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Marine megaherbivore grazing may increase seagrass tolerance to high nutrient loads

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Summary

1. Populations of marine megaherbivores including green turtle (*Chelonia mydas*) have declined dramatically at a global scale as a result of overharvesting and habitat loss. This decline can be expected to also affect the tolerance of seagrass systems to coastal eutrophication. Until now, however, simultaneous effects of top–down control by megaherbivore grazing and bottom–up control by nutrient input have not been tested experimentally.

2. We therefore investigated the interacting effects of nutrient (N and P) addition and mimicked green turtle grazing on seagrass and epiphyte productivity, seagrass biomass and nutrient contents in exclosures at a pristine seagrass site in the Indo-Pacific region (Kalimantan, Indonesia).

3. Grazing almost doubled leaf biomass production rates, while nutrient addition (N + P, slow-release granules) did not have an effect on these rates. Rhizome biomass was, however, strongly reduced by nutrient addition. In contrast to phosphorus, tissue nitrogen contents increased after nutrient addition, showing that nitrogen was not limiting primary productivity. Epiphyte growth was, however, strongly correlated with high water column P concentrations, indicating an indirect negative effect of eutrophication when turtle grazing would be absent. We calculated that green turtle leaf grazing leads to substantial exports of N and P, at rates of at least 8% of the standing stock per day equalling the daily seagrass production, up to 13 (N) and 1.4 (P) mg m^{-2} day^{-1}.

4. *Synthesis.* By combining our quantified effects with literature data, we propose a conceptual model of seagrass functioning under megaherbivore leaf grazing and eutrophication. In tropical seagrass systems with high green turtle grazing pressure, grazing alleviates the negative effects of eutrophication by the stimulation of seagrass production and concomitant nutrient uptake, the increased export of nutrients and the indirect prevention of low below-ground biomass. Similar to the role of terrestrial megaherbivores, these strong top–down controls show the pivotal role of green turtles in current coastal systems, which is lacking in systems where their numbers have greatly declined. These marine megaherbivores do not only drive structure and functioning of their foraging grounds but also increase the tolerance of seagrass ecosystems to eutrophication.

Key-words: *Chelonia mydas*, epiphytes, eutrophication, green turtle, *Halodule uninervis*, herbivory, plant–herbivore interactions, trophic structure, tropical

Introduction

Human overharvesting and habitat loss have led to a dramatic decline of marine megaherbivores including serenians (e.g. *Dugong dugon*) and green turtles (*Chelonia mydas)* (Jackson 2001; Valentine & Duffy 2006). In the Indo-Pacific, the foraging habitats for dugong and adult green turtle are seagrass meadows, and seagrass leaves are their most important food (Bjorndal 1997). These feeding grounds are declining because they are increasingly exposed to the consequences of major changes in land use that increase riverine nutrient loads and sediment run-off, which strongly affect water quality (Abal & Dennison 1996; Orth *et al.* 2010). Understanding how ecosystem functioning may alter as a result of simultaneous

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harvesting of megaherbivores and eutrophication requires the identification of interactions between top–down control by herbivory and bottom–up control by nutrient availability.

The most common mechanism responsible for seagrass decline in shallow coastal areas is eutrophication, which stimulates algal and epiphyte overgrowth and thereby strongly reduces light availability for seagrasses (Burkholder, Tomasko & Touchette 2007). Seagrasses are adapted to oligotrophic environments where their growth is often limited by the supply of nitrogen (terrigenous substrates) or phosphorus (carbonate sediments) (Short, Dennison & Capone 1990; Erftemeijer 1994; Burkholder, Tomasko & Touchette 2007). In general, seagrass species perform better than algae at low nutrient concentrations, because they are also able to take up organic and inorganic nutrients from the sediment (Evrard *et al.* 2005; Vonk *et al.* 2008a). This competitive interaction is however inversed at high nutrient concentrations that favour fast-growing algae (reviewed in Duarte 1995; Burkholder, Tomasko & Touchette 2007).

In general, herbivores have been shown to alter plant productivity, biomass, distribution, community structure and tissue nutrient content (McNaughton 1984; Milchunas & Lauenroth 1993; Augustine & McNaughton 1998; Ritchie *et al.* 1998). Their grazing can enhance nutrient cycling and can have direct effects on nutrient fluxes (Thayer *et al.* 1984; Sirotnak & Huntly 2000). The effects of marine herbivores may include stimulated production of seagrass (Valentine *et al.* 1997; Moran & Bjorndal 2005), changes in seagrass meadow structure (Lal *et al.* 2010) and reduction of the flux of organic matter and nutrients to sediments and plants by short circuiting the detrital cycle (Thayer, Engel & Bjorndal 1982; Ogden *et al.* 1983; Thayer *et al.* 1984; Vonk *et al.* 2008b). In saltmarshes and terrestrial ecosystems, an additional effect of herbivores is the return of nutrients through faeces and urine (Bazely & Jefferies 1985; Hik, Jefferies & Sinclair 1991; Frank *et al.* 2000). In seagrass–green turtle ecosystems, such return is expected to be of minor importance because the produced debris and dung float by entrapped hindgut gasses (Bjorndal 1979) and they are exported to adjacent ecosystems (Balazs, Fujioka & Fujioka 1993). Urine is also not expected to substantially contribute to nitrogen budgets in the grazed plots (Thayer *et al.* 1984; Moran & Bjorndal 2005).

