TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2016) 40: 250-257

doi:10.3906/bot-1404-119

Effects of elevated CO_2 and nutrients on the community metabolism of a *Cymodocea nodosa* bed

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Received: 28.04.2014 • **Accepted/Published Online:** 22.06.2015 • **Final Version:** 08.04.2016

Abstract: We assessed the combined effects of elevated CO_2 and nutrients on the metabolism of a benthic community dominated by the seagrass *Cymodocea nodosa* (Ucria) Ascherson in a mesocosm experiment. *C. nodosa* plants and their associated community were exposed to two CO_2 levels simulating future (700 ppm, pH 7.84) and current (360 ppm, pH 8.12) conditions, and two nutrient levels (enriched and ambient concentration) in a total of four treatments (-C-N, -C+N, +C-N, +C+N). Net community production (NCP) was estimated from changes in the concentration of dissolved inorganic carbon in the seawater in light incubations using benthic chambers. The variation pattern of NCP with the ordinance was consistent for all treatments. Although differences among treatments were not statistically significant, average NCP values were lowest under CO_2 enrichment conditions. NCP was lower at a high CO_2 level and ambient nitrogen concentration compared to when nutrient availability was higher, suggesting that the low nutrient availability may modulate the community response to CO_2 enrichment. The results obtained suggest that the stimulation of the net community production of *C. nodosa* by elevated CO_2 concentrations may be curtailed by low nutrient availability.

Key words: CO_2 enrichment, net community production, nutrients, seagrass

1. Introduction

Oceans are currently absorbing approximately 30% of the atmospheric CO_2, leading to a significant rise in the oceanic CO_2 concentration and a resulting drop in seawater pH by 0.3–0.4 units in 2100 (Orr et al., 2005). The annual average CO_2 concentration in 2013 was calculated to be 395.4 ppm (CDIAC, 2014). The absorption of CO_2 by the ocean alters the carbonate speciation of the seawater (Turley, 2005; Henderson, 2006; Pelejero et al., 2010). Such modifications in the seawater chemistry will positively or negatively affect the ecosystem functioning, influencing competitive interactions between organisms, the community composition, and the biogeochemical cycling of key elements such as carbon, nitrogen, and phosphorus (Vézina et al., 2008).

Seagrass-dominated ecosystems are one of the less studied ecosystems with respect to the effects of CO_2 enrichment/ocean acidification. Seagrasses are marine angiosperms that form extensive meadows in shallow coastal bays and lagoons. Seagrass meadows rank among the most productive ecosystems on earth (Duarte and Chiscano, 1999). The ecological importance of seagrass meadows as areas of high primary productivity and sediment stabilization, and as habitat and nursery areas for many marine juvenile finfish, shellfish, and mammals, is well recognized (Heck et al., 2003; Orth et al., 2006). Seagrass communities act as net sinks for CO_2 in the biosphere and tend to be net autotrophic (Duarte et al., 2010). Because seagrass commonly displays carbon-limited photosynthetic rates at the current seawater CO_2 concentration (Thom, 1996; Zimmerman et al., 1997; Invers et al., 2001), increases in oceanic CO_2 may prove beneficial for these important marine plants. In the absence of nutrient limitation, rising oceanic CO_2 is expected to increase seagrass primary productivity and growth. The few available studies on the effects of CO_2 enrichment on seagrass productivity have reported positive photosynthetic and growth responses (Palacios and Zimmerman, 2007; Hall-Spencer et al., 2008; Jiang et al., 2010), confirming this hypothesis. Such acceleration of growth and increase of biomass would require an additional input of nutrients to sustain increased seagrass productivity, which can be met by increasing nutrient uptake rates. As a result of the coupling of C and N during primary production, if a CO_2-induced increase in plant C storage is not accompanied by an increase in plant N, progressive N limitation may occur. The stimulating effects of CO_2 enrichment on growth and productivity of

