capacities, whereas Rubisco activity sets the maximum rate of photosynthesis (P_max) at saturating irradiances (Henley and Ramus 1989a, Turpin 1991, Markager and Sand-Jensen 1994). It is generally believed that a trade-off exists between these two N-rich compounds in plants acclimated to high- and low-light environments (Raven 1984, Henley and Ramus 1989a). Acclimation from shade to sun typically involves an increase in the synthesis of Rubisco, whereas plants acclimated to lower irradiances usually increase pigment levels by changing the size and/or number of photosynthetic reaction centers. Because ephemeral macroalgae growing in estuarine and intertidal environments are more likely to be exposed to variable irradiances than to full sunlight or deep shade for prolonged periods of time, they may not be expected to develop photosynthetic characteristics typical of “sun” or “shade” plants. Henley and Ramus (1989b) followed the photosynthetic response of the macroalga Ulva rotundata to irradiance changes and suggested that this species is adjusted to an intermediate light acclimation state. It is also likely that the mechanisms of light acclimation are related to the algal N status (Henley et al. 1991).

Nitrogen deficiency typically causes decreases in both photosynthetic pigments and Rubisco content/activity that correspond to marked declines in α (Falkowski and Owens 1980) and P_max normalized to biomass (Lapointe and Duke 1984, Falkowski et al. 1989, Turpin 1991). Allocation of C to biosynthesis or storage also typically varies as a function of macroalgal N status (Chapman and Craigie 1977, Bird et al. 1982, Rosenberg and Ramus 1982, Vergara et al. 1995). Carbon that is fixed during photosynthesis either is incorporated into organic molecules synthesized for growth, stored as sugars, starch, or other carbon compounds (e.g. laminaran) or is ultimately respired to support C maintenance costs. Most N-deficient green algae accumulate C mainly as starch reserves, which can later be mobilized by respiration to provide the carbon skeletons necessary for N assimilation when N is resupplied (Elrifi and Turpin 1986, Weger and Turpin 1989, Vergara et al. 1995).

The response of macroalgae to variable nutrient and irradiance regimes involves both the physiological limits of acclimation to different conditions and the rate at which physiological adjustments occur. In the present study, we describe the effect of growth irradiance on the photosynthesis-nitrogen relationship and carbon allocation patterns in Chae
tomorpha linum, a thin-structured, bloom-forming macroalga that often dominates shallow eutrophic estuaries (Lavery and McComb 1991, Rissgaard et al. 1995, McGlathery et al. 1997). We evaluated both the effect of N supply on the photosynthetic performance of C. linum acclimated to either saturating or limiting irradiances and the non-steady-state relationships between photosynthesis and tissue C and N pools in response to abrupt changes in N availability. Whereas other studies have focused on the coupling of carbon and nitrogen metabolism in algae on seasonal time scales or as rapid (minutes to hours) transient responses to changes in N availability (Chapman and Craigie 1977, Rosenberg and Ramus 1982, Williams and Herbert 1989, Vergara et al. 1995, Pedersen and Borum 1996), we were interested in interactions occurring over time scales of hours to days that match the variability of nutrient and light regimes in estuarine environments.

MATERIALS AND METHODS

Experimental design. Algae for the laboratory experiment were collected from Roskilde Fjord, a shallow Danish estuary (3 m average water depth). About 75 g fresh weight (FW) of C. linum were placed in each of eight aquarium tanks (15 L) filled with filtered seawater (0.45 μm) at 15° C. Background levels of nutrients in the incubation water were <1 μM inorganic nitrogen (NH₄NO₃, NO₂⁻, NO₃⁻) and 4 to 8 μM inorganic phosphorus (PO₄³⁻), reflecting in situ concentrations (Pedersen and Borum 1996). The water was replaced with freshly collected filtered seawater every second day throughout the experiment. Circulation in the aquaria was provided by submersible aquarium pumps (6 mL.min⁻¹).