A regime of intense and regular megaherbivore grazing of seagrass meadows has probably existed over at least the last 50 million years (Domning 2001). Hence, megaherbivore grazing may have had strong ecological and evolutionary impacts on seagrass stands, thereby selecting for seagrass strategies that allow plants to cope with this grazing pressure (Valentine & Heck 1999; Valentine & Duffy 2006). The importance of marine herbivores in determining the productivity of seagrass meadows may therefore equal the role reported for herbivores in terrestrial grasslands (cf. McNaughton 1984; Valentine & Duffy 2006). However, until now, there has been no experimental proof for the effect of green turtles on seagrass ecosystems threatened by eutrophication.

In this paper, we hypothesize that under increasing nutrient pressure, megaherbivore grazing may alleviate the effects of eutrophication on seagrasses. These alleviating effects could include increased production and concomitant uptake and export of nutrients, but also reduction of harmful algal overgrowth as has been found for mesoherbivores (Boyer *et al.* 2004; Hays 2005). Although other studies have addressed the interactive effects of nutrient availability and herbivory on marine primary producers (reviewed in Hughes *et al.* 2004; Tewfik, Rasmussen & McCann 2005; Burkepile & Hay 2006; Heck & Valentine 2007), these did not involve megaherbivores. These studies focused on the effects of the dominant mesoherbivores present, including fish and small invertebrates such as urchins, amphipods, isopod crustaceans, hermit crabs and gastropod molluscs (Valentine & Duffy 2006). These smaller grazers typically remove $< 30\%$ of leaf production, have a modest impact on seagrass growth (Cebrian *et al.* 1997; Poore, Campbell & Steinberg 2009) and induce a higher plant resistance than larger herbivores in marine macrophytes (Toth & Pavia 2007). This implies that the majority of studies on eutrophication have been conducted after the dramatic decline in the numbers and diversity of larger marine consumers due to centuries of extensive harvesting. As large herbivores can remove a substantially greater part of the leaf production (Dayton *et al.* 1995; Jackson *et al.* 2001; Heck & Valentine 2007), there is a strong need for information on the top–down control by megaherbivores that were historically present in high densities and are expected to have considerable effects on seagrass stands.

The aim of our study, carried out on pristine Indo-Pacific seagrass meadows, was threefold. First, we assessed the effects of high nutrient loads and high megaherbivore grazing pressure and their interactions on seagrass biomass production and nutrient contents. For this, we conducted an experiment inside green turtle exclosures, in which we manipulated both grazing pressure (by artificial leaf clipping) and nitrogen and phosphorus levels (by adding fertilizer) to simulate coastal eutrophication. Leaf grazing by green turtle, *Chelonia mydas*, was mimicked, because this is the dominant herbivore species that grazes year-round. Our second objective was to estimate the export of plant biomass and its incorporated nitrogen and phosphorus as a result of green turtle grazing. Third, we wanted to assess the long-term impact of intense leaf grazing pressure on leaf standing biomass by the analysis of leaf biomass data gathered over a 6-year period. Our main hypothesis was that grazing of natural megaherbivore populations, as generally present before strong anthropogenic pressure, would increase the tolerance of seagrass stands to high nutrient inputs.

Materials and methods

STUDY AREA

The seagrass study area was located 400 m to the north-east of Derawan Island, situated 17 km from the mount of the Berau River on the mainland of East Kalimantan, Indonesia (2°17′19′N, 118°14′53′E)

