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Tolerance responses to simulated herbivory in the seagrass *Cymodocea nodosa*

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ABSTRACT: Herbivory causes both direct and indirect damage to plants, with negative consequences for plant performance and fitness. Plants have thus evolved strategies to counteract or mitigate such negative effects. The strategies used by aquatic plants to cope with herbivore pressure are of key importance to better understand ecological and evolutionary processes. However, little is known about such strategies. To help fill this gap, and to better understand induced responses to herbivory in aquatic plants, we simulated grazing at various intensities in the seagrass *Cymodocea nodosa* for ca. 4 mo, and measured plant responses in terms of shoot density, aboveground biomass, leaf growth, total nitrogen and carbon content in tissues, total non-structural carbohydrates in rhizomes and total phenolic content in leaves. Most of these plant attributes showed changes under both low and high simulated herbivory at the end of the experiment, indicating that *C. nodosa* is able to change a suite of plant traits to compensate for biomass losses. At least 3 tolerance strategies were involved in this process: growth compensation and overcompensation, increased nitrogen content (either from uptake or through reclamation from rhizome pools) and remobilization of carbohydrates stored in the rhizomes. Phenolic content decreased in the low-intensity treatment but was similar to control plants in the high-intensity herbivore treatment, indicating the role of phenolic compounds in the tolerance response.

KEY WORDS: Plant–herbivore interactions · Induced responses · Tolerance · Compensatory growth · Phenolic compounds · Mediterranean Sea

INTRODUCTION

Herbivory is probably one of the most pervasive and influential interactions in the biosphere. Beyond its role in trophic fluxes, herbivory has profound effects on vegetation structure, composition and productivity, and has probably been a strong evolutionary driver since the dawn of life (McNaughton 2001). Herbivory seriously affects plant performance and fitness in different ways (e.g. by reducing photosynthetic surfaces, injuring parts of key importance such as meristems, removing flowers or seeds), to the point that it is probably among the main forces shaping both plant and herbivore evolution and co-evolution (Rausher 2001). The long evolutionary history of plant–herbivore interactions is reflected in the large panoply of adaptive mechanisms and strategies displayed by plants to avoid consumption by herbivores or to mitigate its consequences (e.g. Karban & Myers 1989, Karban & Baldwin 1997, Bingham & Agrawal 2010).

Such mechanisms fall into 2 broad categories, constitutive (a constant trait) and inducible (a trait expressed in the presence of herbivores; Karban et al. 1997). In turn, they are based on 2 defense strategies: those reducing the probability or severity of herbivore attack (resistance strategy), and those allowing plants to withstand grazing (tolerance strategy; Agrawal 2000). Resistance-induced responses are generally based on changes in the properties of plant

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tissues, making them less palatable and/or attractive to herbivores, or reducing their performance. This is often achieved through the production of secondary metabolites that act as repellents, toxins or agents that reduce plant digestibility (Lattanzio et al. 2006, Wu & Baldwin 2010), although changes in tissue toughness or in other mechanical properties are also common (Lucas et al. 2000). Tolerance responses attenuate the negative effects of herbivores, by minimizing the loss in plant fitness after herbivore attack, and their nature varies with plant type, developmental status and the part of the plant damaged. A suite of tolerance responses following natural or simulated hervibory has been described, including compensatory growth, increased photosynthetic rate, increased branching, changes in nutrient allocation pattern and increased capacity to shunt carbon reserves from belowground organs to shoots after damage (Strauss & Agrawal 1999, Tiffin 2000). Indeed, several studies have shown that in many plants, primary production can be maintained (compensatory growth) or stimulated (overcompensatory growth) in response to grazing (Gadd et al. 2001), illustrating some of the potential positive effects of herbivory on grazed plants (Agrawal 2000, Ruiz et al. 2008). Both tolerance and resistance strategies entail costs and benefits. Different and at times controversial hypotheses have been proposed about their relationship (Restif & Koella 2004). Apparently, tolerance and resistance are not mutually exclusive and may coexist in plant populations, although trade-offs between them may appear (Mauricio et al. 1997, Leimu & Koricheva 2006).