Half the tanks received incident light of 300 μmol-photons.m⁻².s⁻¹ (high-light treatment) sufficient to saturate C. linum photosynthesis, and half were illuminated at a level that was limiting to photosynthesis, 100 μmol-photons.m⁻².s⁻¹ (low-light treatment). The algae were acclimated to these irradiances (16:8 h LD [light:dark] cycle) and to the background nutrient levels for 3 days prior to the initiation of the experiment. The experiment consisted of a 13-day N-enrichment period, followed by a 13-day period without N supply, and finally a 4-day N-resupply period. During the enrichment periods, N (as NH₄NO₃) was added continuously from a concentrated stock solution throughout the light and dark cycles in sufficient quantity to saturate macroalgal growth demand (calculated as the product of measured growth rates and tissue N content). Surplus phosphorus was added as KH₂PO₄ continuously during both the enrichment and the depletion periods to prevent P limitation during the experiment. Daily loading rates per tank were 90 mg N-day⁻¹, 9 mg P-day⁻¹ in high light, and 30 mg N-day⁻¹ and 3 mg P-day⁻¹ in low light. Nutrients were measured daily in the incubation water to ensure that supplies exceeded the macroalgal demand.

Photosynthetic characteristics (P vs. I) and growth. Photosynthesis and dark respiration of C. linum were measured four times during the N-saturation and depletion periods (days 0, 2, 6, and 13) and once during N resupply, as oxygen evolution in 120-mL glass bottles containing about 0.5 g FW of algal material. The bottles were placed on a rotating wheel (60 cm diameter, 12 rpm) and illuminated at six different irradiances (0-480 μmol photons.m⁻².s⁻¹) for 2 h at 15° C. To conserve algal material during the experiment, two algal samples were taken from each of the four replicate tanks for each treatment and randomly assigned to three of the six light levels. The algae were incubated in three
series using different layers of nylon screens over individual bottles to achieve the desired light level for each bottle; this randomization process resulted in four replicate measurements at each light level. Filtered seawater blanks were illuminated at the highest light intensity and used to correct all light incubations. For each bottle for each series, dissolved O$_2$ concentrations were determined by duplicate micro-Winkler titrations (precision: ±0.01 mg O$_2$ L$^{-1}$), and rates of photosynthesis and respiration were expressed on either a dry-weight (DW) basis as mg O$_2$ g$^{-1}$ DW h$^{-1}$ or normalized to chlorophyll a+b content (mg O$_2$ mg$^{-1}$ chlorophyll a+b). The photosynthetic-irradiance (P-I) curves for each light treatment were made by pooling the data from the four replicate measurements at each irradiance. Because the randomization procedure resulted in four measurements at each irradiance but not four separate P-I curves, we could calculate only one value for $\alpha$ (representing photosynthetic efficiency at low light). This was determined by linear regression as the slope of the initial linear part of the P-I curve ($<108 \mu$mol photons m$^{-2}$ s$^{-1}$). The rate of light-saturated photosynthesis ($P_{\text{max}}$) was calculated as the mean of the four oxygen production rates at the highest photon flux density ($\sim 480 \mu$mol photons m$^{-2}$ s$^{-1}$).

Growth rates were determined as the 1- to 5-day change in algal biomass four times during the experiment. The entire biomass in each tank was harvested at the beginning and end of each growth period, spun dry, and weighed to the nearest 0.01 g. Specific growth rates are reported as the increase in algal fresh weight assuming exponential growth, $\mu = (\ln B_i - \ln B_o)/t$, where $B_i$ and $B_o$ are the algal biomass before and after $t$ days of growth.