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seagrass-dominated systems may therefore be curtailed by nutrient limitation, as shown for terrestrial plants (Stitt and Krapp, 1999). To date, no information exists on the interactions between the nitrogen and carbon metabolisms of seagrass-dominated systems under increased CO_2 even though seagrasses control the elemental cycles of many coastal systems. At the plant level, Alexandre et al. (2012) investigated the effects of CO_2 enrichment on both carbon and nitrogen metabolism of the seagrass *Zostera noltii* in a mesocosm experiment. In the present study, we used a similar mesocosm setup. We investigated the effects of both CO_2 and nutrient enrichment on the metabolism of a benthic community dominated by the seagrass *Cymodocea nodosa* in a mesocosm experiment where plants and their associated community were exposed for 1 month to two levels of CO_2, simulating current vs. future seawater CO_2 concentrations, and two levels of nutrients (ambient vs. enriched) that were combined to obtain four treatments. The following hypotheses were tested: 1) the net community production of *C. nodosa* will be stimulated by elevated CO_2 concentrations, 2) the stimulation of net community production (NCP) at future CO_2 concentrations will be curtailed by low nitrogen-availability. This study is one of the first investigating the effects of combined oceanic CO_2 and nutrient enrichment, therefore providing critical information on how seagrass communities will respond to these changing conditions that reflect the global trend for the next decades.

2. Materials and methods

2.1. Plant collection and experimental design

Cymodocea nodosa plants were collected from a subtidal seagrass meadow in March 2011 in the Ria Formosa lagoon, South Portugal (37°00′N, 7°58′W) (Figure 1), using a core of 20 cm in diameter. Cores were carefully collected in order to preserve the community associated with *C. nodosa*. The plant's belowground structures were intact and epiphytes were not removed from the leaves. *C. nodosa* plants and the associated seagrass community were transferred to terracotta pots of similar diameter (17 cm). Each pot contained an average of 10 plant shoots. The pots were transported to an outdoor mesocosm at the Centre of Marine Sciences (CCMAR) field station located near the donor meadow. In the mesocosm, the plants and the associated community were exposed to two levels of CO_2 (current vs. future) and two levels of nutrients (ambient vs. enriched) that were combined to obtain four treatments: current CO_2 and ambient nutrient level (-C-N), current CO_2 and enriched nutrient level (-C+N), future CO_2 and ambient nutrient level (+C-N), and future CO_2 and enriched nutrient level (+C+N).

The mesocosm consisted of two flow-through open systems running in parallel, one with seawater at the current CO_2 concentration (360 ppm) and the other with two-fold the current CO_2 concentration (700 ppm). Each of these systems consisted of one head tank (1500 L) and six independent tanks (200 L), three with ambient nutrient level (2 µM NH_4^+/NO_3^-; 0.5 µM PO_4^-) and three with enriched nutrient level (≈20 µM NH_4^+/NO_3^-; 5 µM PO_4^-). Each of these independent tanks was conserved as a single unit containing 3 replicate pots with *C. nodosa* plants and the associated community. The seawater was pumped from the lagoon, passing through a sand filter, to the head tanks. The experimental CO_2 concentrations (360 and 700 ppm) were achieved through the gradual addition of CO_2 (bubbles) into the head tanks from a CO_2 tank. The rate of CO_2 injection into the system was controlled by the pH level of the seawater using pH probes connected to CO_2 controllers (EXAtx 450, Yokogawa, Tokyo, Japan) (Alexandre et al., 2012). CO_2 and nutrient concentrations

