

Effects of nutrient enrichment on seagrass population dynamics: evidence and synthesis from the biomass–density relationships

Susana Cabaço¹, Eugenia T. Apostolaki², Patricia García-Marín³, Renee Gruber⁴, Ignacio Hernández³, Begoña Martínez-Crego¹, Oriol Mascaró⁵, Marta Pérez⁵, Anchana Prathep⁶, Cliff Robinson⁷, Javier Romero⁵, Allison L. Schmidt⁸, Fred T. Short⁹, Brigitta I. van Tussenbroek¹⁰ and Rui Santos^{1*}

¹Marine Plant Ecology Research Group (ALGAE) Centre of Marine Sciences (CCMAR), University of Algarve, Campus of Gambelas, 8005-139 Faro, Portugal; ²Institute of Oceanography, Hellenic Centre for Marine Research, PO Box 2214, 71003 Heraklion, Crete, Greece; ³Division of Ecology, Department of Biology, Faculty of Marine and Environmental Sciences, University of Cadiz, 11510 Puerto Real (Cadiz), Spain; ⁴New South Wales Office of Environment and Heritage, Scientific Services Division, Coastal Catchments Unit, 59-61 Goulburn Street, Sydney, NSW 2000, Australia; ⁵Departamento de Ecología, Facultad de Biología, Universidad de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain; ⁶Seaweed and Seagrass Research Unit, Center of Excellence for Biodiversity of Peninsular Thailand, Department of Biology, Faculty of Science, Prince of Songkla University, HatYai, Songkhla, Thailand; ⁷Marine Protected Areas Research Group, Department of Geography, University of Victoria, Victoria, BC, V8W 3P5 Canada; ⁸Dalhousie University, Department of Biology, 1355 Oxford Street, Halifax, NS B3H 4R2, Canada; ⁹Aquatic Resources Division, Washington State Department of Natural Resources, Olympia, WA 98504, USA; and ¹⁰Unidad Académica de Sistemas Arrecifales/Puerto Morelos Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Apdo. Postal 1152, Cancún, Quintana Roo, México

Summary

1. The available data from experimental and descriptive studies on seagrass biomass and density responses to nutrient enrichment were analysed to assess the intraspecific mechanisms operating within seagrass populations and whether biomass–density relationships can provide relevant metrics for monitoring seagrasses.
2. The response of shoot biomass and density to nutrient enrichment was dependent on the type of study; the short-term positive response of biomass and density in experimental studies reveals context-specific nutrient limitation of seagrasses. The long-term negative response of descriptive studies probably results from ecosystem-scale events related to nutrient enrichment such as increased turbidity, algal blooms, epiphyte loads and anoxia.
3. Most seagrass species analysed lie in the nonthinning part of the theoretical biomass–density curves. A simultaneous increase in biomass and decrease in density, evidence of self-thinning, were only observed in 4 of 28 studies. The analysis of both the static and the dynamic biomass–density relationships revealed that the slopes increase under nutrient enrichment. Surprisingly, the species-specific slopes (log B–log D) were higher than one, revealing that the B/D ratio, that is, the average shoot biomass, increases with density in all seagrass species analysed. Nutrient enrichment further enhanced this effect as biomass–density slopes increased to even higher values. The main drivers behind the increasing biomass–density slopes under nutrient enrichment were the increase in shoot biomass at densities above a species-specific threshold and/or its decrease below that threshold.
4. *Synthesis.* Contrasting short- and long-term responses of both biomass and density of seagrasses to nutrient enrichment suggest that the former, positive ones result from nutrient limitation, whereas the later, negative ones are mediated by whole ecosystem responses. In general, shoot biomass of seagrasses increases with density, and nutrient enrichment enhances this effect. Experimental testing of facilitation processes related to clonal integration in seagrasses needs to be done to reveal whether they determine the low incidence of self-thinning and the intriguing biomass–density relationships of seagrass species. The increasing slopes and decreasing

*Correspondence author. E-mail: rosantos@ualg.pt

intercepts of the species-specific dynamic biomass–density relationships of seagrasses and the decreasing coefficients of variation of both biomass and density constitute relevant, easy-to-collect metrics that may be used in environmental monitoring.

Key-words: biomass–density relationship, facilitation, intraspecific competition, monitoring, nutrient enrichment, plant population and community dynamics, seagrass

Introduction

The relationships between plant biomass and density ultimately reflect the competitive mechanisms operating within populations and how they respond to the environment (Weller 1987). Changing environmental conditions (e.g. nutrient availability) may modify such competitive mechanisms, affecting the biomass–density relationships of plant populations (Morris & Myerscough 1985, 1991; Morris 1995, 1999, 2002, 2003; Steen & Scrosati 2004; Cabaço, Machás & Santos 2007; Chu *et al.* 2010). These relationships may be structured along the same or along different biomass–density lines as the environment varies (Morris 2003). Matching biomass–density lines indicates that the biomass packing does not change with environmental conditions, even though the rate of propagation along the line may vary. Different biomass–density lines resulting from higher availability of resources reflect different competition processes occurring within populations as a consequence of biomass accumulation (Morris 2003). Ultimately, increased intraspecific competition due to increased biomass of individuals in crowded plant populations may result in a density decrease, a process known as self-thinning (Yoda *et al.* 1963; White 1981; Westoby 1984; Weller 1987). In addition to competition, facilitation, that is, the positive effect of plants on the establishment or growth of neighbouring plants (Brooker *et al.* 2008), may also be involved. Chu *et al.* (2008) demonstrated that facilitation could also affect the biomass–density relationships, playing an important role in plant–plant interactions and in the population dynamics outcome. Intraspecific facilitation is common in clonal plants such as seagrasses, where clonal integration results in particularly active spatial and temporal dynamics involving the continuous recruitment and mortality of shoots within the same individual (Duarte *et al.* 2006).