**Tissue N and C pools.** Algal material for the analysis of total N and C and the various N and C pools was collected from each tank ($n = 4$ for each treatment) 10 times during both the N-addition and the N-depletion period. Algae were sampled with decreasing frequency during each period: after 0, 3, and 9 h during the first day and at the end of days 1, 2, 4, 6, 8, 11, and 13. Samples were also taken one to three times during the N-resupply period. Tissue N and C contents were determined using a Carlo Erba NA-1500 CNS analyzer on duplicate freeze-dried samples from each tank. Two N pools that influence photosynthetic activity, chlorophyll and protein (of which the enzyme Rubisco comprises up to 25%; Evans and Seeman 1989), were analyzed. Chlorophyll a+b content was determined using the method of Wintermann and DeMotts (1965) on duplicate freeze-dried and ground samples that were extracted overnight in 96% ethanol, homogenized, and measured spectrophotometrically. Protein content was determined on duplicate 0.1-g fresh weight (FW) algal samples from each incubation tank using the BCA (bicinchoninic acid) assay (Smith et al. 1985). Frozen samples were thawed, ground with a mortar and pestle in 5 mL of 1% (w/v) sodium bicarbonate, and extracted overnight in a refrigerator. The extracted samples were centrifuged for 5 min (2600 × g), and a 0.1-mL aliquot of the supernatant was used for the colorimetric determination of protein against a bovine serum albumin standard.

Two carbon pools, ethanol-soluble carbohydrates (mainly sugar) and insoluble carbohydrates (starch reserves and cell wall polysaccharides), were determined on duplicate 100-mg samples from each tank. The samples were freeze-dried, ground, extracted for 1 h in 5 mL of 80% ethanol at 60°C, and then centrifuged (5 min at 2600 × g). Soluble sugar concentrations were measured colorimetrically using 2.5 mL of the supernatant diluted to 25 mL with demineralized water after adding 1 mL of 5% (v/v) phenol and 5 mL of concentrated (96%) sulfuric acid and incubating the sample in a 25°C to 30°C water bath for 30 min. The remaining ethanol-insoluble pellet was dried and used for analysis following the same procedure after first extracting the insoluble carbohydrates in 10 mL of 30% (v/v) perchloric acid. The soluble and insoluble carbohydrate concentrations for each sample were corrected for absorbance by pigment color and initial extraction volume.

**Movement of N pools during starvation.** We determined the flux into or out of the chlorophyll and protein pools during the N-starvation period by correcting the measured pool size at each time interval for the dilution of the pool by the addition of new biomass from growth. Details of these calculations for all tissue N pools (inorganic + organic) are given in McGlathery et al. (1996). Briefly, we assumed that because no new N was available during the N-saturation period, the exponential depletion of total tissue N reflected dilution by the addition of new biomass from growth. Likewise, the exponential depletion of the individual N pools represented the decline in N concentration from both dilution by growth and movement between the pools. At each time step (3 h) in our calculation, we applied the growth dilution factor calculated from the total N-depletion curve to the pool size (determined from the exponential fit to the measured values). Thus, the changes in the pool sizes corrected for growth at each successive time interval equals the difference between the flux into and out of the pool. We assumed that proteins and chlorophyll are end products of N assimilation.

**Statistical analysis.** Four replicates were measured per treatment for all analyses at each time period, and means and standard errors are reported. Treatment differences (high light vs. low light) during the N-saturation and -depletion periods for photosynthetic characteristics and tissue constituents were determined using one-way analysis of variance (ANOVA). Comparisons of growth rates and of individual parameters at the end treatment periods were made using $t$-tests.

**RESULTS**

**Photosynthesis-nitrogen relationship.** The maximum photosynthetic rate ($P_{\text{max}}$) of $C$. linum was positively correlated with the tissue N content for both high- and low-light treatments (high light: $r = 0.52, P \leq 0.01$; low light: $r = 0.48, P \leq 0.01$, ANOVA) over the range of tissue N concentrations tested (1.2%–4.7% DW for high-light algae and 1.7%–3.7% for low-light algae; Fig. 1). The regression lines for the two light treatments show that for a given tissue N content, low-light algae tended to have a higher $P_{\text{max}}$ than high-light algae ($P \leq 0.01$, ANOVA test of coincidence of lines; Fig. 1). Both $P_{\text{max}}$ and $\alpha$ normalized to dry weight were positively correlated over the range of tissue N concentrations for both light treatments ($r = 0.90, P < 0.001$, ANOVA; Fig. 2).
Photosynthesis and growth. When N was supplied in surplus, the P-I relationship based on dry weight was similar for both high- and low-light algae and varied little for either treatment during the 13-day N-saturation period (Fig. 3a, b). Rates of Pmax of the two treatments were not significantly different ($P > 0.05$, ANOVA), but respiration was about 40% lower in the low-light algae ($P \leq 0.001$, ANOVA; Fig. 3, Table 1). The initial slope ($\alpha_{\text{dw}}$) of the P-I curve also was similar for the algae throughout the N-saturation period regardless of light exposure ($P > 0.05$, ANOVA; Fig. 3, Table 1). Both $P_{\text{max}}$ and respiration normalized to chlorophyll were significantly higher in the N-saturated high-light plants (Table 2).