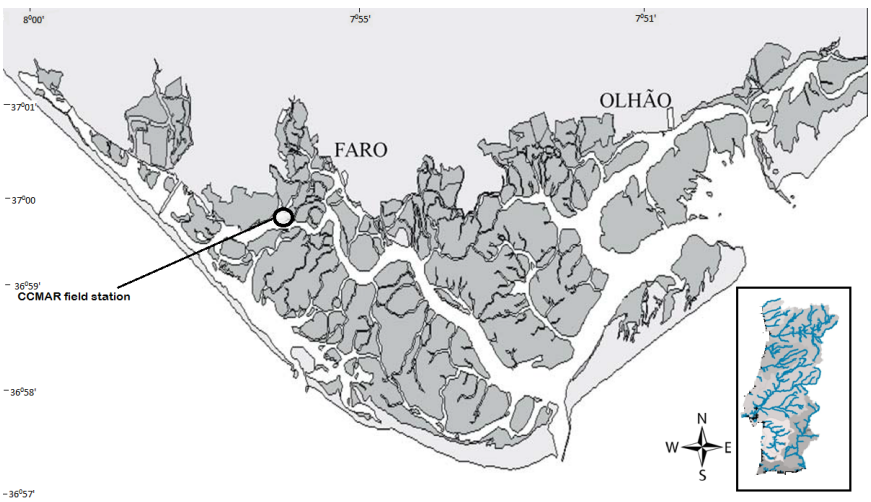

Figure 1. Ria Formosa lagoon with location of the CCMAR field station.

were kept constant at the indicated levels throughout the experiment. The concentration of nutrients in the ambient nutrient level treatments (-N) was adjusted and maintained by continuous addition of stock solutions of NH_4NO_3 (0.125 M) and K_2HPO_4 (0.025 M) using a dosing pump, whereas the concentration of nutrients in the enriched nutrient level treatments (+N) was maintained by adding slow-release fertilizer pellets directly to each replicate tank. Nutrient concentrations were analyzed using a loop-flow analyzer (μMac-1000, Systea, Anagni, Italy). The temperature variation of the seawater in the mesocosm (the difference between the daily maximal and minimum) did not exceed 3 °C and ranged between 17 °C and 20 °C.

2.2. Measurement of net community production of *C. nodosa*

The NCP of *C. nodosa* was assessed from differences in total dissolved inorganic carbon (DIC) in incubations using dome-shaped, UV-transparent Plexiglas chambers (17 cm diameter; 20 cm height) that were fitted to the sediment in the pots. In order to obtain NCP values at a range of underwater irradiances, several incubations were performed throughout the day on different days. Incubations were performed simultaneously for all treatments. Air bubbles that formed inside the chambers during chamber setting were completely removed before incubation. Incubations lasted 2 h. Triplicate water samples were collected at the beginning and at the end of incubations through a sampling port in the chamber. The underwater photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}) was measured every 30 min with an underwater PAR measuring device (LI-COR LI-192 Underwater PAR Sensor, USA). Community respiration was assessed using dark incubation chambers following the procedure described above for light incubations. Dark incubations lasted only 1 h because preliminary experiments showed that changes in DIC concentrations inside the chambers were faster compared to light incubations. The volume of incubated seawater in the chambers was determined using the equation $V = (\pi R^2) \times h + 1.45$ after measuring the height of the incubation chamber once inserted into the sediment, where $\pi = 3.14R$ is the radius of the chamber (cm), h is the height above the sediment (cm), and 1.45 is the volume (L) of the bell-shaped part on top of the chamber. The concentration of total dissolved inorganic carbon (DIC = the sum of CO_2, HCO_3^-, and CO_3^{2-}) of the water samples before and after incubations was calculated from the total alkalinity, pH, temperature, and salinity of the seawater using the Excel-based program CO_2SYS.XLS (Lewis and Wallace, 1998). Total alkalinity (TA) was determined by directly measuring pH with a probe (Multimeter 340, WTW, Germany) in 5 mL of seawater before (pHb) and after (pHa) acidification of the sample with 1 mL of 0.01 M HCl (Semesi et al., 2009). TA was calculated from the pHa according to the 'rapid electrometric determinations of the alkalinity' sensu Anderson and Robinson (1946) as described by Parsons et al. (1984). Salinity was measured using a refractometer, whereas temperature was measured using a combined pH + temperature probe (SenTixHWS, WTW).