Herbivory is considered to be stronger in aquatic systems than in terrestrial ones (Cyr & Pace 1993). Although less studied than in their terrestrial counterparts, the mechanisms of defense against herbivores are also widespread among aquatic plants (e.g. Toth & Pavia 2007, Miler & Straile 2010, Morrison & Hay 2011). The presence of secondary metabolites deterring grazing in tissues of aquatic macrophytes seems to be an important strategy to protect against consumers that is found in producers from different taxonomic groups (i.e. micro- and macroalgae, angiosperms) and environments (McClintock & Baker 2001, Pohnert 2004, Prusak et al. 2005). The tolerance strategy, in contrast, seems to be less common, and this may be because it rarely occurs among algae (but see, for instance, Wai & Williams 2005). In macroalgae, the lack of a complex morphological and functional organization such as that of higher plants may prevent the existence of tolerance responses. In contrast, angiosperms and, specifically, marine angiosperms (i.e. seagrasses) possess the same functional traits (basal meristems, clonal integration, storage organs; Marbà et al. 2006) that favor tolerance in terrestrial plants. Indeed, compensatory growth has been demonstrated in seagrasses as a response to defoliation (Tomasko & Dawes 1989, Valentine et al. 1997, Moran & Bjorndal 2005, Vergés et al. 2008).

Seagrasses are considered to be among the most important components of marine submersed vegetation for the goods they produce and the services they provide (Barbier et al. 2011). Their extensive meadows constitute a key habitat in the littoral system, and are relevant to the global carbon cycle. Recent evidence has proved that grazing in seagrasses is by far more important than previously thought (Heck & Valentine 2006, Valentine & Duffy 2006), affecting their population dynamics, composition, distribution and production (Valentine & Heck 1999, Tomas et al. 2004, Moran & Bjorndal 2005). For these reasons, seagrasses are excellent model species to explore mechanisms of defense against grazing. However, the responses of seagrasses to the high herbivory pressure they may suffer have, to date, scarcely been explored. On the one hand, it is known that seagrasses produce secondary metabolites, such as phenolic compounds (Steele et al. 2005, Grignon-Dubois et al. 2012, Ragupathi Raja Kannan et al. 2012), and there is evidence that some of these metabolites, such as condensed tannins, are induced following simulated herbivory (Arnold et al. 2008). However, the precise resistance-induced metabolites produced in response to herbivore attack remain in general poorly known, and it seems that the total phenolic content is not a good predictor of induced defense (Vergés et al. 2007, Steele & Valentine 2012, Sieg & Kubanek 2013). On the other hand, there is evidence that seagrasses can tolerate grazing by means of both intensifying recruitment of new shoots (Valentine et al. 1997) and compensatory growth of existing shoots (Tomasko & Dawes 1989, Moran & Bjorndal 2005). This compensatory growth could be achieved in part by the use of carbon reserves stored in the belowground organs (Eklöf et al. 2008), and supported by increased nitrogen (N) metabolism (N resorption or uptake; Valentine et al. 2004, Alcoverro & Mariani 2005). These studies have provided insights into the defense strategies against herbivores in marine plants. However, most were conducted in tropical species, especially *Thalassia testudinum*, thus narrowing the generality of the findings. Despite recent studies (Vergés et al. 2008, Burnell et al. 2013) demonstrating the existence of

compensatory growth in the temperate genus *Posidonia*, our knowledge of seagrass–herbivore interactions, which have both ecological and evolutionary importance, remains poor.

The aim of the present study was thus to assess phenotypic changes in the seagrass *Cymodocea nodosa* caused by simulated macroherbivore attacks to detect possible tolerance responses. *C. nodosa* is a small, fast-growing species with a wide ecological range and high phenotypic plasticity (Pérez & Romero 1994, Marbà et al. 1996, Cancemi et al. 2002, Mascaró et al. 2009), which is subjected to relatively high levels of herbivory (Cebrián et al. 1996), and can be temporally overgrazed in some coastal lagoons (Fernandez et al. 2012). In this study, we attempt to expand the knowledge of tolerance responses of seagrasses to herbivory, and assess their generality or specificity. Our approach was based on a field experiment consisting of repeatedly clipping the seagrass leaves and measuring subsequent plant responses in terms of changes in density, biomass, leaf growth, carbon and nitrogen content in tissues and total non-structural carbohydrates (TNC). In addition, we measured the total phenolic content in leaves to explore whether or not they participate in the tolerance response rather than in defense mechanisms, as suggested by Vergés et al. (2008).

MATERIALS AND METHODS

Study site

The study was carried out in the southern bay (Alfacs Bay) of the Ebro river delta (northeastern coast of Spain; 40° 35′ N, 0° 41′ E), where extensive shallow meadows of *Cymodocea nodosa* develop in the sandy platforms (<1.5 m depth) surrounding the bay (Pérez & Romero 1994). The study site selected was on the southern shore, where meadows have a good ecological status and are away from the influence of the freshwater entering the bay on its northern shore (Oliva et al. 2012). At this site, shoots show fast turnover (average shoot life span: 2–4 yr) and reach a maximum density (around 2500–3000 shoots m^{-2}) in May–June (Mascaró et al. 2014). Herbivory is supposed to be low within the bay (Cebrián et al. 1996), although scattered populations of sea urchins (*Paracentrotus lividus*) have been detected (authors' pers. obs.). The experimental site was chosen to be at a distance from these populations (>500 m), so as to ascertain low natural herbivory pressure throughout the experiment. This made it unnecessary to deploy cages to protect plots against grazing.