When the external N supply was removed, a clear difference was seen in the photosynthetic response for algae grown in the two light treatments. Algae acclimated to low light showed no significant change in the P-I relationship based on dry weight over the 13-day N-depletion period, even though the tissue N decreased from 3.1% to 1.7% DW ($P > 0.05$, ANOVA; Fig. 3c, Table 1). In contrast, both $P_{\text{max}}$ and $\alpha_{\text{dw}}$ decreased immediately following the removal of the N supply and continued to decline throughout the N-depletion period (Fig. 3d). Within the first 2 days, $P_{\text{max}}$ decreased by 20% and $\alpha_{\text{dw}}$ by more than 30% despite high tissue N levels (4.0%–5.1% DW, Fig. 4a). By the end of the N-depletion period, $P_{\text{max}}$ was reduced by more than 60% (Table 1), and $\alpha_{\text{dw}}$ decreased by over 50% (Fig. 3d, Table 1). Dark respiration rates increased by 25% during the first 2 days of N depletion but then remained constant (Fig. 3d). On a chlorophyll basis, $P_{\text{max}}$, respiration, and $\alpha$ all were higher in high-light
alga at the end of the N-starvation period because of the lower chlorophyll content of high-light plants (Table 2, Fig. 4).

Nitrogen resupply caused no significant change in P$_{\text{max}}$ on a dry-weight basis in the low-light algae (P > 0.05, ANOVA, Table 1, Fig. 3e), but P$_{\text{max}}$ nearly doubled in algae acclimated to high light (Table 1, Fig. 3f). During resupply, $\alpha_{\text{chl}}$ increased by 20% in the high-light algae and decreased by about the same amount in the low-light algae (Table 1). Respiration rates for C. linum in both light treatments did not change significantly during N resupply (Table 1). On a chlorophyll basis, high-light algae had higher rates of P$_{\text{max}}$, respiration, and photosynthetic efficiency (Table 2).

The relative growth rates in our laboratory experiment ranged from 0.03 to 0.15·day$^{-1}$, which is in the range of reported growth rates for C. linum measured in situ at the collection site (0.01-0.22·day$^{-1}$; Pedersen and Borum 1996). Growth rates were higher in the algae acclimated to high irradiance throughout the N-saturation and -depletion periods (Table 3). When N starved, growth rates declined in the algae in the high-light treatment, but remained relatively constant in algae grown in the low-light treatment (Table 3).

**Tissue nitrogen pools.** The N content of freshly collected C. linum was N 2.4% to 2.8% DW (Fig. 4). When surplus N was supplied, the N content increased to about 4% DW in algae grown at high irradiance, whereas it remained relatively constant at about 3% DW in algae grown at low irradiance. The N content declined exponentially in algae from both treatments when the external N supply was removed (Fig. 4a), reflecting the dilution of tissue N content by growth, as no new N was available during this period. During the N-saturation period, the protein pools showed a similar increase in algae from both treatments; chlorophyll was constant in low-light plants but decreased by about 25% in high-light plants (Fig. 4b, c). Both pools declined exponentially when no external N was supplied (Fig. 4b, c). Protein concentrations were significantly higher in algae grown at low irradiance when external N was supplied (P ≤ 0.001, ANOVA) but were similar during the starvation period regardless of light treatment (P > 0.05, ANOVA). Chlorophyll concentrations were always higher and less variable in the algae acclimated to low irradiance (P ≤ 0.001, ANOVA). During the N-resupply period, tissue N increased more than three-fold to 4.7% DW in the high-light algae and doubled to 3.7% DW in the low-light algae (Fig. 4a). Protein concentrations increased by 50% in the high-light treatment and 25% in the low-light treatment (Fig. 4b). Chlorophyll concentrations continued to decline in low-light al-

### Table 1. Maximum rate of light-saturated photosynthesis (P$_{\text{max}}$), respiration rate, and photosynthetic efficiency at low light (a; normalized to dry weight of biomass) for Chaetomorpha linum during sequential periods of N supply or N starvation. Significant differences (t-test of treatment effects) determined between high and low light; * P ≤ 0.05; ** P ≤ 0.01.