The NCP (μmol C m^{-2} h^{-1}) was estimated according to the following equation:

$NCP = (DIC_i - DIC_f) \times V)/t/A$,

where DIC_i and DIC_f are the initial and final dissolved inorganic carbon in the chamber (μM), V is the volume of the chamber (L), t is the length of the incubation (h), and A is the benthic incubated area (m^2). The standard deviation of the estimated NCP was calculated by error propagation. The standard deviation (dz) of net community production (Z value) was calculated using the online calculator package laffers.net, according to the following equation:

Z value ± dz = (X value ± dx) + (Y value ± dy),

where X is the DIC_i, dx is the standard deviation of X (which corresponds to the error of the method), Y is the DIC_f in the chamber, and dy is the standard deviation of Y.

The average NCP was calculated as the sum of the NCP obtained in light incubations divided by the total number of incubations. Differences in the average NCP among treatments were tested with one-way analysis of variance (statistical software Sigma Plot 11.0). Effects of treatments were considered statistically significant at a level of P = 0.05.

3. Results

The NCP of *Cymodocea nodosa* varied with irradiance in all treatments (Figure 2). In general, NCP increased up to 700–1000 μmol m^{-2} s^{-1}, when it reached its maximum. At higher irradiances, NCP decreased due to photoinhibition of the primary producers (*C. nodosa* plants and associated

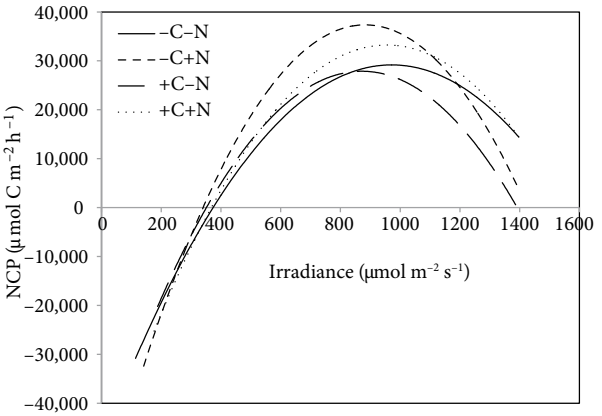

Figure 2. Comparison of the net community production (NCP) of *Cymodocea nodosa* with irradiance among the different treatments. Data were fitted to polynomial quadratic functions.

epiphytes). This decrease was sharper in the nutrient-enriched treatments (-C+N and +C+N). Negative NCP values were observed at very low irradiances (<300 PAR) when community respiration exceeded production.

No significant effects of nutrient enrichment were found on the NCP of *C. nodosa* at the current CO_2 ($P = 0.635$) or elevated CO_2 ($P = 0.691$) (Figure 3). However, at current CO_2, NCP values were consistently higher at lower irradiances in the nutrient-enriched treatment (-C+N), whereas at higher irradiances NCP values decreased more rapidly compared to those of the control treatment (-C-N), indicating that NCP was sensitive to nutrient enrichment (Figure 3A). At elevated CO_2, NCP values reached higher values at the highest irradiances in the nutrient-enriched treatment (+C+N) compared to the treatment with ambient nutrient levels (+C-N) (Figure 3B). Similarly, no significant effects of CO_2 enrichment were detected on NCP, both at low ($P = 0.744$) and high ($P = 0.691$) nutrient availability (Figure 4). At low nutrient availability, however, NCP was higher at elevated CO_2 (+C-N) than at current CO_2 levels (-C-N) up to 1000 μmol m^{-2} s^{-1}, but it decreased more rapidly at higher irradiances, indicating that the photoinhibition effect on the NCP was stronger