Experimental design

We simulated low and high grazing pressure by macroherbivores by repeatedly clipping the leaves of *C. nodosa* during spring–summer 2010. After 4 mo, we sampled plants to measure several plant response variables (see next section). This period was chosen because it is the period of maximum activity of both plants and macroherbivores in the NW Mediterranean (Prado et al. 2007, Mascaró et al. 2014). The leaf clipping procedure is aimed at mimicking the feeding behavior of the 2 main macroherbivores in the NW Mediterranean (the sea urchin *P. lividus* and the sparid fish *Sarpa salpa*), as both feed on leaf tips, thus removing the distant part of the leaf blades (Prado et al. 2007). The same approach (i.e. simulating herbivory in stands with low natural levels of grazing) has been used previously and results considered representative of the potential response of the species when grazed (Vergés et al. 2008).

We established 9 plots of 1 m^2 in a *C. nodosa* meadow at 1 m depth, spaced at least 2 m apart. Three treatments, i.e. control, low herbivory (LH) and high herbivory (HH), were randomly assigned to each plot. In the HH treatment plots, the leaf canopy was cut to 10 cm height above sediment level, which corresponds to removal of about 75% of leaf biomass. In the LH treatment plots, only the leaf tips were cut, corresponding to a leaf biomass removal of less than 5%. In the control plots, the leaf canopy was left unmodified, thus remaining at its normal height (ca. 40 cm above sediment level).

The experiment was run from April to late July 2010. During this period, leaves within each plot were clipped periodically. Maintenance (clipping) visits were made every 2 wk, except during the maximum growth period (June and July) when clipping was performed weekly, resulting in a total of 10 clipping events between the start of the experiment and the July sampling. All clipped blades were removed from the plots to avoid any artifact derived from detritus accumulation. At the end of the experimental period, a series of response variables (see next section) were measured. Additional samples were taken for analysis of TNC and phenolic content in October 2010. These samples coincided with the seasonal maximum carbohydrate content (Mascaró et al. 2014). Between July and October, and to maintain the experimental conditions, further clipping visits were made every 2 wk.

Measurement of plant response variables

Biomass and shoot density

At the end of the period of maximum growth (end of July), samples of *C. nodosa* were collected from the central part of each plot using a 16 cm diameter corer. Each sample was thoroughly rinsed *in situ* with seawater until sediment had been completely removed, and stored in plastic bags that were refrigerated for transport. In the laboratory, all shoots were counted, and the leaves were separated from rhizomes and roots, dried at 70°C for 48 h (until constant weight) and weighed, thus obtaining shoot density (shoots m^{-2}) and leaf biomass (g DW m^{-2}, where DW = dry weight), with n = 3 per treatment. Subsamples of each fraction were kept for biochemical analysis.

Leaf growth and leaf number per shoot

Leaf growth was measured using a modified Zieman method (Pérez & Romero 1994). On 20 July 2010, 15 shoots were marked in each plot by punching a hole just above the ligule of the outermost leaf using a hypodermic needle. All marked shoots were collected 9 d later. In the laboratory, the leaves from each one of these shoots were separated, the number recorded, and each leaf divided into 'new' and 'old' tissue (i.e. tissue formed during or before the marking, respectively), dried at 70°C for 48 h (until constant weight) and weighed. Leaf growth was expressed in mg DW $shoot^{-1}$ d^{-1}, and relative growth rate (RGR; d^{-1}) was calculated as shoot leaf growth divided per shoot biomass. Both variables thus had 15 subsamples per plot and 3 replicates per treatment.

Tissue biochemical analysis

Dried leaves, rhizomes and roots from the core samples were ground to a fine powder. The carbon and nitrogen content in all tissues was measured in subsamples using a Carlo-Erba elemental autoanalyzer (Scientific and Technical Services of the University of Barcelona). TNC (sucrose plus starch) content was measured in rhizomes, using a modified method from Alcoverro et al. (1999). Ground samples were dissolved in 96% (v/v) ethanol, sonicated for 5 min and heated at 80°C for 15 min to extract soluble carbohydrates. This process was repeated 3 times. Starch was extracted from the remaining ethanol-insoluble pellet by dissolving it in 0.1 N NaOH at room temperature overnight. Sucrose content was determined using a resorcinol assay standardized to sucrose, and starch content was analyzed by spectrophotometry using an anthrone assay with sucrose as a standard. TNC content was the sum of the 2 fractions.