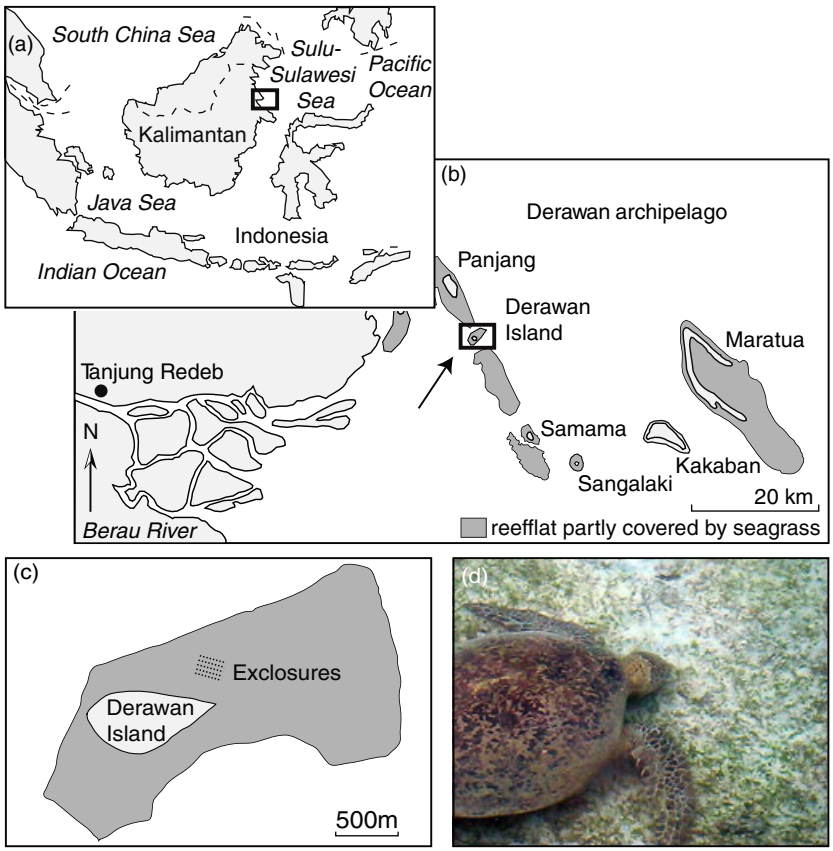

Fig. 1. (a) Map of the a Indo-Pacific Ocean with (b) The Derawan Archipelago and the (c) location of the exclosures on Derawan Island (2°17′19′N, 118°14′53′E). (d) Leaves are intensively grazed by green turtles, and a detritus layer is absent.

(Fig. 1). The island was surrounded by a shallow subtidal mono-species seagrass meadow characterized by *Halodule uninervis* (Ehrenberg, Ascherson) growing on carbonate substrate. The density of *H. uninervis* was 3710 ± 271 shoots m^{-2}; leaves were short (41 ± 2.5 mm) and narrow-leaved (1.2 ± 0.1 mm) and had a specific leaf area (SLA) of 290 ± 5.6 mm^2. Rhizomes had short internodes (18 ± 2.6 mm), and there were only 1.8 ± 0.1 leaves per shoot on average. A detritus layer was absent (Fig. 1d).

High densities of green sea turtles (*Chelonia mydas*; 2008: 15 ± 2.2 ha^{-1}, unpublished data of the authors) intensively grazed on the seagrass meadow during daylight hours. *H. uninervis* is an early successional species (Vermaat *et al.* 1995; Aragones *et al.* 2006) and is highly digestible and preferred by megaherbivores (De Iongh *et al.* 2007). Observations of intensive grazing, stomach contents of stranded dead green turtles and contents of fresh floating green turtle dung at this location imply that the green turtles in this area foraged almost entirely on seagrass biomass. The density of common seagrass mesograzers was extremely low when compared to fauna densities of seagrass meadows in the Spermonde Archipelago where *C. mydas* is almost absent. This is most likely due to the low structural complexity of the short-leaved meadow as a result of turtle grazing. Densities of shrimp *Alpheus sp.*, fish *Calotomus spinidens* and *Leptoscarus vaigiensis*, and sea urchin *Tripneustes gratilla* were, respectively, 15, 200 and 100 times lower at our study site (Vonk, Christianen & Stapel 2008c; D. Kneer unpublished data).

During the experiment, water depth was minimally 0.10 m, with a maximum tidal amplitude of 2.9 m. Pore water pH was on average 7.7 (±0.06), while water column pH was on average 8.3 (±0.01). Oxygen concentration of the water column (90 ± 4.6%) was higher than oxygen concentrations of the pore water (21 ± 2.7%), because of tidal flushing, currents and respiration in the sediment. Temperature (29.8 ± 0.4 °C) and salinity (32.9 ± 0.03‰) were similar for surface water and pore water.

EXPERIMENTAL DESIGN

We tested the combined effects of nutrient addition (i.e. 'fertilizer' or 'no fertilizer') and green turtle leaf grazing (i.e. 'clipping', 'natural grazing' or 'no clipping') in a full-factorial design using exclosures. This resulted in six different treatments that were replicated five times. Four treatments were tested on experimental plots inside exclosures, i.e. fertilizer/no clipping, fertilizer/clipping, no fertilizer/no clipping, no fertilizer/clipping, and two treatments were tested on experimental plots without exclosures, i.e. fertilizer/natural grazing and no fertilizer/natural grazing. The experimental plots were selected on homogeneous seagrass substrate, with minimum distances of 10 m to prevent nutrient cross-contamination. To test for possible effects due to increasing distance from the shore, we placed the replicates for each treatment in a randomized block design, with each block at increasing distance parallel to the shore (270, 290, 320, 340, 370 m, on average). These experiments were located in a zone with minimal differences in water depth (40 cm) and were fronted with a 500-m-wide large intertidal area; consequently, differences in light penetration and hydrodynamics were minor. All plots were subdivided into five circular subplots of 43 cm in diameter to facilitate plant harvesting during five points in time.

EXCLOSURE DESIGN

The exclosures (1.5 × 1.5 × 0.3 m) were effective to exclude grazing of green turtles and designed to maximize light passage. They consisted of fishing net (mesh size 5 cm, black colour to ensure visibility to green turtles) attached to the tops of four steel poles that were connected by ropes. Cage inspection, cleaning and repair were conducted thrice a week to ensure enclosure integrity. Four short steel poles on the corners marked the plots with the natural grazing treatment to allow turtle grazing. We could, however, not totally exclude grazing by other herbivores, but based on their extremely low densities (D. Kneer, unpublished data), their seagrass consumption is assumed insignificant compared to turtle grazing.