<table>
<thead>
<tr>
<th>Day</th>
<th>Condition</th>
<th>Treatment</th>
<th>P$_{\text{max}}$ (mg O$_2$·g$^{-1}$DW·h$^{-1}$)</th>
<th>Respiration (mg O$_2$·g$^{-1}$DW·h$^{-1}$)</th>
<th>$\alpha_{\text{chl}}$ (mg O$_2$·mg$^{-1}$chl·h$^{-1}$)</th>
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<tbody>
<tr>
<td>0</td>
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<td>12.93 ± 1.08</td>
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<td>9.83 ± 0.98</td>
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<td>10.94 ± 0.15</td>
<td>1.00 ± 0.02*</td>
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Value calculated from pooled P-I curve for that day.

### Table 2. Maximum rate of light-saturated photosynthesis (P$_{\text{max}}$), respiration rate, and photosynthetic efficiency at low light (a; normalized to chlorophyll) for Chaetomorpha linum during sequential periods of N supply or N starvation. Significant differences (t-test of treatment effects) determined between high and low light; * P ≤ 0.05; ** P ≤ 0.01.

<table>
<thead>
<tr>
<th>Day</th>
<th>Condition</th>
<th>Treatment</th>
<th>P$_{\text{max}}$ (mg O$_2$·mg$^{-1}$chl·h$^{-1}$)</th>
<th>Respiration (mg O$_2$·mg$^{-1}$chl·h$^{-1}$)</th>
<th>$\alpha_{\text{chl}}$ (mg O$_2$·mg$^{-1}$chl·h$^{-1}$)</th>
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<tbody>
<tr>
<td>0</td>
<td>initial</td>
<td>high light</td>
<td>1.83 ± 0.15</td>
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<tr>
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<td></td>
<td>low light</td>
<td>1.64 ± 0.053**</td>
<td>0.087 ± 0.014**</td>
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<td>2.63 ± 0.032*</td>
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Value calculated from pooled P-I curve for that day.
FIG. 4. Time-course changes in total tissue N content (A), protein (B), and chlorophyll (C) for light-saturated (V) and light-limited (v) C. linum during N-saturation, N-depletion, and N-resupply periods. Values represent means ± SE (n = 4).

gae when N was resupplied but tended to increase in the high-light algae (Fig. 4c).

The change in total pool sizes of chlorophyll and protein during N starvation corrected for the amount that was due to growth dilution are shown in Figure 5 (calculated over the 13-day N-depletion period). Algae grown at low irradiance lost N from the chlorophyll pool immediately after removing the external N supply, and this efflux continued throughout the N-depletion period (Fig. 5a). In contrast, the synthesis of chlorophyll in algae grown at high irradiance continued until day 6 of the N-depletion period, after which an efflux from the chlorophyll pool occurred (Fig. 5a). A continuous flux into the protein pool was seen in both high- and low-light algae, indicating that protein was being synthesized during the entire N-depletion period (Fig. 5b) and that the decrease in concentration on a dry-weight basis was due to pool dilution by the addition of new biomass from growth. The rate of protein synthesis changed little in macroalgae grown at low irradiance compared to the three-fold change in algae acclimated to high irradiance.