The total phenolic content of leaves was analyzed using a modified Folin-Ciocalteau method (Bolser et al. 1998). Each sample was extracted in 1 ml 50% methanol at 4°C for 24 h. Then, 0.1 ml of the supernatant was added to 7.9 ml distilled water, mixed and combined with 0.5 ml Folin-Ciocalteau reagent. After 2 min, 1.5 ml $NaCO_3$ solution was added to the sample. Two hours were allowed for color development, and absorbance was spectrophotometrically measured at 765 nm and compared with that of a standard curve for gallic acid. Although samples for phenolic analysis were taken in both July and October, the samples from July were lost due to technical problems in the analytical procedure.

Statistical analysis

For the variables shoot density and leaf biomass, the significance of differences among treatments (3 levels: control, LH and HH) was assessed using 1-way ANOVA. For the remaining variables, differences between treatments were analyzed using a 2-way nested univariate ANOVA, considering treatment as a fixed effect and plot (3 levels) as a random effect nested within treatment. To test for between-treatment differences for each variable, we used Tukey's HSD post hoc tests.

All variables were individually checked for normality, homogeneity of variance and outliers using first exploratory data analysis procedures (e.g. QQ plots), and parametric tests (Lilliefors and Shapiro-Wilks tests for normality, Cochran test for homoscedasticity) for assessing whether or not the ANOVA assumptions were met. No outliers were removed. Where necessary, data were transformed to achieve normality, as indicated in the 'Results'.

RESULTS

Most of the plant traits investigated responded to the simulated herbivory (Fig. 1). Shoot density increased significantly with clipping intensity (Table 1, Fig. 1a), and was, at the end of the experimental period, >50% higher in the HH treatment plots than

in the control plots, while LH treatment plots displayed intermediate values. Leaf growth was significantly higher in LH plots (ca. 20% higher) than in control and HH plots (Table 2, Fig. 1c), while the relative growth rate increased significantly in both the LH and HH treatments (Table 2, Fig. 1d). The average number of leaves per shoot increased slightly but significantly in the HH treatment (Table 2, Fig. 1e), relative to the other 2 treatments. In addition, it should be noted that no bite marks made by herbivores were observed in the sampled leaves, thus confirming the low levels of herbivory at the experimental site, and the absence of interferences between natural and simulated herbivory.

In terms of biochemical traits (Figs. 2–5), the HH treatment caused an overall reduction (relative to the control) in nitrogen content, significantly affecting leaves, rhizomes and roots (Table 2, Fig. 2a–c). In contrast, nitrogen content was higher in the leaves of plants from plots subjected to LH, relative to control plots (Table 2, Fig. 2a). Carbon content in leaves and rhizomes tended to be lower in the HH treatment than in the other treatments (Table 2, Fig. 2d,e). Overall, these changes resulted in increased C:N ratios in all 3 organs in the HH treatment (Table 2, Fig. 3a–c). TNC content in rhizomes measured in July in the HH treatment was 50% lower than in the control (Table 2, Fig. 4a). The TNC content increased more than 2-fold from July to October, when the differences among treatments disappeared (Table 2, Fig. 4b). The responses of sucrose and starch were similar. The total phenolic concentration in leaves collected in October was significantly lower (40%) in the LH treatment, relative to both the control and HH treatments (Table 2, Fig. 5).

Table 1. One-way ANOVA assessing differences in density and leaf biomass of *Cymodocea nodosa* between treatments. **Bold** indicates significant ($p < 0.05$) differences

Source	df	MS	*F*	p
Shoot density				
Treatment	2	2451340	6.111	**0.035**
Error	6	401083		
Leaf biomass				
Treatment	2	9519.5	3.020	0.123
Error	6	3151.4		