To check for possible effects of cage structure on light availability at the canopy level, we measured photosynthetic active radiation (PAR) between 10 and 11 AM on a sunny day (4π Underwater Quantum Sensor; LI-COR, Lincoln, Nebraska, USA). After the maximum time before cleaning (3 days), PAR was 19% lower inside cages (1088 ± 28 μmol m^{-2} s^{-1}) than outside cages (1343 ± 11 μmol m^{-2} s^{-1}; $n = 30$; $P < 0.001$), but there was no difference between grazing or nutrient treatments. Because PAR measurements were well above the reported light saturation levels for *H. uninervis* of 300 μmol m^{-2} s^{-1} (Beer & Waisel 1982), it was very unlikely that light reduction caused by the exclosures negatively affected *H. uninervis* and epiphyte growth. The large mesh size was also effective in minimizing differences in hydrodynamics within and outside the cages, which was confirmed by repeatedly monitoring movements of test beads that were put on the sediments.

NUTRIENT ADDITION

Plots assigned to 'fertilizer' treatment (2.25 m^2) were fertilized with 2 kg osmocote slow-release fertilizer at the start of the experiment (g/g ratio N:P:K; 18:9:10). The osmocote fertilizer was inserted into four panty hoses placed on top of the sediment, between each of the four poles of the cages. In case of the natural grazed vegetation where cages were absent, panty hoses were fixed to the short poles used to mark the plot. This set-up was chosen to maintain fertilization independent of tidal flow direction and to enable the nutrients to reach both the water column and the pore water. Loading rates were between 136–181 mmol N m^{-2} day^{-1} and 30–41 mmol P m^{-2} day^{-1}, based on dry weight loss of the osmocote beads.

MIMICKED GRAZING

To mimic green turtle leaf grazing ('clipping' treatment), we clipped the above-ground canopy after 21 days initially (t_1) and repeated this every 14 days ($t_2 - t_4$). Based on literature values, the 14-day interval coincides with the time that is needed for the *H. uninervis* shoot to regrow 5 cm above the blade sheath junction, the size of an average green turtle bite (Bjorndal 1980; Ogden *et al.* 1983; Williams 1988; Moran & Bjorndal 2007). However, based on biomass regrowth following clipping in exclosures, we assessed that this interval in our meadow was smaller (see Results). The true natural grazing interval (day^{-1}) was estimated by:

$$\text{Natural grazing interval} = \frac{\text{total standing stock}}{\text{daily biomass export}} \qquad \text{eqn 1}$$

where daily biomass export equals the amount of consumed biomass and some minor spillage of leaf fragments while eating (g DW m^{-2} day^{-1}), and in our study, this also equalled seagrass production rates (see results). We clipped all the leaves within the subplots (43 cm Ø), but only harvested a smaller core (23 cm Ø) in the centre of the subplot to prevent edge effects by longer leaves. We chose to clip by hand instead of using scissors (used by Moran & Bjorndal 2007) as (i) prior tests in the field showed no differences in biomass left after grazing between both the methods and (ii) behavioural observations on *Chelonia mydas* grazing showed that our standardized hand clipping strongly resembles the natural leaf grazing behaviour. That is, on Derawan Island, turtles graze on the thin leaves of *H. uninervis* by pulling the short fragile leaves (Videos S1 and S2), causing it to break off at the thinnest point, the leaf–sheet border (personal observations). As this grazing by pulling was closely mimicked by hand clipping, we expect that possible grazing induced production of secondary metabolites and other elicitors will be equal after natural grazing and our mimicked grazing, unlike the use of scissors. Ten plots were left untreated ('cage – no grazing') and another ten plots marked by short poles were not enclosed ('no cage – natural grazing'), to allow for natural leaf grazing by green turtles.

SAMPLING REGIME

The experiment was conducted during 63 days (1 June–3 August 2008). Seagrass and epiphytes were sampled after 0, 21, 35, 49 and 63 days ($t_{0,\ 1,\ 2,\ 3,\ 4}$, respectively). The harvest at day 0 (t_0) was to establish baseline conditions, before any treatment was applied. Epiphytes were only present at $t_{0,\ 2\ \text{and}\ 3}$ and therefore only harvested thrice. Nutrient concentrations in plant tissue, pore water and surface water (water column) were determined in each plot for each sampling period. Pore water was sampled by random placement of two rhizon pore water samplers (0.2 μm pore size, 10 cm; Eijkelkamp Agri-Search Equipment, Giesbeek, the Netherlands) in the sediment of each plot, connected to a 60-mL syringe. The two samples were pooled. Samples from the water column above each plot were taken by placing the same rhizon units within the leaf canopy. Temperature, salinity, pH and $\%O_2$ of the water samples were measured immediately after sampling with a multiprobe meter (556 Multi Probe System; YSI, Yellow Springs, OH, USA). Water samples were frozen and transported to Nijmegen (the Netherlands) for nutrient analysis. To check whether treatments affected photosynthetic performance, fluorescence measurements (photosynthetic yield; diving PAM; Walz, Effeltrich, Germany) were performed on all plants at the final day of the experiment.