Tissue carbon pools. The concentration of total C varied from 27% to 32% DW, which is within the range reported for benthic marine macroalgae and sea grasses (15%–48%; Atkinson and Smith 1983). The carbon content always was highest in macroalgae acclimated to high irradiance (P ≤ 0.01, ANOVA; Fig. 6a). In both high- and low-light algae, tissue C content increased when surplus N was supplied and decreased during N starvation. The concentrations of soluble and insoluble carbohydrates re-

<table>
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<th>Day</th>
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<th>High light</th>
<th>Low light</th>
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<tr>
<td>3</td>
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<td>9</td>
<td>+N</td>
<td>0.10 ± 0.01</td>
<td>0.05 ± 0.01*</td>
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<tr>
<td>13</td>
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<td>0.08 ± 0.01</td>
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<tr>
<td>21</td>
<td>−N</td>
<td>0.07 ± 0.01</td>
<td>0.03 ± 0.01*</td>
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</table>

Table 3. Relative growth rates (per day) for Chaetomorpha linum during periods of N supply or N depletion. Significant differences (t-test of treatment effects) determined between high and low light; *P ≤ 0.05.
Carbon allocation patterns reflect a trade-off between storage and biosynthesis. We estimated the distribution of C among the different pools on samples taken at the start of the experiment and then at the end of the N-saturation, -depletion, and -resupply periods by determining the proportion of tissue carbon contained in the C storage pools and the pools of nonstorage organic compounds using a molar C:N of 4:1 for proteins (Sprent 1987), 5:1 for other organic N compounds (mainly amino acids; range 2:1–9:1; Sprent 1987, Jones et al. 1996), and 13.5:1 for chlorophyll. The contents of these concentrations of N pools are reported in McGlathery et al. (1996). Initial samples and N-saturated algae had a similar distribution of C among the different pools regardless of light treatment (Fig. 7). By the end of the 13-day N-supply period, total tissue C was allocated about equally to C storage and biosynthesis in algae from both light treatments. Allocation to C storage in the insoluble carbohydrate pool was higher in N-starved algae, and this occurred to a greater extent in algae acclimated to high irradiance. Insoluble carbohydrates comprised about 70% of total C in N-depleted high-light algae but only 57% in N-depleted low-light algae. After 4 days of N resupply, the pools of C in nonstorage organic compounds were about equal for the two light treatments, but the starch stores decreased to a greater extent in the high-light algae.

Carbon versus nitrogen assimilation. Variation in the C:N (molar) ratio during the experiment reflected the balance between carbon and nitrogen assimilation as a function of N saturation and depletion. The C:N ratio was similar in high- and low-light algae throughout the entire experiment (P > 0.05, ANOVA; Fig. 8) despite different growth rates, until the high-light algae became severely N depleted at the end of the starvation period (P ≤ 0.001, t-test; Fig. 8).

DISCUSSION

Changes in the photosynthetic metabolism and carbon allocation in C. linum occurred rapidly in response to variations in the external nitrogen supply. These responses are consistent with our previous results showing similar rapid (hours to days) changes in the size of intracellular nitrogen pools and feedback control from these pools on nitrogen uptake rates of C. linum (McGlathery et al. 1996). Although many macroalgae are known to adjust C and N allocation in response to changing nutrient and light levels, most previous studies have considered this on a seasonal time scale or under steady-state laboratory conditions (e.g. Chapman and Craige 1977, Rosenberg and Ramus 1982, Lapointe and Duke 1984, Pedersen and Borum 1996). Because nutrient and light availabilities may vary as much daily as seasonally in estuaries and lagoons, our results suggest that the ability of fast-growing, ephemeral macroalgae such as C. linum to regulate C and
N allocation on this time scale may be critical to maintaining balanced growth in these environments. Other studies have shown that rapid but transient response to changes in external nutrient supplies do occur in some macroalgae (Ramus and Venable 1987, Fujita et al. 1988, Pedersen 1994, McGlathery et al. 1996).