Nutrients affect both the structure and dynamics of the populations of seagrasses mainly through changes in plant architecture, morphology and mortality (Short 1983; Romero *et al.* 2006; Fertig, Kennish & Sakowicz 2013) and therefore may have an effect on the biomass–density relationships. The meta-analysis of Hughes *et al.* (2004) revealed that experimental additions of inorganic nutrients to sediments generally stimulate seagrass growth, suggesting nutrient limitation of plant production. However, the excessive growth of epiphytes, macroalgae and phytoplankton under high nutrient loads decreases seagrass growth and survival (Lee, Park & Kim 2007; Schmidt *et al.* 2012). Excessively high nutrient regimes also result in built-up of organic matter, which may result in conditions unfavourable to seagrasses, such as sediment anoxia or sulphide toxicity (Koch

2001; Koch *et al.* 2006). Direct nutrient toxicity effects on seagrass growth and survival have also been reported (van Katwijk *et al.* 1997; Brun *et al.* 2002; Burkholder, Tomasko & Touchette 2007). These have been considered the major factors contributing to seagrass decline world-wide (Short & Wyllie-Echeverria 1996; Ralph *et al.* 2006; Waycott *et al.* 2009).

To date, self-thinning processes have not been explicitly reported for seagrasses in established, natural populations.

Here, we analyse how the biomass–density relationship of seagrass meadows responds to nutrient enrichment. As changes to the biomass–density relationship are determined by the growth and survival responses of individual plants, this relationship may reveal the competitive and resource allocation mechanisms operating within seagrass meadows under increasing nutrient loads. The only report on this subject is Cabaço, Machás & Santos (2007), who observed that the slope of the biomass–density relationship of *Zostera noltii* increased along a gradient of anthropogenic nutrient enrichment and that this was mainly driven by biomass changes. Our analysis is based on a world-wide data set of biomass and density responses of seagrass species both under small-scale, controlled nutrient additions (experimental studies) and under large-scale contrasting nutrient levels in natural settings (descriptive studies), obtained from published and unpublished data sources. We analyse the effects of nutrient enrichment on biomass and density separately, as well as on the biomass–density relationships. We also investigate whether the life strategy of different species influences the population's response to nutrients. Size and growth of seagrasses are linked to their life-history strategy as small-size species tend to have high growth rates and large-size species tend to have low growth rates (Duarte *et al.* 2006). In order to test this, the species responses were scaled to their specific shoot weight, rhizome diameter, leaf length, and both horizontal and vertical rhizome elongation rates.

The biomass and density of seagrass populations are easily measurable and, in fact, have been widely used both in regional-scale monitoring programs (e.g. Mediterranean basin; Lopez y Royo *et al.* 2010) and in global-scale monitoring programs (e.g. SeagrassNet, www.seagrassnet.org). If the biomass–density relationships vary with nutrient loadings, reflecting the outcome of the competitive mechanisms operating within populations, this relationship can constitute a sound metric for coastal monitoring based on seagrass stands. The biomass–density relationships could then be used as early warning indicators of the negative effects of excessive nutrient loadings on coastal ecosystems dominated by seagrasses.

Materials and methods

RESPONSE OF SEAGRASS BIOMASS AND DENSITY TO NUTRIENT ENRICHMENT

Data on the effects of nutrient enrichment on both the above-ground biomass and shoot density (per square metre) of monospecific seagrass meadows were compiled both from the literature and from unpublished data sources (Table 1). Both descriptive studies and field experiments were included in the analysis. The sources of nutrient enrichment in descriptive studies were mainly urban wastewater, aquaculture and agriculture (Table 1). Most of the experimental studies were performed by enriching the sediment with slow-release fertilizers (Table 1). Only the studies reporting both the above-ground biomass and density responses for the same sites and time period were included in the analysis. Mesocosms and laboratory experiments were not considered in this study as they generally involve plant manipulations and we consider that they do not represent natural conditions. As well, these data are not expressed per unit of area as required for the analysis.

Nutrient levels were classified as low and high because the absolute nutrient concentrations varied widely, with the differences between undisturbed (low) and enriched (high) conditions ranging from 2- to 97-fold (Table 1). When more than one experiment per study was conducted on the same species in different locations (sites), the mean values were used in the analysis.

The biomass and density responses to nutrient enrichment were quantified for each study case as the percentage change $((H-L)/L) \times 100$, where H is the biomass or density at high nutrient levels and L is the biomass or density at low nutrient levels. The response is negative when biomass or density of seagrass meadows declines with nutrient enrichment and positive when they increase. Linear regression analyses were used to examine the effects of seagrass growth or size on the biomass or density responses to nutrient enrichment (Sokal & Rohlf 2012). The species-specific average values of individual shoot weight (g DW), rhizome diameter (mm) and leaf length (cm) were used as size parameters, and the horizontal and vertical rhizome elongation rates (cm yr^{-1}) as growth parameters. All these parameters were derived from literature data (see Table 2 for details).

BIOMASS–DENSITY RELATIONSHIPS

The 'static interspecific biomass–density relationship' (Weller 1989; Scrosati 2005) was obtained using data collected a single time, the season of maximum above-ground biomass. The static relationships under low and high nutrient levels were compared. When the temporal variation of biomass and density of a species at low and high nutrient levels was available, the 'dynamic biomass–density relationship' was analysed. This was possible for seven seagrass species, *Cymodocea nodosa* (Spain), *Halophila ovalis* (Thailand), *Thalassia hemprichii* (Thailand), *Thalassia testudinum* (Belize, Colombia and Mexico), *Zostera capricorni* (Australia), *Zostera marina* (USA) and *Zostera noltii* (Portugal), in a total of nine study cases. These were all descriptive studies (Table 1). The linear log–log relationships between biomass and density were determined by principal component analysis (PCA), because both variables are random (Weller 1987; Scrosati 2005). The PCA yields an orthogonal regression, which minimizes deviations perpendicularly to the fitted line and therefore does not rank variables as independent or dependent. PCA was performed on the covariance matrix with the linear fit corresponding to the first eigenvalue (Manly 1986; Jackson 1991). The slope was estimated by

dividing the biomass loading by the density loading. The linear dependence of the biomass–density relationships was measured by Pearson's correlation coefficient, and its statistical significance was determined by testing the null hypothesis that log biomass and log shoot density were uncorrelated (Sokal & Rohlf 2012).