Seagrass biomass samples (Ø 23 cm) were taken randomly in one of the five subplots (Ø 43 cm) within each experimental plot. To minimize edge effects of the exclosure net, the outer margin of 40 cm inside the cage was not sampled. Within the core (Ø 23 cm), all above-ground and below-ground seagrass biomass was manually removed and carefully collected. Seagrasses were separated from epiphytes (filamentous algae) after which the plants were split up into four fractions; leaves, sheaths, rhizomes and roots. The following morphological characteristics were determined: presence of leaf tips, number of shoots, rhizome length, sheath length, number of leaves, leaf length and leaf width. Subsequently, dry weights of all fractions were determined after drying at 70 °C for 48 h. Organic matter contents of the upper 5 cm of the sediment were analysed by determining the weight loss of a dry weight sample on ignition at 550 °C.

To assess long-term impacts of intense turtle grazing on standing biomass, present measurements were compared to earlier and later biomass data. Seagrass biomass data were available for October 2003, June–August 2008 and July–November 2009. The data are highly comparable, as all data were collected at the same location by the same researchers, using the same methods.

NUTRIENT ANALYSIS

The concentrations of ortho-phosphate and ammonium in all water samples were measured colorimetrically, using ammonium molybdate and salicylate (Bran & Luebbe Autoanalyser III, Nordstedt, Germany) (Lamers, Tomassen & Roelofs 1998). Nitrate was determined by sulfanilamide after reduction of nitrate to nitrite in a cadmium column (Wood, Armstrong & Richards 1967). Total nitrogen and total phosphorus in the water column and pore water were measured as nitrate and ortho-phosphate after digestion with persulphate (Koroleff 1983). A homogenized portion of 3 mg dry plant material was used to determine carbon and nitrogen contents by a carbon–nitrogen–sulphur analyser (type NA1500; Thermo Fisher Scientific, Waltham, MA, USA). To analyse phosphorus and other plant nutrients, a homogenized portion of 50 mg dry plant material was digested with 1 mL HNO_3 (65%) and 1 mL H_2O_2 (30%), using an Ethos D microwave lab station (Milestone srl, Sorisole, Italy). Digestates were diluted, and concentrations of phosphorus were determined with an ICP Spectrometer (IRIS Intrepid II; Thermo Electron Corporation, Franklin, MA, USA). Stable isotope compositions for carbon and nitrogen were measured with an elemental analyser (type NA1500; Carlo Erba Thermo Fisher Scientific), coupled online via an interface (Finnigan Conflo III) to a mass-spectrometer (Thermo – Finnigan DeltaPlus). Carbon and nitrogen isotope ratios were expressed in the delta notation ($\delta^{13}C$, $\delta^{15}N$) relative to Vienna PDB and atmospheric nitrogen. Average reproducibility based on replicate measurements for $\delta^{13}C$ and $\delta^{15}N$ was 0.15‰.

LEAF PRODUCTION

Leaf biomass production was calculated for the 'no fertilizer/clipping' and 'no fertilizer/no grazing' treatment inside exclosures during the first 21 days of the experiment. The 21 days of the first clipping interval was less than the minimum leaf turnover rate of *H. uninervis* (36 days: Brouns 1987; Masini, Anderson & McComb 2001). Therefore, we assumed that loss of seagrass by detachment of dead leaves in this period was minimal. To calculate (compensatory) leaf production of 'clipping' treatment (g DW m^{-2} day^{-1}), using standing biomass (g DW m^{-2}), we used the following equation:

$$\text{Leaf production}_{\text{clipping}} = \frac{(\text{biomass}_{t1} - \text{biomass}_{t0\,\text{after initial clipping}})}{\Delta\text{days}_{t1-t0}} \quad \text{eqn 2}$$

Where $\text{biomass}_{t0\ \text{after initial clipping}}$ and biomass_{t1} are the leftover standing biomass after initial clipping on day 1 and standing biomass after 21 days (g DW m^{-2}), respectively. For the 'control – no grazing' treatment, the production (g DW m^{-2} day^{-1}) was measured as the extra growth of the seagrass from the moment of placing the exclosures over the shortly grazed seagrass shoots in 21 days as:

$$\text{Leaf production}_{\text{ungrazed}} = \frac{(\text{biomass}_{t1} - \text{biomass}_{t0})}{\Delta\text{days}_{t1-t0}} \quad \text{eqn 3}$$