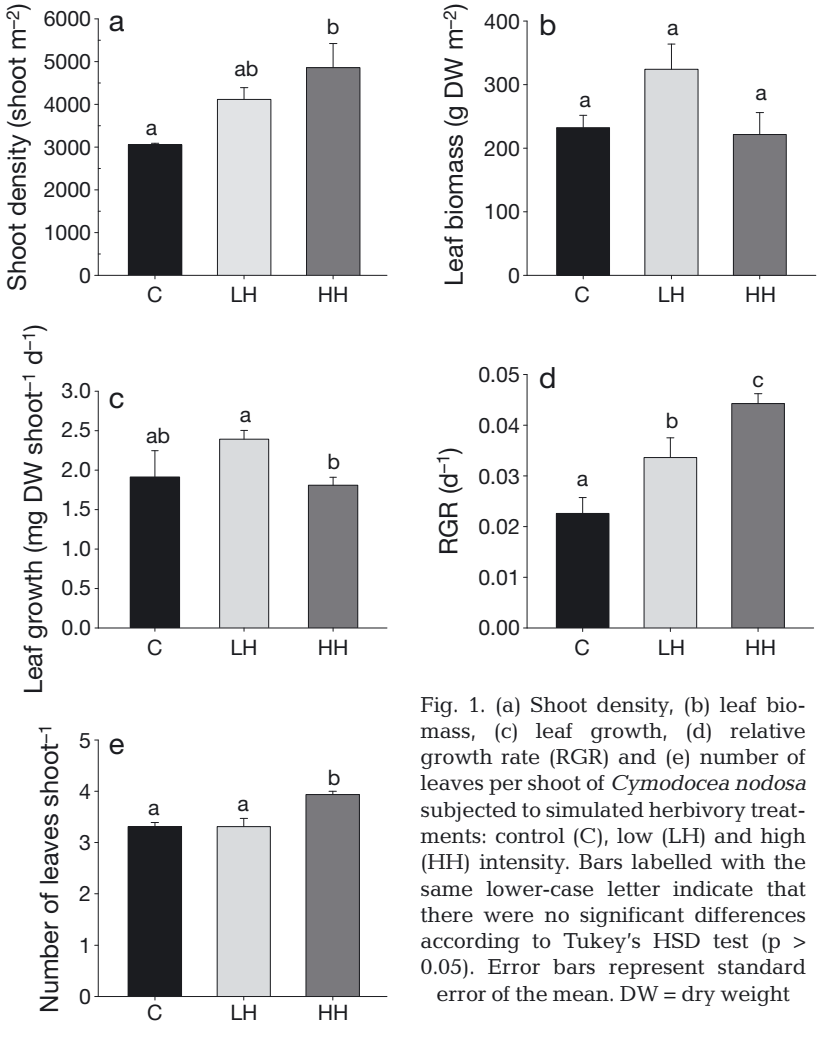

Fig. 1. (a) Shoot density, (b) leaf biomass, (c) leaf growth, (d) relative growth rate (RGR) and (e) number of leaves per shoot of *Cymodocea nodosa* subjected to simulated herbivory treatments: control (C), low (LH) and high (HH) intensity. Bars labelled with the same lower-case letter indicate that there were no significant differences according to Tukey's HSD test ($p > 0.05$). Error bars represent standard error of the mean. DW = dry weight

DISCUSSION

This study demonstrates that simulated herbivory causes several responses in the seagrass *Cymodocea nodosa*. While some of these responses seem a simple and direct consequence of defoliation, others appear to attenuate the detrimental effects of consumer damage and are thus suggestive of adaptive tolerance responses. After a 4 mo defoliation period, a suite of plant trait changes, including changes in leaf growth, shoot recruitment, nutrient content and carbohydrate content, were observed, all of them suggestive of nutrient reallocation and mobilization of carbon reserves that either compensated or overcompensated for biomass losses.

Table 2. Two-way nested ANOVA assessing differences between treatments and plots on different traits of *Cymodocea nodosa*. **Bold** indicates significant ($p < 0.05$) differences. TNC = total non-structural carbohydrates

Source	df	MS	*F*	p
Leaf growth[a]				
Treatment	2	0.077	4.838	**0.009**
Plot		0.037	2.347	**0.035**
Error	126	0.016		
Leaf relative growth rate				
Treatment	2	0.002	32.858	**<0.001**
Plot	6	0.000	2.508	**0.025**
Error	126	0.000		
Number of leaves per shoot				
Treatment	2	5.807	10.595	**<0.001**
Plot	6	0.548	1.780	0.108
Error	126	0.308		
Nitrogen content in leaves				
Treatment	2	0.513	119.72	**<0.001**
Plot	6	0.016	3.94	**0.010**
Error	18	0.004		
Nitrogen content in rhizomes				
Treatment	2	0.226	104.42	**<0.001**
Plot	6	0.040	18.69	**<0.001**
Error	18	0.002		
Nitrogen content in roots				
Treatment	2	0.048	6.745	**0.006**
Plot	6	0.032	4.475	**0.006**
Error	18	0.007		
Carbon content in leaves				
Treatment	2	12.78	100.1	**<0.001**
Plot	6	0.94	7.3	**<0.001**
Error	18	0.13		
Carbon content in rhizomes				
Treatment	2	12.42	27.51	**<0.001**
Plot	6	1.16	2.58	0.055
Error	18	0.45		
Carbon content in roots				
Treatment	2	4.12	1.88	0.180
Plot	6	4.17	1.9	0.135
Error	18	2.19		
C:N ratio (leaves)				
Treatment	2	14.349	133.56	**<0.001**
Plot	6	0.493	4.59	**0.005**
Error	18	0.107		
C:N ratio (rhizomes)				
Treatment	2	209.70	50.582	**<0.001**
Plot	6	42.38	10.223	**<0.001**
Error	18	4.15		
C:N ratio (roots)				
Treatment	2	0.048	6.745	**0.006**
Plot	6	0.032	4.475	**0.006**
Error	18	0.007		
TNC content in rhizomes (July)				
Treatment	2	15.721	42.72	**<0.001**
Plot	6	0.734	1.997	0.126
Error	16	0.368		
TNC content in rhizomes (October)[b]				
Treatment	2	1.212	0.599	0.560
Plot	6	4.818	2.379	0.071
Error	16	2.025		
Phenolic content in leaves				
Treatment	2	3.478	65.933	**<0.001**
Plot	6	0.565	10.711	**<0.001**
Error	18	0.052		