The short-term adjustments in C and N metabolism in *C. linum* resulted in a positive correlation between light-saturated photosynthesis (P_max) and tissue nitrogen over the range of experimental conditions. These results agree with the general correlation between P_max and tissue N that has been shown for phytoplankton, other aquatic macrophytes, and terrestrial plants in field populations and laboratory cultures (Field and Mooney 1986, Turpin 1991, McGlathery et al. 1992). This correlation in algae has been explained by variations in Rubisco activity (Kuppers and Weidner 1980, Lapointe and Duke 1984). Our results also show that *C. linum* acclimated to high irradiance had lower net production per unit tissue N than macroalgae acclimated to low irradiance. This difference is probably because macroalgae in the high-light treatment had a higher tissue N content and accumulated more N in nonphotosynthetic components (i.e. inorganic storage pools of NH_3 and NO_3, nucleic acids and proteins associated with cell regulation and respiration; McGlathery et al. 1996). If this is a general characteristic of ephemeral macroalgae, it suggests that unless severely N limited (i.e. tissue N content below critical levels of 1.1%–1.2% of dry weight; La-very and McComb 1991, Pedersen and Borum 1997), macroalgae growing at low irradiances have the capacity for increased production for a given N content if exposed to higher irradiances. This would be especially beneficial in shallow coastal waters, where unattached, ephemeral macroalgae experience variable irradiance conditions because of physical movement in the water column or rapid changes in light availability following episodic turbidity events.

Light-saturated photosynthesis and \( \alpha \) (normalized to dry weight) were positively correlated over the range of tissue N levels (1.1%–4.8% DW) for both light treatments, indicating that chlorophyll and Rubisco were equally limiting to photosynthesis and that light harvesting and C fixation were coupled (Falkowski 1980). Our data suggest that a strategy to optimize both \( \alpha \) and P_max may be a general characteristic of opportunistic thin-structured algae that represents a compromise between maximizing light-harvesting and carbon-fixation capacities when exposed to variable light and nutrient regimes. This is possible because \( \alpha \) and P_max can vary to the same degree in plants with a thin structure (e.g. phytoplankton and sheetlike or filamentous macroalgae) because absorbance can increase roughly in proportion to chlorophyll density (Augusti et al. 1994, Enriquez et al. 1996). Correlations between \( \alpha \) and P_max have been observed in general comparisons among phytoplankton species (Harding et al. 1987) and Mediterranean marine macrophytes (Enriquez et al. 1995) and in within-species comparisons for phytoplankton (Richardson et al. 1983, Barlow and Alberte 1985; but see Falkowski and LaRoche 1991) and for macroalgae grown at low-light levels (*Ulva*...
and Rubisco is expressed and that a trade-off between N allocation to chlorophyll pools of NO and simple organic N compounds was calculated at a faster rate in high-light algae but the comparison to low-light algae, protein always was synthesized with less energy and reductant away from C fixation. Probably because the slower growth rates diverted (2–2 weeks) of high light and low nitrogen supply that a trade-off between N allocation to chlorophyll and Rubisco is expressed and α and P_{max} become uncoupled.

Differences in the photosynthetic response of C. linum to changes in external N availability between the two irradiance treatments reflected differences in growth rates and the size of internal N pools. For algae acclimated to low irradiance, both α and P_{max} varied little over the depletion phase even after a 50% reduction in tissue N from 3.1% to 1.7% of dry weight, whereas algae acclimated to saturating irradiance showed an immediate and continued decline in both α and P_{max} during N deprivation. Although this rapid response to N starvation in high-light algae is similar to the decline in photosynthesis observed in other algae after long-term exposure to steady-state nutrient-limiting conditions (e.g. Rosenberg and Ramus 1982, Lapointe and Duke 1984), the short-term response of C. linum in our experiment was not the result of incipient nutrient limitation because tissue N levels (2.3%–3.1% of dry weight) were well above the tissue N content required for maximum growth (1.1%–1.2% of dry weight; Lavery and McComb 1991, Pedersen and Borum 1997). Instead, this response reflects the diversion of energy and reductant away from C fixation to amino acid and protein synthesis to support high algal growth rates. Competition between N assimilation and C fixation also has been shown to influence both P_{max} and α of N-deprived microalgae (Turpin and Weger 1988) and macroalgae (Williams and Herbert 1989) as a transient response to NH_{4}^{+} resupply. We did not observe the same response when C. linum was grown at low irradiance, probably because the slower growth rates diverted less energy and reductant away from C fixation. Compared to low-light algae, protein always was synthesized at a faster rate in high-light algae but the protein pool declined more rapidly as the storage pools of NO_{3}^{-} and simple organic N compounds were depleted (McGlathery et al. 1996). Respiration rates also increased immediately following the removal of the external N supply, probably to support reduction of stored NO_{3}^{-} and to provide the carbon skeletons for protein synthesis (Elrifii and Turpin 1986, Curtis and Megard 1987). In low-light algae, the lack of a photosynthetic response to N deprivation may also reflect that the active pool sizes of chlorophyll and Rubisco remained relatively constant during the starvation period. Even though chlorophyll was degraded (outward flux) during the N-depletion period, the lack of a corresponding change in α (on a chlorophyll or dry-weight basis) indicates that excess N in antenna pigments was reallocated without a change in electron transport capacity (Turpin 1991). These algae also may have accumulated excess N as Rubisco during the N-saturation phase, as has been shown for terrestrial plants in low-light environments (Millard 1988). This may partly account for both the high rate of biomass-normalized photosynthesis at light saturation and the lack of a significant response in P_{max} during the N-depletion period.