BIOMASS AND NUTRIENT EXPORT VIA GRAZING

Tall vegetation, as found inside the exclusion cages, was not present in any un-caged area of the seagrass meadow. All plants outside the exclosures had the same short length representing maximum bite range (Bjorndal 1980; Williams 1988) so we assumed that green turtles consumed 100% of the daily primary leaf production of the whole meadow (but see paragraph 3 in the Discussion section for a more elaborated explanation). This assumption allowed us to calculate the daily export of leaf biomass relative to the total leaf standing stock (in % day^{-1}) and of its incorporated nutrients, nitrogen and phosphorus (mg N m^{-2} day^{-1}) (Table 3):

$$\text{Relative export biomass} = \frac{\text{leaf production}_{\text{clipping}}}{\text{total leaf biomass}_{t0}} \times 100 \quad \text{eqn 4}$$

$$\text{Export nitrogen} = \text{leaf production}_{\text{clipping}} \times \left(\frac{\%\,\text{nitrogen}}{100}\right) \times 1000 \quad \text{eqn 5}$$

$$\text{Export phosphorus} = \text{leaf production}_{\text{clipping}} \times \left(\frac{\%\,\text{phosphorus}}{100}\right) \times 1000 \quad \text{eqn 6}$$

STATISTICAL ANALYSES

A one-way ANOVA showed no significant block effect for all variables for every time step ($P > 0.12$), and therefore, the factor block was not included in further statistical models (Hines 1996). A repeated measures ANOVA (three-way RM-GLM) was conducted (time as within factor) to analyse the effects of the factors nutrient addition and grazing in time on seagrass biomass and growth, epiphyte biomass and growth, and water and plant nutrients (Table 1). If Mauchly's criterion indicated rejection of the compound symmetry assumption, adjusted probability values were presented using Greenhouse–Geiser correction. Two-way ANOVA was used to analyse the effects of leaf grazing and nutrient addition on all relevant variables for the final harvest (t_4) (Table 2). For the comparison of leaf production between grazing treatments (Fig 2a,b), we used a one-way ANOVA for the first harvest (t_1). To evaluate the differences between grazing treatments in the two-way ANOVAS for t_1 and t_4, we used Bonferroni post hoc tests for which we reported SPSS Bonferroni adjusted p-values. Data were log-transformed when necessary to meet assumptions of the ANOVAS. Differences with $P < 0.05$ were considered significant. SPSS (14.0; Chicago, IL, USA) was used for all analyses.

Results

We did not find a significant interaction between grazing and nutrient addition neither in time (RM-ANOVA; Table 1) nor on the final harvest (two-way ANOVA; Table 2) for all measured variables. Therefore, the effects for both factors are described separately.

EFFECT OF GRAZING

Under mimicked leaf clipping, *Halodule uninervis* leaf production increased significantly by 73%, from 0.32 ± 0.10 g DW m^{-2} day^{-1} (no grazing) to 0.54 ± 0.09 g DW m^{-2} day^{-1} (clipping) ($P = 0.042$) (Fig. 2a). Comparison between leaf biomass sampled in this study (June–August 2008), and samples collected in October 2003 and during July–November 2009, showed that the leaf biomass was low and constant, and measured 6.9 ± 0.1 g DW m^{-2} (Fig. 2c). Exclosures successfully prevented green turtles from seagrass grazing, as the

Table 1. Results of the repeated measures ANOVA ($t_{0,\,1,\,2,\,3,\,4}$), for all relevant variables for *Halodule uninervis*. *F* values and significance levels from the repeated measures ANOVA are shown for all main effects and their interactions *0.01 ≤ *P* ≤ 0.05, **0.001 ≤ *P* ≤ 0.01, ****P* < 0.001, ns = not significant, *n* = 5. When Mauchly's *P* < 0.05, Greenhouse–Geisser estimates were used to correct for sphericity. Above-ground biomass = leaf + sheet, below-ground biomass = rhizome + root. *T* = time, G = grazing, F = fertilizer