[a]Data log $(x + 1)$ transformed to satisfy parametric test assumptions
[b]Data $1/x$ transformed to satisfy parametric test assumptions

Despite the repeated and massive defoliation to which it was submitted in the HH treatment, leaf biomass of *C. nodosa* in HH-treated and control plots at the end of the experimental period were very similar. HH-treated plants compensated for defoliation by the addition of new modules (leaves and shoots), while leaf growth remained similar to that found in control plants. Under the much more benign defoliation performed in LH plots, the response was slightly different. In LH-treated plants, besides the addition of new modules (only shoots), we also found a compensatory leaf growth, that, in the long term, could have led to an overcompensatory biomass response (Belsky 1986). These compensatory mechanisms described above have been reported in the tropical seagrass *Thalassia testudinum* (Valentine et al. 1997, Moran & Bjorndal 2005), and in the temperate species *Posidonia oceanica* (Vergés et al. 2008) and *P. sinuosa* (Burnell et al. 2013). Overcompensation is a common response to damage in terrestrial, freshwater and marine plants (e.g. Oba et al. 2000, Li et al. 2010), and is considered more common in fast- than in slow-growing species (Coley et al. 1985, Haukioja & Koricheva 2000; but see Soti & Volin 2010). This is consistent with the characterization of *C. nodosa* as a fast-growing and plastic species (Pérez et al. 1994, Mascaró et al. 2009). However, the slow-growing *P. oceanica* has also shown overcompensation for leaf growth (Vergés et al. 2008) but not for shoot recruitment. In this respect, it should be noted that the observed variability in growth compensatory responses of plants is often attributed to extrinsic factors such as nutrients (Li et al. 2010), light availability and damage frequency and intensity (Eklöf et al. 2008).

Our observations stress the importance of nutrients in plant–herbivore interactions. In effect, the observed compensatory growth of *C. nodosa* seems, to some extent, to be related to changes in nutrient content. Our results show that modest defoliation (LH treatment) caused an increase in N concentration in leaves, possibly accounting for the increase in leaf elongation. This increase is more likely to be due to uptake stimulation than to reserve mobilization, as the N concentration in roots and rhizomes remained unaltered. In contrast, intense defoliation (HH treatment) caused an overall reduction in N content (in leaves, rhizomes and roots). Although N uptake stimulation due to defoliation is a common response elsewhere (Jaramillo & Detling 1988, McNaughton et al. 1996), such stimulation did not occur or was unable to compensate for the N losses in *C. nodosa*. The decrease in N content not only in leaves, but also in rhi-