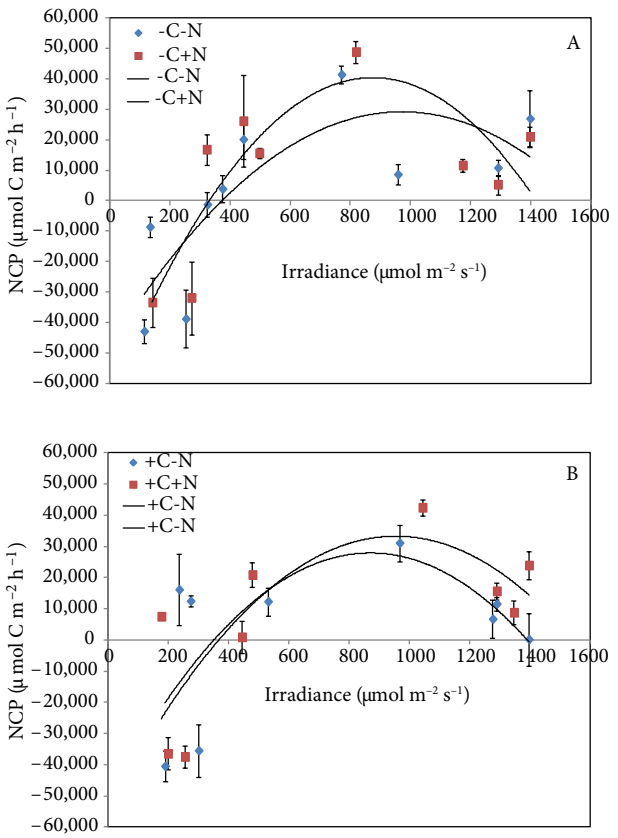

Figure 3. Effect of nutrient enrichment on the net community production (NCP) of *Cymodocea nodosa* at (A) current and (B) elevated CO_2 concentrations.

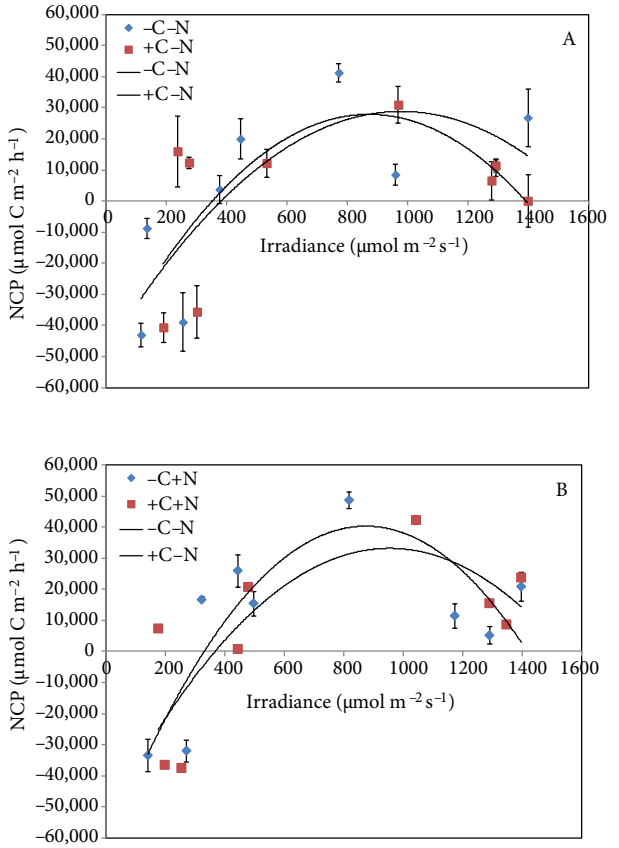

Figure 4. Effect of CO_2 enrichment on the net community production (NCP) of *Cymodocea nodosa* at (A) ambient and (B) elevated nutrient concentrations.

under elevated CO_2 conditions (Figure 4A). In contrast, NCP values at high nutrient availability were higher at low CO_2 conditions (-C+N) up to 1100 μmol m^{-2} s^{-1} (Figure 4B).