To estimate the variance of the slopes and intercepts of PCA regressions of the static interspecific biomass–density relationship, so that the differences between low and high nutrient levels could be tested, a bootstrap resampling technique (random sampling with replacement) was done 50 times to the original set of biomass–density variables and a PCA was performed to each bootstrap sample. Fifty bootstrap samples are considered adequate to estimate standard errors (Timmerman, Kiers & Smilde 2007). The variables of interest, that is, the slope and intercept, were estimated for each PCA, and their average values (\pm SE) were calculated. A Student's t -test was used to assess the significant differences between the mean slope and intercept of the static interspecific biomass–density relationship obtained under low and high nutrient levels.

For the dynamic intraspecific biomass–density lines, a chi-square test was used to investigate the response trends to nutrient enrichment of the slopes and intercepts, testing the null hypothesis that the number of cases showing increasing or decreasing responses was equal. Statistical analyses were performed using SYSTAT. Levels of significance were established at $\alpha = 0.05$.

Results

Responses of above-ground biomass and shoot density to increasing nutrient loads were obtained from 28 studies (17 descriptive and 11 experimental) of 14 species (Table 1), including a wide range of plant sizes and geographical distribution. In 22 of the studies, biomass and density covaried simultaneously, either increasing together (six studies, all experimental, Fig. 1 upper right quarter) or decreasing together (three experimental plus 13 descriptive studies, Fig. 1 lower left quarter). Only in four studies were there increases in biomass accompanied by density decrease as expected under self-thinning (two descriptive and two experimental). Interestingly, descriptive and experimental studies showed opposite trends in the biomass and density responses to nutrients. A simultaneous decrease in biomass and density was the most common response in descriptive studies (13 of 16 cases, 76%, Fig. 1) as opposed to experimental studies (2 of 11 cases, 18%). No descriptive studies reported simultaneous increases in biomass and density. In experimental studies, biomass increased in 8 of the 11 cases (72%).

The magnitude of the biomass–density responses to nutrient enrichment (Fig. 1) was much higher in the species showing a positive response (up to 269% for biomass and 125% for density) than in the species showing a negative response (down to –84% and –79%, respectively). The biomass–density regression line is above the 1:1 line (Fig. 1) showing that, overall, the above-ground biomass of seagrasses responds more than density to nutrient increase.

The analysis of the static interspecific relationship between biomass and density shows that the slope of the line is significantly higher under high nutrient conditions in experimental studies (Fig. 2a), but not in descriptive studies (Fig. 2b) due to higher variability of biomass and density. The intercept of

Table 1. List of seagrass reports of monospecific meadows for which the biomass–density relationships at low and high nutrient levels were available. Nutrient sources, enrichment levels (x fold) and responses of above-ground biomass and density ('+' for increase and '-' for decrease) of descriptive (D) and experimental (E) studies are presented

Species (abbreviation)	Location	Nutrient source	Enrich. (× fold)	Response		Reference
				Biomass	Density	
<i>Cymodocea nodosa</i> (Cn)	Alfacs Bay, Spain	Agriculture run-off (rice paddy fields)	D 30	-	-	O. Mascaró, M. Pérez & J. Romero unpubl. data
<i>Cymodocea rotundata</i> (Cr)	Cape Bolinao, NW Philippines	Slow-release fertilizer in sediment	E 18	+	+	Agawin, Duarte & Fortes (1996)
<i>Cymodocea serrulata</i> (Cs)	Moreton Bay, Australia	Slow-release fertilizer in sediment	E 97	-	-	Udy & Dennison (1997a)
<i>Halophila ovalis</i> (Ho)	Trang and Satun, Thailand	Mangrove run-off	D 3	-	-	A. Prathep unpubl. data
<i>Heterozostera tasmanica</i> (Ht ^a)	Port Phillip Bay, Australia	Slow-release fertilizer in sediment	E 6	+	+	Bulthuis, Axelrad & Mickelson (1992)
<i>Heterozostera tasmanica</i> (Ht ^b)	Westport, Australia	Agriculture and urbanization	D 2	-	-	Miller, Campbell & Scudds (2005)
<i>Halodule uninervis</i> (Hu ^a)	Moreton Bay, Australia	Slow-release fertilizer in sediment	E 97	+	+	Udy & Dennison (1997a)
<i>Halodule uninervis</i> (Hu ^b)	Green Island (GBR), Australia	Slow-release fertilizer in sediment	E 41	+	+	Udy <i>et al.</i> (1999)
<i>Posidonia australis</i> (Pa)	Rottne Island, Australia	Slow-release fertilizer in sediment	E 6	-	-	Udy & Dennison (1999)
<i>Posidonia oceanica</i> (Po ^a)	Aegean Sea, Greece	Fish farm	D 4	-	-	Apostolaki <i>et al.</i> (2009), E.T. Apostolaki unpubl. data
<i>Posidonia oceanica</i> (Po ^b)	NE Spain	Urbanization, sewage effluents, agriculture	D 4	-	-	Martínez-Crego <i>et al.</i> (2008), Romero <i>et al.</i> (2007)
<i>Syringodium isoetifolium</i> (Si)	Green Island (GBR), Australia	Slow-release fertilizer in sediment	E 41	-	-	Udy <i>et al.</i> (1999)
<i>Thalassia hemprichii</i> (Th ^a)	Cape Bolinao, NW Philippines	Slow-release fertilizer in sediment	E 18	+	+	Agawin, Duarte & Fortes (1996)
<i>Thalassia hemprichii</i> (Th ^b)	Trang, Thailand	Mangrove run-off	D 3	-	-	A. Prathep unpubl. data
<i>Thalassia testudinum</i> (Tt ^a)	Sarasota Bay, Florida, USA	Run-off, baseflow, point sources, septic tanks, rainfall	D 12	-	-	Tomasko, Dawes & Hall (1996)
<i>Thalassia testudinum</i> (Tt ^b)	St. Joseph Bay, Florida, USA	Slow-release fertilizer in water	E 13	+	+	Heck <i>et al.</i> (2000)
<i>Thalassia testudinum</i> (Tt ^c)	CCB and LLM, Texas, USA	Ammonium enrichment in sediment	E 9	+	+	Lee & Duntoon (2000)
<i>Thalassia testudinum</i> (Tt ^d)	Puerto Morelos, Mexico	Mangrove run-off	D 2	+	-	B.I. van Tussenbroek unpubl. data
<i>Thalassia testudinum</i> (Tt ^e)	Lighthouse Atoll, Punta Gorda and Placencia, Belize	Run-off, water pollution	D 5	-	+	SeagrassNet unpubl. data
<i>Thalassia testudinum</i> (Tt ^f)	Tayrona and Rosario Is., Colombia	Human waste in nearby developments	D 2	-	-	SeagrassNet unpubl. data
<i>Zostera capricorni</i> (Zc ^a)	Moreton Bay, Australia	Slow-release fertilizer in sediment	E 97	+	+	Udy & Dennison (1997a)
<i>Zostera capricorni</i> (Zc ^b)	Moreton Bay, Australia	Sewage, septic effluent, prawn-farm effluent, river discharge	D 28	-	+	Udy & Dennison (1997b)
<i>Zostera capricorni</i> (Zc ^c)	Lake Macquarie and Tuggerah Lakes, NSW, Australia	Agriculture run-off, urban stormwater	D 4	+	-	R. Gruber unpubl. data
<i>Zostera marina</i> (Zm ^a)	Gulf of St. Lawrence, Canada	Bivalve aquaculture, fish processing plants, peat mining, agriculture	D 20	-	-	Schmidt <i>et al.</i> (2012)
<i>Zostera marina</i> (Zm ^b)	British Columbia, Canada	Urbanization	D 7	-	-	Robinson, Yakimishyn & Dearden (2011)
<i>Zostera marina</i> (Zm ^c)	Humboldt Bay, California, USA	Aquaculture, agriculture run-off	D 3	-	-	SeagrassNet unpubl. data
<i>Zostera noltii</i> (Zn ^a)	Ria Formosa, Portugal	Urban wastewater effluent	D 49	-	-	Cabaço, Machás & Santos (2007), Cabaço, Santos & Sprung (2012), Peralta <i>et al.</i> (2005)
<i>Zostera noltii</i> (Zn ^b)	Cádiz Bay, Spain	Fish aquaculture effluent	D 4	-	-	García-Marín <i>et al.</i> (2013)