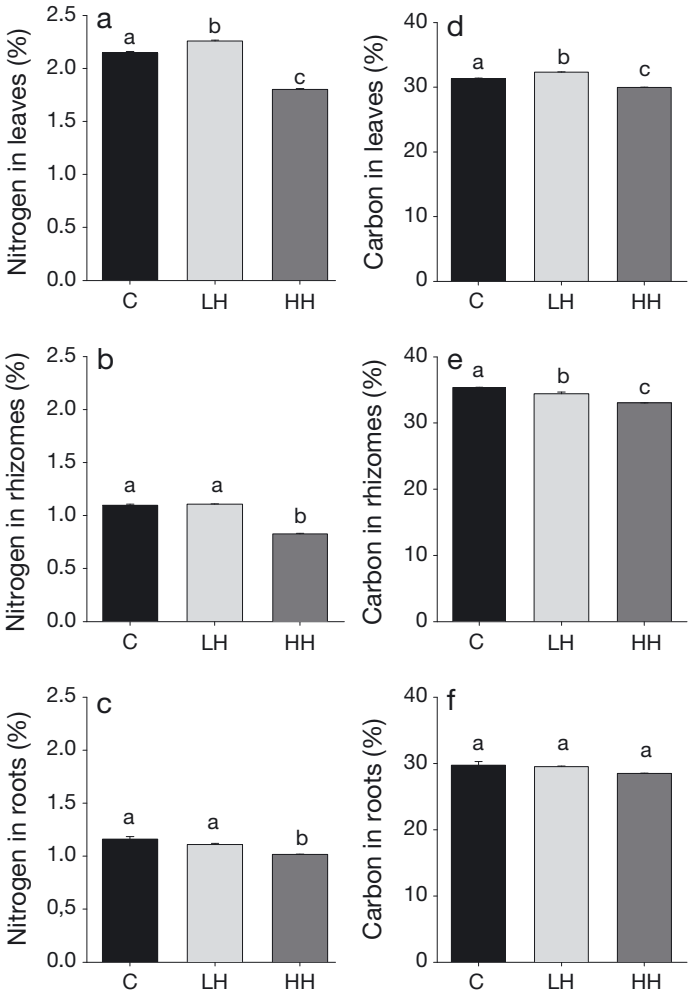

Fig. 2. (a–c) Nitrogen and (d–f) carbon content in leaves, rhizomes and roots of *Cymodocea nodosa* subjected to simulated herbivory treatments: control (C), low (LH) and high (HH) intensity. Bars labelled with the same lower-case letter indicate that there were no significant differences according to Tukey's HSD test ($p > 0.05$). Error bars represent standard error of the mean

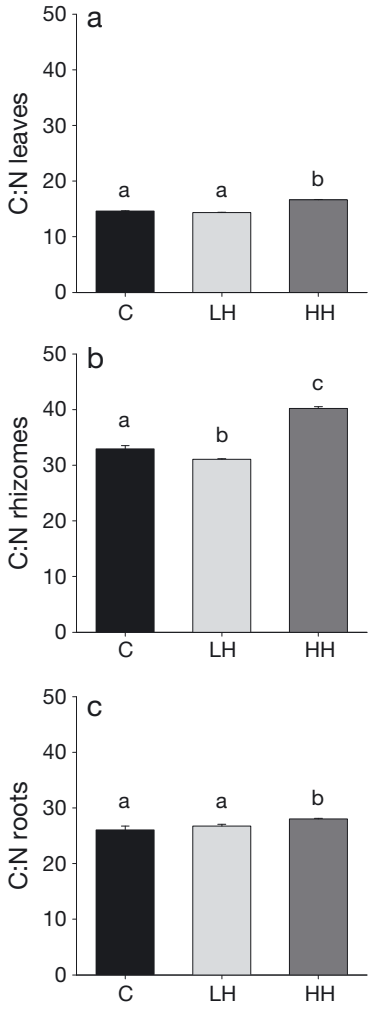

Fig. 3. C:N ratio in (a) leaves, (b) rhizomes and (c) roots of *Cymodocea nodosa* subjected to simulated herbivory treatments: control (C), low (LH) and high (HH) intensity. Bars labelled with the same lower-case letter indicate that there were no significant differences according to Tukey's HSD test ($p > 0.05$). Error bars represent standard error of the mean

zomes and roots, suggests a mobilization of nutrients from the belowground organs to the aboveground parts to maintain leaf growth rates and to support the production of new modules (leaves and shoots), resulting in a dilution into the new biomass of N pools and increasing aboveground primary production (Valentine et al. 1997). Incidentally, this depletion of N may have consequences for the palatability of the tissues, as the intense defoliation caused a decline in the nutritional quality of leaves and rhizomes by increasing C:N ratios. Plant quality (often expressed as C:N ratio) has been shown to play a central role in determining herbivore feeding patterns in marine habitats (Cebrián & Duarte 1998, Barile et al. 2004, Duarte et al. 2011), and some authors have suggested that a low leaf N concentration can act as a plant defense against grazing (Augner 1995). To what extent this reduction in plant nutritional quality is an adaptive response or a mere consequence of nitrogen loss and dilution, as explained above, remains unclear. In any case, it should be noted that nutrient availability may play an important role in determining the capacity of a plant to compensate for tissue loss (Hay et al. 2011), as has been demonstrated, among others, by Li et al. (2010), who reported that individuals of *Vallisneria spiralis* growing in nutrient-rich habitats were better able to compensate for damage than those plants growing in nutrient-limited habitats, where they were unable to acquire the necessary amount of nutrients to replenish biomass loss.