The pattern of NCP versus irradiance throughout the day was consistent for all treatments. In general, NCP increased rapidly with irradiance during the morning, reaching a maximum before 1100 hours, and decreased afterwards until around 1330 hours, when irradiances were highest. NCP reached another maximum in the afternoon, between 1700 and 1730 hours, before decreasing again as daylight decreased (Figures 5 and 6). In general, the increase in NCP was sharper during the morning. Nutrient addition increased the NCP values of the afternoon peaks, both at ambient and elevated CO_2 conditions, from 25,000 to 30,000 μmol C m^{-2} h^{-1} and from 20,000 to 35,000 μmol C m^{-2} h^{-1}, respectively. The elevated CO_2 and nutrient addition treatment (+C+N) resulted in higher NCP values in the morning and the afternoon peaks than in any other treatment. NCP was higher in the afternoon than in the morning, except for the +C-N treatment, which reveals a continuity of the community production along the day.

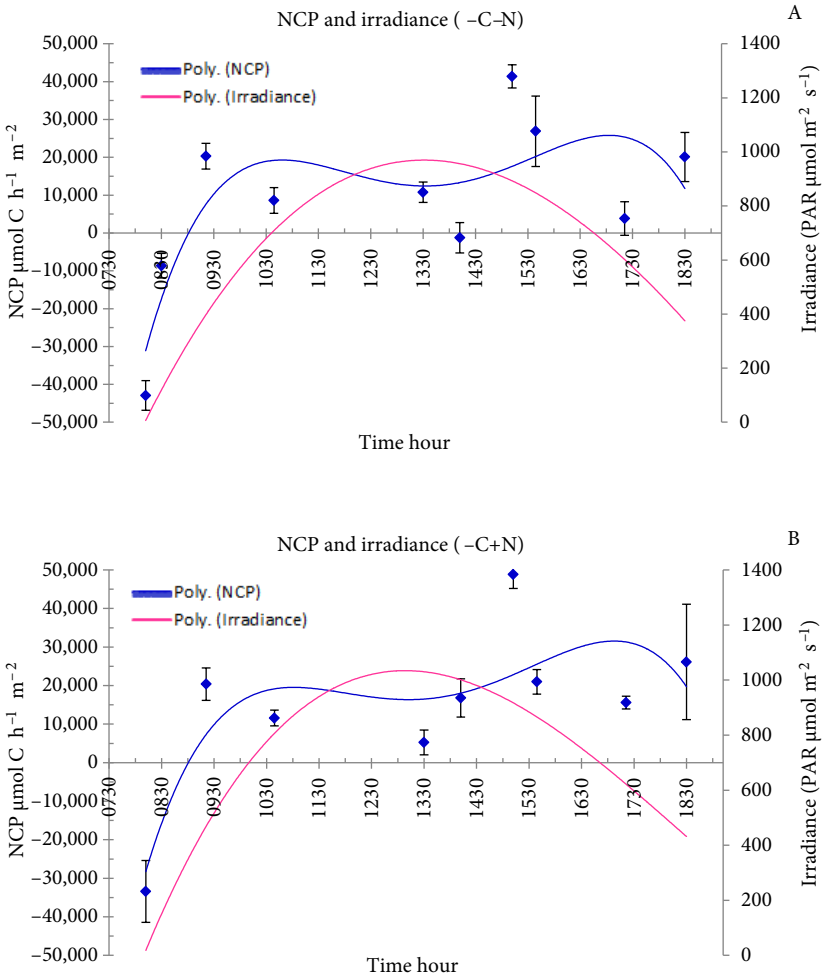

Figure 5. Variation of the net community production (NCP) with irradiance throughout the day at (A) current CO_2 levels and ambient nutrient concentrations and (B) current CO_2 levels and enriched nutrient concentrations.

On average, NCP during the day was higher in the treatment with enriched nitrogen levels at current CO_2, although no significant differences were found among treatments (P = 0.725) (Figure 7).