Table 2. Growth and size characteristics of the seagrass species examined. HE, horizontal rhizome elongation rate (cm yr⁻¹); VE, vertical rhizome elongate rate (cm yr⁻¹); RD, rhizome diameter (mm); SW, shoot weight (g DW); LL, leaf length (min.-max., cm); n/a, data not available

Species	HE	VE	RD	SW	LL
<i>Cymodocea nodosa</i>	40.2	1.43	2.73	0.09	10–30
<i>Cymodocea rotundata</i>	209.9	1.53	2.44	0.065	7–15
<i>Cymodocea serrulata</i>	153.0	13.10	2.78	0.121	6–15
<i>Halophila ovalis</i>	357.5		1.30	0.016	1–4
<i>Heterozostera tasmanica</i>	102.8	8.91	1.74	0.080	7–35
<i>Halodule uninervis</i>	101.2	4.10	1.37	0.027	6–15
<i>Posidonia australis</i>	9.3	1.42	7.21	0.875	30–60
<i>Posidonia oceanica</i>	2.8	2.70	10.75	0.657	40–50
<i>Syringodium isoetifolium</i>	109.1	8.55	1.74	0.038	7–30
<i>Thalassia hemprichii</i>	54.1	3.25	3.63	0.156	10–40
<i>Thalassia testudinum</i>	69.3	3.89	5.96	0.238	10–60
<i>Zostera capricorni</i>	78.0		1.40	0.091	7–50
<i>Zostera marina</i>	26.1		3.50	0.323	n/a
<i>Zostera noltii</i>	68.4		1.54	0.011	6–22

Data compiled by Duarte (1991), Marbà & Duarte (1998), Duarte *et al.* (1998), Hemminga & Duarte (2000), and Marbà & Duarte (2003). Data on seagrass leaf length (minimum and maximum leaf length) were obtained from Phillips & Meñez (1988) and Kuo & den Hartog (2001). Data on the horizontal rhizome elongation rate for *Zostera capricorni* were obtained from Turner *et al.* (1996) and Turner (2007). Updated data on *Zostera noltii* by Cabaço, Santos & Duarte (2008) were used.

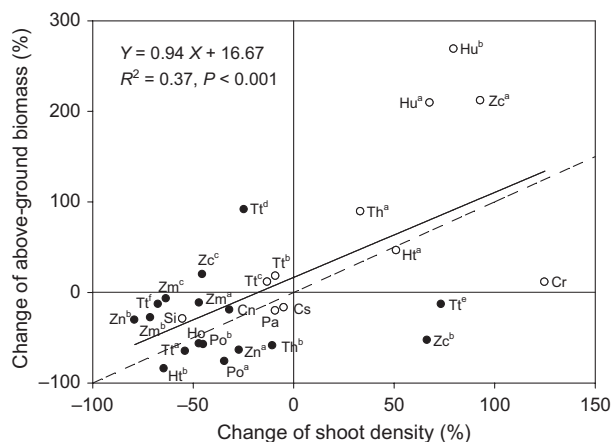


Fig. 1. Relationship between the percentage of change of seagrass above-ground biomass and shoot density in response to nutrient enrichment. The coefficient of determination (R^2) and significance (P) of linear regression is shown. Dashed line represents the 1:1 biomass–density line. Open and closed circles represent experimental and descriptive studies, respectively. See Table 1 for species name abbreviations.

the biomass–density relationship shows the opposite response, decreasing with nutrient enrichment in both experimental and descriptive studies.

The slopes of the intraspecific, dynamic biomass–density relationships (in log scale) for the nine species of seagrasses from which data were available were all positive and higher than one (Table 3). This revealed, respectively, that data are

in the nonthinning part of the theoretical biomass–density curves and that the B/D ratio increases with density in all seagrass species tested, that is, that the average biomass of each individual increases with density. Nutrient enrichment further increased the slopes and thus the positive effect of density on the average biomass of each seagrass shoot. This was observed in all reported cases, except one of *Thalassia testudinum* where the slope decreased from 1.38 to 1.09 with nutrients (Tt^d, Table 3). The intercept of the dynamic biomass–density line showed the opposite trend of the slopes. The coefficients of variation of density and biomass decreased with nutrient enrichment in seven of nine cases (Table 3).