	Rep measures GLM4 ($t_{0,1,2,3,4}$)								
	Mauchly	Time		*T* × *G*		*T* × *F*		*T* × *G* × *F*	
	P	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Seagrass									
Above-ground biomass (g DW m^{-2})	0.20	27.09	***	1.70	ns	0.56	ns	0.60	ns
Below-ground biomass (g DW m^{-2})	0.75	17.72	***	0.20	ns	3.34	*	0.79	ns
Root:shoot (g:g)	0.17	8.75	***	1.49	ns	3.24	*	1.44	ns
Leaf length (mm)	0.06	9.17	***	7.82	ns	1.51	ns	0.68	ns
Leaf width (mm)	0.04	2.20	***	1.58	ns	0.22	ns	0.84	ns
Shoot density (m^{-2})	0.14	10.22	***	0.85	ns	0.40	ns	0.27	ns
Seagrass nutrients									
C:N of leaf	0.00	3.36	*	0.32	ns	0.56	ns	0.56	ns
C:N of rhizome	0.09	33.82	***	2.11	ns	6.48	***	1.52	ns
N:P of leaf	0.29	9.88	**	1.31	ns	1.05	ns	2.01	ns
N:P of rhizome	0.08	4.96	*	4.39	ns	4.20	*	0.70	ns
Nitrogen content of leaf (%)	0.11	4.60	**	0.45	ns	3.04	*	1.51	ns
Nitrogen content of rhizome (%)	0.01	14.54	***	1.57	ns	4.89	**	1.10	ns
Phosphorus content of leaf (%)	0.42	24.18	***	1.66	ns	0.07	ns	1.64	ns
Phosphorus content of rhizome (%)	0.79	19.80	***	3.37	ns	0.14	ns	1.85	ns
Carbon content of leaf (%)	0.00	3.93	*	0.58	ns	0.34	ns	0.37	ns
Carbon content of rhizome (%)	0.00	13.74	***	0.94	ns	1.40	ns	1.04	ns
δ^{15}N of leaf	0.00	61.05	***	1.78	ns	7.55	***	0.55	ns
δ^{15}N of rhizome	0.20	126.43	***	0.78	ns	0.33	ns	0.73	ns
Epiphyte									
Biomass (g DW m^{-2})	0.04	2.38	ns	0.27	ns	1.27	ns	1.25	ns
Nitrogen content (%)	0.27	15.11	***	2.39	ns	0.55	ns	0.74	ns
Carbon content (%)	0.07	50.91	***	0.98	ns	0.46	ns	0.57	ns
δ^{15}N epiphyte	0.18	126.78	***	0.92	ns	7.29	**	0.14	ns
Pore water									
NH_4 (μmol L^{-1})	0.00	10.77	***	1.03	ns	3.55	*	0.57	ns
NO_3 (μmol L^{-1})	0.00	3.85	*	0.39	ns	2.73	*	0.16	ns
o-PO_4 (μmol L^{-1})	0.00	10.42	***	0.70	ns	0.45	ns	0.68	ns

'no grazing' treatments showed significantly higher biomass at t_4 (Fig. 2b, Table 2) and intact leaf tips for 83.2% of the leaves. During the experiment, the standing leaf biomass within the control plot (no cage; natural grazing) initially increased during the first month and levelled off to a stable level. At the end, the biomass of the 'no grazing' plots was 67% higher than in naturally grazed plots outside the experimental plots (Fig. 2c, Table 1). We think that the corner poles have probably visually disturbed green turtles, which may have decreased natural grazing frequency between the corner poles of these plots.

Compared to the clipping treatment, the natural grazing treatment resulted in a lower biomass (clipping: natural grazing; 14.9 ± 1.5: 11.2 ± 2.1 g DW m^{-2}) and shorter leaves (clipping : natural grazing; 38.8 ± 1.5: 27.7 ± 1.6 mm) (Table 2). This indicated that the frequency of natural green turtle leaf grazing was higher than our 14-day clipping interval. Using eqn 1, we assessed the grazing frequency needed to reach natural steady-state leaf biomass to be around 12 days (Table 3). Leaf tip presence was significantly decreased by leaf grazing, from 83.2 ± 4.1% in no grazing, to 51.9 ± 3.1% in clipping and 50.1 ± 5.2% in natural grazing treatments. On average, there were two leaves per shoot; one full-grown and one developing leaf. For the clipped treatment, this suggests that all fully grown leaves had been clipped at the previous sampling and clipping event.

EFFECT OF NUTRIENT ADDITION

Under increased nutrient availability, below-ground biomass was 17.3% lower after 63 days and was reduced from 57.2 ± 4.1 g DW m^{-2} (no fertilizer) to 47.2 ± 2.2 g DW m^{-2} (fertilizer) (P = 0.013, Fig. 3b, Table 2). The root-to-shoot ratio became significantly lower in time (P = 0.015, Fig. 3i, Table 1). *H. uninervis* leaf nitrogen content (Fig. 3c), rhizome nitrogen content (Fig. 3d) and rhizome N:P ratio (Table 2) had all significantly increased after 63 days of nutrient addition, and leaf δ^{15}N (Fig. 3g) and rhizome C:N ratio (Table 2) had significantly decreased (P < 0.05, repeated measures ANOVA). Leaf δ^{15}N values were 1.63 ± 0.15 (no fertilizer) and 0.05 ± 0.32 (fertilized, P = 0.0008) (Fig. 3g). The averages at the end of the

Table 2. Results of the two-way ANOVA analysis for the final harvest (t_4) and averages for all relevant variables for *Halodule uninervis*. *F* values and significance levels from the two-way ANOVA are shown for all main effects and their interactions *$0.01 \leq P \leq 0.05$, **$0.001 \leq P \leq 0.01$, *** $P < 0.001$, ns = not significant, $n = 5$. Bonferroni-corrected significance levels of the post hoc test are shown with [a, b, c]. Values from the same row followed by the same letter are not significantly different ($P < 0.05$). Above-ground biomass = leaf + sheet, below-ground biomass = rhizome + root. G = grazing, F = fertilizer