4. Discussion

The results of this study showed that the NCP of *C. nodosa* responded positively to nutrient enrichment irrespective of the CO_2 level. On the other hand, the net production of the community was lowest under CO_2 enrichment both in the presence of low or high nutrient concentrations. This result contradicts our initial hypothesis that the NCP of *C. nodosa* would be stimulated under high CO_2 concentrations and contrasts with recent observations of significantly higher NCP of *C. nodosa* meadows in CO_2-enriched sites compared to control sites (Apostolaki et al., 2014). Community metabolism of seagrass meadows is also generally reported to be higher than that of unvegetated communities (Stutes et al., 2007; Apostolaki et al., 2010). Average NCP was lowest in the treatment with CO_2 enrichment at ambient nutrient level, suggesting that production of the community may be curtailed by nutrient limitation as hypothesized. Similar observations were reported for terrestrial plants (Stitt and Krapp, 1999). The long-term stimulation of photosynthesis and growth by CO_2 enrichment, which has been reported for seagrass (Palacios and Zimmerman, 2007; Hall-Spencer et al., 2008; Jiang et al., 2010), is expected to depend on the availability of nutrients and the way in which they are utilized by the plants. Plants may use nutrients more efficiently and/or may increase the rate at which nutrients are taken up and assimilated. In our study, when nutrients were supplied at higher concentrations in addition to high CO_2 levels, NCP increased slightly, suggesting that the seagrass community will respond positively to high CO_2 levels if the supply of nutrients is less limited.

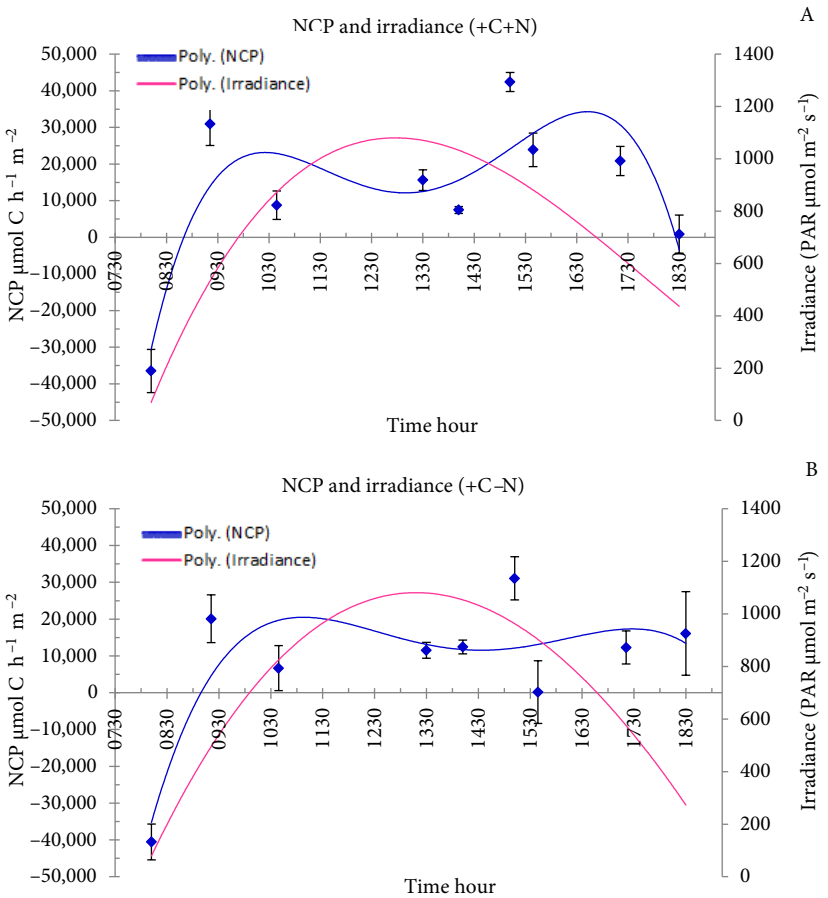

Figure 6. Variation of the net community production (NCP) with irradiance throughout the day at (A) enriched CO_2 levels and ambient nutrient concentrations and (B) enriched CO_2 levels and enriched nutrient concentrations.