Figure 3 shows how the biomass–density data fit around the intraspecific, dynamic biomass–density relationships to help understanding the main drivers of the slope responses to nutrient enrichment (Table 4). In species where the lines intercept within the range of data dispersal (Fig. 3), the biomass–density ratio (B/D), that is, the biomass of each shoot, decreased below a certain density threshold, whereas above that threshold it increased. This was observed in five of the nine seagrass species. However, the density thresholds are near the range limits of the observed density values in four species. Consequently, the main driver of the slope response to nutrient enrichment was a progressive increase in B/D with density, observed in *Thalassia hemprichii* (Th^b), *Thalassia testudinum* (Tt^f), *Zostera capricorni* (Zc^c) and *Zostera marina* (Zm^c). In *Cymodocea nodosa*, *Halophila ovalis* and *Thalassia testudinum* (Tt^e), there was a progressive reduction in the B/D ratio at lower densities, whereas in *Thalassia testudinum* (Tt^d), B/D increased progressively at lower densities. In *Zostera noltii*, shoot biomass decreases with density at densities lower than 8511, whereas it increases above that threshold (Table 4). This is the only species where the density threshold is in the middle of the density distribution range (Fig. 3).

The analysis of the interspecific responses of biomass and density to different levels of nutrient enrichment shows that biomass is significantly related to nutrients (Fig. 4A), only when biomass increased with nutrients (most experimental studies). In descriptive studies, where biomass decreased with nutrients (negative values of change, Fig. 4A), there was no significant relationship between biomass and nutrient levels. In contrast, density was not related to nutrient enrichment both in the positive and negative sets of responses (Fig. 4B).

Neither the seagrass biomass nor the shoot density responses to nutrients were related to seagrass size or growth characteristics analysed (Table 5). Therefore, the hypothesis that the species-specific life strategy of seagrasses (expressed as size and growth) influences the populations' response to nutrients was not supported.

Discussion

The analysis of the available data on the effects of nutrient enrichment on seagrass biomass and density revealed that the most common reaction was a synchronized response of both variables to increasing nutrients, which was reflected in the significant relationship between biomass and density

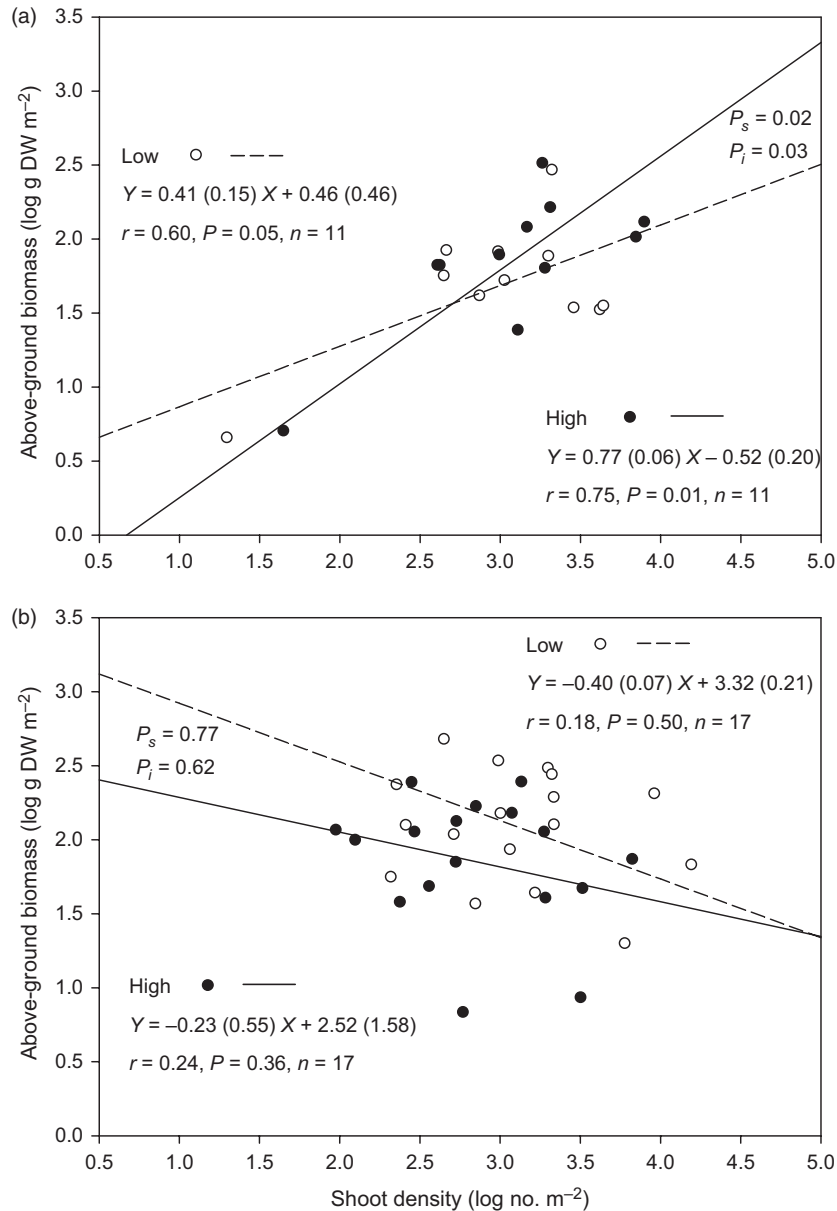


Fig. 2. Static interspecific relationship between above-ground biomass and shoot density (both log-transformed) of seagrasses at low and high nutrient levels for experimental (a) and descriptive (b) studies. Mean slopes and intercepts (\pm SE) were obtained using PCA bootstrapping ($n = 50$); P in italics shows the significance levels of the t -tests between the low and high nutrient levels for the slope (s) and intercept (i). Pearson's correlation: r – correlation coefficient, P – significance level of linear relationship and n – number of studies included in the analysis.

responses (Fig. 1). The analysis also revealed two opposite responses that corresponded to the type of study performed. Biomass and density tended to increase simultaneously under high nutrient levels in short-term experimental studies, whereas they tended to decrease simultaneously in descriptive studies where the seagrass populations were exposed to the long-term effects of nutrient increase. Experimental and descriptive studies may reveal different time frames of the nutrient enrichment response curve, indicating that experiments are context-dependent and that the limited temporal scales of experimental approaches may result in conclusions that cannot be extrapolated to a long-term ecosystem scale.