Despite the large and immediate changes in photosynthetic capacity during periods of N depletion in high-light algae, growth irradiance had little effect on the allocation of carbon to pools of carbohydrates and organic compounds until the end of the N-starvation period. It was only when the internal NO_{3}^{-} storage pool was depleted completely and the concentration of simple organic N compounds (mainly amino acids) reached its minimum pool size (McGlathery et al. 1996) that a significant increase in the starch pool in the high-light algae was seen. Our results agree with previous findings from longer-term studies that starch stores accumulate as algae become N deficient (Chapman and Craigie 1977, Bird et al. 1982, Rosenberg and Ramus 1982), presumably because the C skeletons produced by photosynthesis are no longer being diverted to the synthesis of structural polysaccharides and proteins (Vergara et al. 1995). Resupply of N resulted in a rapid decrease in insoluble carbohydrate reserves even though rates of C fixation nearly doubled during this period, indicating that respiration of reserve carbohydrates supplemented recently fixed transient sugars to meet the increased demands for N assimilation and growth. An increased flow of C from carbohydrates to organic N compounds (amino acids and proteins) also was shown for the red alga Gracilaria lemaneiformis as a transient response to NH_{4}^{+} assimilation (Vergara et al. 1995). The dynamic nature of the insoluble carbohydrate pool on removal and resupply of N in our study is analogous to rapid changes in starch reserves found in U. rotundata following transfer between saturating and limiting irradiances (Henley and Ramus 1989b). Surplus N and light limitation apparently have the same effect of an immediate decrease in starch reserves to support growth; transfer to high irradiance replenishes starch pools just as N deprivation does because in both cases C fixation capacity exceeds N assimilation into organic compounds.

Overall, the variation in the C:N ratio observed for C. linum grown at either saturating or limiting irradiance is consistent with other studies showing variation in C:N as a function of algal N status (Lapointe and Duke 1984, McGlathery et al. 1992, Ver-
However, our data suggest that C. linum exposed to different growth irradiances adjusts internal allocation to acquire carbon and nitrogen resources in approximately constant proportions when exposed to changes in the external N supply. Carbon and nitrogen were assimilated proportionately throughout the N saturation period for both light treatments regardless of differences in photosynthetic rates, as shown by the similar C:N ratios. During this period, photosynthesis was capable of supplying all the C required for the synthesis of organic molecules, such that both CO2 and N assimilation were directly coupled to the photosynthetic electron flow. When the external N supply was removed and the algae became dependent on the internally stored N to support growth, C:N ratios were similar for both light treatments even though growth rates differed. This lasted until the final few days of the starvation period, when the algae grown under high light had depleted their internal N reserves completely and thallus N was being diluted as cells continued to divide (McGLathery et al. 1996).

This pattern implies that algal growth rate acts as a feedback regulator to maintain balanced C:N metabolism, except under extreme conditions of high irradiance and low N supply. Such self-regulation may minimize the costs of growth as in terrestrial plants (Bloom et al. 1985, Chapin et al. 1990) and may be especially beneficial to algae living in environments that are characterized by a high degree of temporal and spatial variability in irradiance and nutrient supply.

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