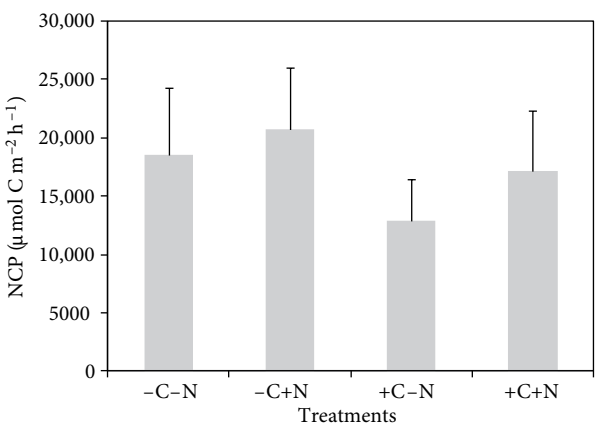

Figure 7. Average net community production (NCP) of *Cymodocea nodosa* during light incubations. Values are mean ± SD (n = 7, except -C-N, where n = 6).

Like terrestrial plants, seagrasses adjust their photosynthetic activity as a function of changes in the photosynthetic active irradiance throughout the day (Ralph et al., 1998). Typically, photosynthesis increases with irradiance up to a threshold irradiance level, after which photoinhibition may occur as a result of an adjustment in the plant's photosynthetic processes to increase again when irradiances become lower (Abal et al., 1994). In all treatments, the net community production of *C. nodosa* peaked twice during the day, in the morning and in the afternoon, showing photoinhibition at irradiances higher than 900 µmol m^{-2} s^{-1}. Excessive irradiances, however, may damage the photosynthetic apparatus, leading to the destruction of the photosynthesizing pigments (Shao et al., 2014). Photoinhibition at high irradiances is a common mechanism of photoprotection of plants (Turan, 2012), which is known to occur in seagrasses (Ralph and Burchett, 1995). In all treatments, NCP increases during the morning were more pronounced than during

the afternoon, just after the photoinhibition process, as shown by the differences in the slopes of NCP versus irradiance obtained from the morning and the afternoon incubations. It is important to note that NCP values may vary seasonally, since the length of the day, irradiance, and temperature, which are factors known to affect metabolic rates (MacLeod and Barton, 1988; Lee et al., 2007; Kim et al., 2012), will all vary as there is variation in hours of light, light intensity, and temperature. Therefore, future studies assessing the net community production and community metabolism of seagrass meadows should include the seasonal variation. Epiphytes on seagrass leaves can reduce the photosynthetic rate by acting both as a barrier to carbon uptake and by reducing light intensity (Sand-Jensen and Borum, 1984; Björk et al., 2008). In addition, daily seawater temperature in the mesocosm did not vary much (from a minimum of 17 °C to a maximum of 20 °C). Therefore, respiration, which is also affected by temperature, must have been nearly constant throughout the incubations. Future experiments should consider a higher range of temperature so that significant responses of the metabolic processes may eventually be detected. Even though the number of shoots was equal in all pots, the biomass in each pot varied because plant shoots had a different number of leaves, and leaves had different lengths. This might have caused a significant difference in the DIC concentrations inside the chambers during incubations. Consequently, the effect of a higher biomass in some pots on the CO_2 consumption during light incubations and production during dark incubations was probably higher than the effect of the experimental treatments. The lack of replicate incubations and the impact of the epiphytes in the leaves might have accounted for the lack of significant differences among treatments. Because the community in each incubation chamber will always differ, the number of replicate incubations should be increased in future experiments assessing the NCP of seagrasses in order to increase the power to detect significant differences among treatments. The present study generated some important preliminary findings on the metabolism of a benthic community dominated by the seagrass *C. nodosa*; however, it also revealed a number of issues concerning the conceptual experimental design that need improvement in future studies.

Acknowledgments

The authors thank João Silva for his valuable comments on the experimental incubations. FK was supported by the International Master of Science in Marine Biodiversity and Conservation (EMBC) program. AA was funded by a PhD grant from Fundação para a Ciência e a Tecnologia (SFRH/BD/21487/2005), co-funded by POCI 2010 and FSE. We would also like to thank the Editor in Chief and the anonymous reviewers for their valuable comments that significantly helped to improve the quality of the manuscript.

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