The short-term responses observed in experimental studies suggest a general nutrient limitation of seagrasses in the systems where these experiments were carried out. These responses were driven primarily by changes in biomass, rather than density as shown both by the statistically significant response of seagrass biomass to different nutrient levels, which was not observed for density (Fig. 4), and by the observation that the linear regression of biomass and density responses is above the 1:1 line (Fig. 1). Nutrient enrichment in nutrient limited conditions will result in higher biomass per shoot, increasing potential competitive interactions (Morris 2003), for example, for light. A similar trend was reported for terrestrial herbaceous clonal plants that responded to increasing nutrients

Table 3. Dynamic relationship between above-ground biomass and shoot density (both log-transformed) for seagrass species (descriptive studies) at low and high nutrient levels obtained using PCA. *r*, correlation coefficient, *P*, significance level of linear relationship and *n*, number of samples included in the analysis. Coefficient of variation (CV,%) of density (D) and biomass (B) is shown. See Table 1 for species name abbreviations

Species	Nutrients	Slope		Intercept		<i>r</i>	<i>P</i>	<i>n</i>	CV _D		CV _B	
Cn	Low	2.55		-6.09		0.81	< 0.001	36	6.46		24.67	
	High	2.71	↑	-6.63	↓	0.64	< 0.001	36	5.20	↓	17.02	↓
Ho	Low	1.27		-3.63		0.87	< 0.001	242	9.30		46.79	
	High	1.36	↑	-4.00	↓	0.64	< 0.001	916	7.28	↓	37.92	↓
Th ^b	Low	1.18		-1.59		0.69	< 0.001	65	7.19		11.67	
	High	1.23	↑	-1.61	↓	0.76	< 0.001	40	9.19	↑	15.42	↑
Tt ^d	Low	1.38		-2.38		0.57	< 0.001	72	4.87		10.99	
	High	1.09	↓	-1.15	↑	0.77	< 0.001	78	4.10	↓	6.57	↓
Tt ^e	Low	1.98		-2.89		0.68	< 0.001	126	11.70		28.59	
	High	3.23	↑	-6.41	↓	0.44	< 0.001	99	9.82	↓	28.23	↓
Tt ^f	Low	2.37		-5.13		0.47	< 0.001	144	11.29		25.95	
	High	2.68	↑	-5.15	↓	0.41	0.002	58	10.93	↓	23.57	↓
Zc ^c	Low	1.02		-1.50		0.10	0.033	127	10.18		18.37	
	High	1.72	↑	-3.10	↓	0.56	< 0.001	116	9.01	↓	18.08	↓
Zm ^c	Low	1.91		-2.61		0.69	< 0.001	356	17.30		38.43	
	High	2.02	↑	-1.98	↑	0.55	< 0.001	333	15.52	↓	25.10	↓
Zn ^a	Low	1.02		-1.89		0.65	< 0.001	283	8.18		15.70	
	High	2.23	↑	-6.64	↓	0.38	< 0.001	252	9.53	↑	28.39	↑

Table 4. Drivers of the slope responses to nutrient enrichment based on the biomass–density data point distribution of seagrass species presented in Fig. 3. See Table 1 for species name abbreviations

Species	Log D intercept	D intercept (shoots m ⁻²)	Driver of slope response
Cn	3.41	2570	B/D ratio decrease at D < 2570 and increase at D > 2570
Ho	4.35	22387	B/D ratio decrease at D < 22387 and increase at D > 22387
Th ^b	out of range	out of range	B/D ratio increase with density
Tt ^d	out of range	out of range	B/D ratio increase with lowering density
Tt ^e	2.80	631	B/D ratio decrease at D < 631 and increase at D > 631
Tt ^f	out of range	out of range	B/D ratio increase with density
Zc ^c	2.28	191	B/D ratio decrease at D < 191 and increase at D > 191
Zm ^c	out of range	out of range	B/D ratio increase with density
Zn ^a	3.93	8511	B/D ratio decrease at D < 8511 and increase at D > 8511

by increasing their biomass (Müller, Schmid & Weiner 2000). These findings are also supported by the meta-analysis done by Hughes *et al.* (2004), which revealed that experimental additions of inorganic nutrients to sediments generally stimulated seagrass growth. On the other hand, the long-term negative responses of both biomass and density of seagrasses observed in descriptive studies probably result from ecosystem-scale events related to nutrient enrichment such as increased turbidity, algal blooms, epiphyte loads and anoxia (Ralph *et al.* 2006). The major role of these factors on seagrass decline is well known (Burkholder, Tomasko & Touchette 2007; Ralph *et al.* 2007; Fertig, Kennish & Sakowicz 2013).

Most seagrasses analysed here lie in the nonthinning part of the theoretical biomass–density curves. A simultaneous increase in biomass and decrease in density, evidence of self-thinning (Yoda *et al.* 1963; White 1981; Westoby 1984; Weller 1987), was only observed in 4 of 28 studies (Table 1). As well, the maximum biomass values across seagrass species, 2.67 at low nutrients and 2.51 log g DW m⁻² at high, are well

under the 'ultimate biomass–density line' (4.87 log g DW m⁻²; Scrosati 2005), which describes the maximum biomass possible at any plant density and constrains all plant populations (Weller 1989; Scrosati 2005). This suggests that density-dependent mortality is not common in seagrasses, probably because of flexible clonal growth patterns or facilitative interactions associated with the species clonal integration (discussed below). Three of these cases were documented on *Thalassia testudinum* (Heck *et al.* 2000; Lee & Dunton 2000; B.I. van Tussenbroek, unpubl. data) and one in *Zostera capricorni* (R. Gruber, unpubl. data), which indicates that self-thinning may occur at least on these species. *T. testudinum* shows a unique regulation of shoot density involving shoots that become dormant at high densities (van Tussenbroek, Galindo & Marquez 2000). Increased dormancy was related to biomass increase (B.I. van Tussenbroek, pers. comm.) as expected in a self-thinning demographic process. Additionally, experimental mesocosm studies have shown a process of self-thinning in *Zostera marina* (Short, Burdick & Kaldy 1995). The species density decreased with reduced light