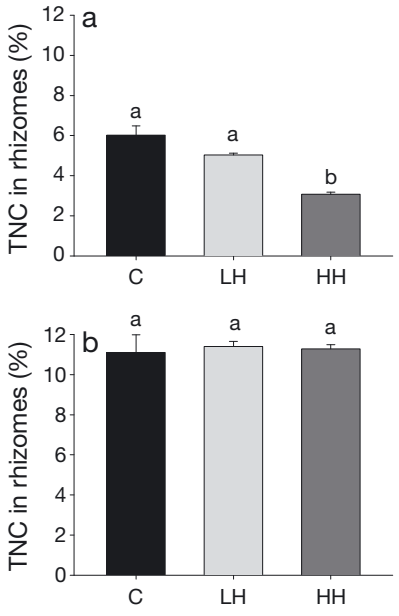

Fig. 4. Total non-structural carbohydrates (TNC) content in rhizomes measured in (a) July and (b) October in *Cymodocea nodosa* subjected to simulated herbivory treatments: control (C), low (LH) and high (HH) intensity. Bars labelled with the same lower-case letter indicate that there were no significant differences according to Tukey's HSD test ($p > 0.05$). Error bars represent standard error of the mean

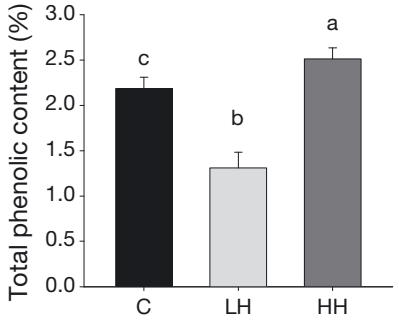

Fig. 5. Total phenolic content in leaves of *Cymodocea nodosa* measured in October subjected to simulated herbivory treatments: control (C), low (LH) and high (HH) intensity. Bars labelled with the same lower-case letter indicate that there were no significant differences according to Tukey's HSD test ($p > 0.05$). Error bars represent standard error of the mean

Nevertheless, the compensatory responses reported are not only facilitated by N mobilization or uptake, but also by the use of carbohydrate reserves. It has been shown that *C. nodosa*, like other seagrasses, has the capacity to store carbohydrates, building up reserves in late summer, and translocating these reserves to support shoot growth from early spring to mid-summer (Mascaró et al. 2014). The depletion of TNC after clipping, by 16% (LH) and 50% (HH) relative to controls, suggests that carbohydrate mobilization took place in response to defoliation, and part of the compensatory leaf growth (LH treatment) and the addition of new modules (LH, only shoots; and HH, leaves and shoots) was supported by these reserves. Indeed, mobilization of carbohydrate reserves appears to play a major role in the ability of plants to withstand disturbances involving the loss of aboveground tissue (Rodgers et al. 1995, Brun et al. 2003, Eklöf et al. 2008, Ruiz et al. 2009). However, the magnitude of the contribution of carbohydrates to regrowth may depend on both storage capacity and physiological integration of the plant. It should be emphasized that, despite the significant depletion of TNC in July following defoliation (especially in the HH treatment), TNC recovered, and the values in October, which were much higher than in July (ca. 2-fold, in agreement with the seasonality of the plant; Mascaró et al. 2014), were very similar among treatments.

While our results clearly indicate the ability of *C. nodosa* to develop diverse induced tolerance responses against both low and high simulated herbivory, the results for total phenolic content, which were either lower than (in the LH treatment) or equal to (in the HH treatment) control values, corroborated previous findings that this variable is not a good indicator of defense mechanisms (Sieg & Kubanek 2013). In this respect, it should be acknowledged that total phenolic content is uninformative about the deterrent capacity of a given tissue, as phenolic compounds participate in a huge number of plant functions besides deterrence (e.g. antioxidant: Hodzic et al. 2009; antimicrobial: Vergeer & Develi 1997; antifungal: Jensen et al. 1998). However, the fact that plants from the LH treatments had a 40% lower total phenolic content than controls, whereas plants from the HH treatment had similar values to controls, suggests a negative relationship between phenolic content and leaf growth. This underlines the role of phenolic compounds as primary metabolites, particularly in cell wall construction during plant growth (Abdulrazzak et al. 2006). Part of the compensatory leaf growth found in plants from the LH plots could thus have been achieved using carbon from the phenolic pool, as suggested by Vergés et al. (2008) based on results very similar to ours obtained in *P. oceanica.*

In conclusion, under low levels of defoliation, leaf losses seem to act as a stimulating cue, triggering overcompensatory responses, apparently using internal carbon sources, to which re-use of phenolic compounds seems to contribute, and external N sources. In contrast, under high levels of defoliation, leaf elon-

gation rates are maintained, while the number of leaves and shoots increases; this compensatory response seems to be supported, at least in part, by internal carbon sources (i.e. carbohydrates supplied by the rhizome reserves) and internal N sources (i.e. N remobilization from belowground organs). At the same time, the C:N ratio increases, potentially lowering the nutritional quality of leaves. All these mechanisms contribute to the tolerance of *C. nodosa* to grazing, reducing the negative effects of herbivore consumption on plant fitness.

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