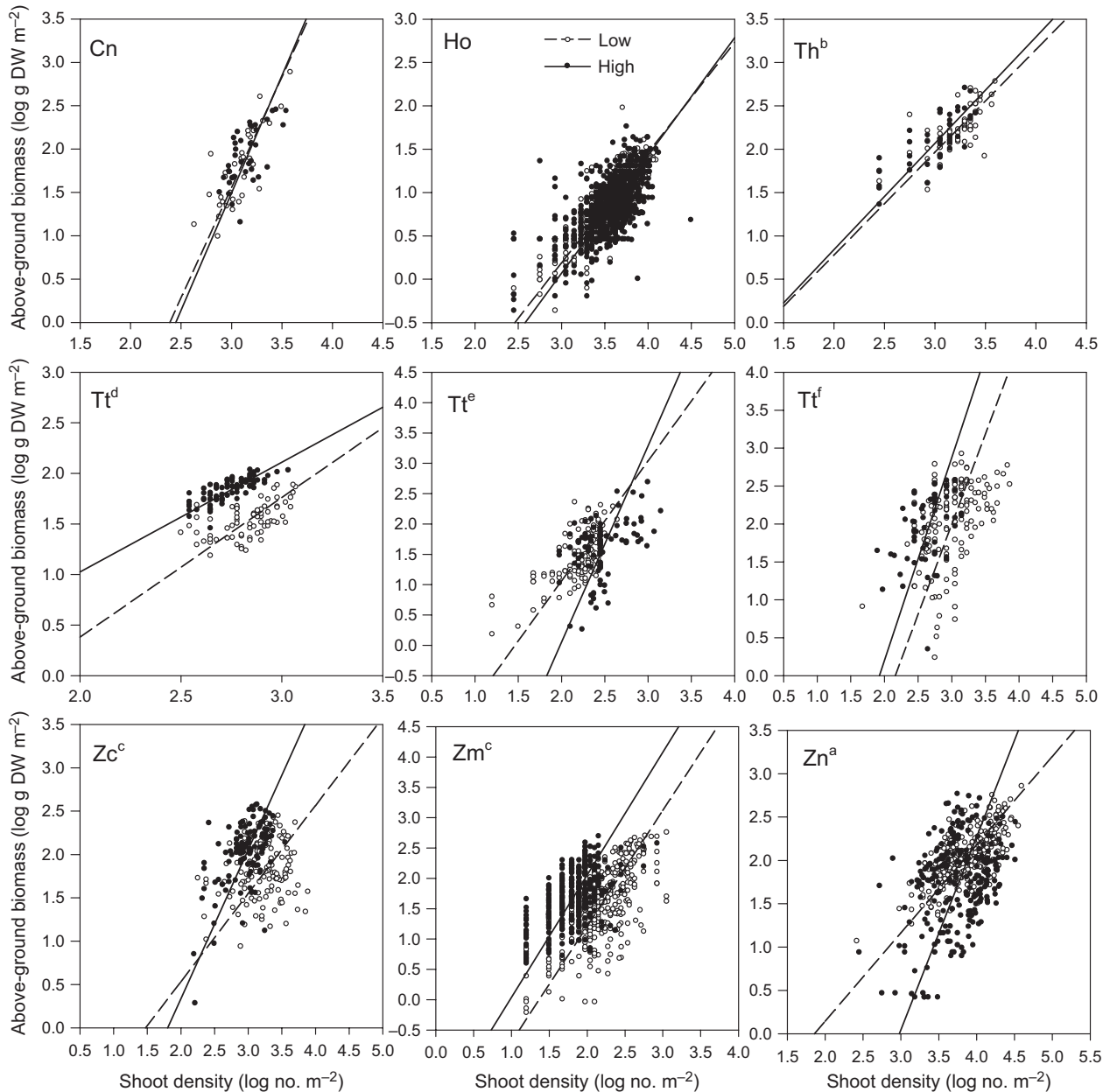


Fig. 3. Intraspecific, dynamic biomass–density relationships in seagrass species. See Table 1 for species name abbreviations.

conditions as expected under self-thinning. This response did not change under nutrient enrichment conditions.

The analysis of both the static and the dynamic biomass–density relationships of seagrasses revealed that the slopes of the biomass–density linear relationships increase under nutrient enrichment. In particular, increasing slopes and decreasing intercepts of the dynamic biomass–density relationship of single seagrass species are good integrative indicators of altered environmental condition of seagrass habitats related to nutrient loading. Higher slopes and lower intercepts under high nutrients were also observed elsewhere in *Z. noltii* (Cabaço, Machás & Santos 2007), macroalgae (Steen & Scrosati 2004) and terrestrial plants (Morris 2003; Chu *et al.* 2010). As biomass and density of seagrass populations are easily

measurable and have been widely used in monitoring programs, their linear relationship in monospecific communities, particularly the slope and intercept, can be used as metrics that reveal alterations of the intraspecific competitive mechanisms resulting from nutrient disturbances, adding relevant inputs for the assessment of the ecological quality status of coastal and transitional waters.

The seagrass biomass and density variability can also provide relevant information on the species response to nutrient disturbance. In general, the variability of biomass and density decreased with nutrient enrichment, showing an opposite response to the general trend of ecological responses to disturbance-driven changes (e.g. Sousa 1984; Underwood 1992; Turner 2010). However, a decrease in variability may occur

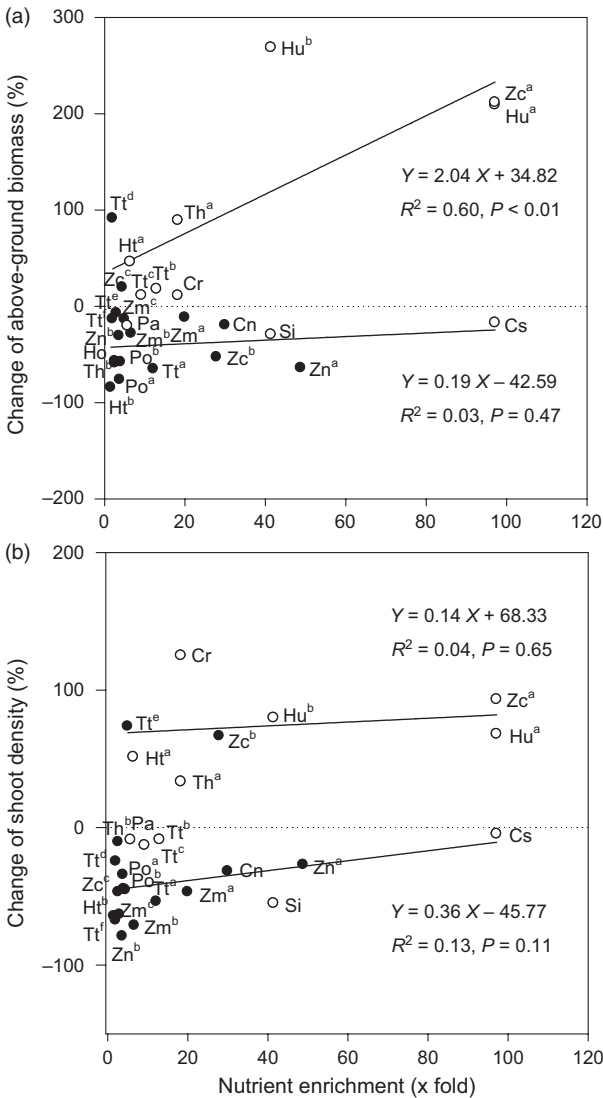


Fig. 4. Relationship between the nutrient enrichment level and the rate of change in seagrass above-ground biomass (a) and in seagrass shoot density (b) in descriptive (black circles) and experimental studies (open circles). R^2 and significance (P) are shown for both the increasing and decreasing biomass–density data sets. See Table 1 for species name abbreviations.

when populations are under chronic rather than discrete disturbances, such as sewage discharge or organic enrichment, due to the absence of response recovery time of continuous disturbance events (Fraterrigo & Rusak 2008).

Even though biomass–density metrics may be useful indicators to monitor seagrass meadows, the intrinsic population mechanisms that drive the biomass–density relationships and their response to nutrients must be understood. The analyses performed here revealed surprising conclusions that need to be tested. The first is that the B/D ratio increases with density in all seagrass species tested (because $\log B - \log D$ slopes > 1), that is, that the average biomass of each individual increases with density. This is intriguing and probably is a consequence of facilitative processes related to clonal integration in seagrasses. Furthermore, our analysis suggests that nutrient enrich-

Table 5. Results of linear regression analysis between the rate of change of both shoot density and above-ground biomass and the seagrass size and growth characteristics. See Table 2 for size and growth abbreviations. anova F statistics and P -significance level are presented. n , number of studies included in the analysis

Change (%)	Size/growth	Regression		ANOVA	
		R^2	n	F	P
Shoot density	HE	0.047	28	1.277	0.269
	RD	0.056	28	1.536	0.226
	SW	0.058	28	1.604	0.217
	VE	0.022	19	0.386	0.543
	LL _{min}	0.040	25	0.952	0.339
	LL _{max}	0.024	25	0.558	0.463
	Above biomass	HE	0.002	28	0.049
RD	0.093	28	2.680	0.114	
SW	0.079	28	2.236	0.147	
VE	0.005	19	0.092	0.766	
LL _{min}	0.074	25	1.829	0.189	
LL _{max}	0.026	25	0.603	0.445	

ment further increases this effect as biomass–density slopes increased to even higher values. The redistribution of photosynthates through clonal integration to shoots receiving less light due to increasing densities leading to a more efficient production performance of shoots could be an explanation for this, but this hypothesis must be experimentally tested.

Facilitation among plants can affect the course of intra-specific competition to self-thinning under abiotic stress (Chu *et al.* 2010). Abiotic stress results in a steeper biomass–density relationship, as generally observed here for seagrasses, but this effect may be reduced by positive interactions among individuals (Chu *et al.* 2010), delaying the onset of density-dependent shoot mortality. Competitive interactions in clonal plants are not solely determined by the resource itself (Schwinning & Weiner 1998), as their clonal nature and implicit internal resources translocation may alleviate competition within populations (de Kroon 1993). This may explain why seagrasses, in general, do not always show self-thinning. In species with low clonal integration such as *Zostera noltii* (Marbà *et al.* 2002; Cabaço, Alexandre & Santos 2005), where lower facilitative interactions are expected, self-thinning is not observed probably because the high biomass per shoot at high nutrient levels is mediated through high growth and turnover rates (Peralta *et al.* 2005). The role of facilitation as a causal mechanism for the lack of self-thinning in seagrasses is worthy of being tested experimentally in the future.

To test the hypotheses emerging from our analysis of the main drivers behind the increasing biomass–density slopes of seagrasses under nutrient enrichment will be challenging. In four of the nine cases assessed, the biomass of individual shoots increased progressively with increasing densities fitting the facilitation hypothesis, for example, that the redistribution of photosynthates through clonal integration to shoots receiving less light leads to a more efficient production performance of shoots. In three other cases, there was a progressive

reduction in shoot biomass at lower densities. This suggests that the potential deleterious effects of excessive nutrient conditions are more effective under lower densities, that is, that facilitation may reduce the negative impacts of excessive nutrients. The combination of both of these processes may explain the observations in *Z. noltii*. This was the only species where the density threshold was well in the middle of the density distribution range and consequently where the biomass of each shoot decreased with density at densities lower than 8500 shoots m⁻², whereas it increased above that threshold. Interestingly, in *T. testudinum* (Tt^d, from Puerto Morelos, Mexico), the only case where the biomass–density slope decreased and that also showed self-thinning, the biomass of shoots increased more at lower densities.

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