	Two-way ANOVA t_4						Average t_4					
	G F	*P*	*F* F	*P*	*G* × *F* F	*P*	Clipping Mean ± SE	Nat. grazing Mean ± SE	No grazing Mean ± SE	No fertilizer Mean ± SE	Fertilizer Mean ± SE	Total Mean ± SE
Seagrass												
Above-ground biomass (g DW m^{-2})	3.25	*	0.00	ns	1.64	ns	$22.78^{ab} \pm 2.01$	$18.20^{a} \pm 2.71$	$27.57^{b} \pm 3.02$	22.89 ± 2.29	22.81 ± 2.37	22.85 ± 1.6
Below-ground biomass (g DW m^{-2})	0.10	ns	3.97	*	0.53	ns	52.97 ± 5.53	50.21 ± 4.32	52.19 ± 4.11	$56.37^{a} \pm 4.13$	$47.21^{b} \pm 2.91$	51.79 ± 2.6
Root:shoot (g:g)	3.03	ns	2.57	ns	1.65	ns	2.33 ± 0.25	2.76 ± 0.39	1.89 ± 0.17	2.46 ± 0.31	2.07 ± 0.18	2.27 ± 0.18
Leaf length (mm)	39.7	***	0.11	ns	2.01	ns	$38.83^{a} \pm 1.47$	$27.66^{a} \pm 1.60$	$55.92^{b} \pm 3.35$	40.38 ± 3.33	41.23 ± 3.87	40.80 ± 2.5
Leaf width (mm)	0.42	ns	0.25	ns	0.59	ns	1.22 ± 0.11	1.36 ± 0.08	1.27 ± 0.11	1.31 ± 0.06	1.25 ± 0.10	1.28 ± 0.0
Shoot density (m^{-2})	0.76	ns	0.18	ns	0.57	ns	4189 ± 437	3565 ± 531	3376 ± 445	3830 ± 384	3591 ± 393	3710 ± 271
Photosynthetic yield	0.33	ns	0.53	ns	0.21	ns	726.4 ± 15.5	739.6 ± 19.1	746.6 ± 17.1	745.1 ± 13.4	730.0 ± 14.4	737.5 ± 9.8
Leaf tip presence (%)	43.3	***	0.01	ns	1.02	ns	51.9 ± 3.10	50.11 ± 5.22	83.2 ± 4.11	$57.8^{a} \pm 4.13$	$65.6^{b} \pm 4.91$	61.7 ± 4.6
Seagrass nutrients												
C:N of leaf	0.33	ns	0.18	ns	0.16	ns	14.81 ± 0.21	15.68 ± 1.77	15.92 ± 0.43	15.71 ± 0.30	15.15 ± 1.08	15.44 ± 0.5
C:N of rhizome	0.26	ns	6.04	*	0.36	ns	29.50 ± 2.86	30.87 ± 1.77	32.89 ± 3.66	$34.86^{a} \pm 2.54$	$26.92^{b} \pm 1.45$	31.04 ± 1.6
N:P of leaf	1.16	ns	0.19	ns	0.65	ns	13.42 ± 0.85	11.95 ± 0.72	13.40 ± 0.71	12.73 ± 0.76	13.12 ± 0.48	12.92 ± 0.4
N:P of rhizome	0.11	ns	1.77	ns	0.09	ns	16.04 ± 2.01	15.47 ± 1.08	15.08 ± 1.04	14.40 ± 1.33	16.67 ± 0.88	15.53 ± 0.8
Nitrogen content of leaf (%)	0.99	ns	1.37	ns	0.98	ns	2.33 ± 0.04	2.22 ± 0.10	2.21 ± 0.04	2.21 ± 0.02	2.29 ± 0.07	2.25 ± 0.0
Nitrogen content of rhizome (%)	1.40	ns	14.26	**	0.11	ns	0.99 ± 0.06	0.86 ± 0.05	0.90 ± 0.07	$0.80^{a} \pm 0.05$	$1.03^{b} \pm 0.04$	0.92 ± 0.0
Phosphorus content of leaf (%)	1.03	ns	0.10	ns	1.51	ns	0.18 ± 0.01	0.19 ± 0.02	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.0
Phosphorus content of rhizome (%)	0.86	ns	0.40	ns	0.40	ns	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.0
Carbon content of leaf (%)	0.69	ns	0.68	ns	1.10	ns	34.40 ± 0.50	33.24 ± 1.58	34.93 ± 0.81	34.65 ± 0.54	33.79 ± 1.03	34.23 ± 0.5
Carbon content of rhizome (%)	0.20	ns	0.27	ns	2.77	ns	27.74 ± 1.38	26.63 ± 1.34	27.26 ± 1.21	26.94 ± 1.03	27.59 ± 1.10	27.25 ± 0.7
$\delta^{15}N$ of leaf	2.67	ns	23.16	***	0.28	ns	0.29 ± 0.40	0.86 ± 0.44	1.37 ± 0.26	$1.63^{a} \pm 0.15$	$0.05^{b} \pm 0.32$	0.84 ± 0.2
$\delta^{15}N$ of rhizome	0.85	ns	0.29	ns	0.06	ns	0.82 ± 0.17	0.76 ± 0.36	1.22 ± 0.19	1.02 ± 0.22	0.85 ± 0.20	0.93 ± 0.1
Epiphyte												
Biomass (g DW m^{-2})	0.37	ns	0.03	ns	0.92	ns	10.09 ± 3.49	9.09 ± 3.09	13.85 ± 6.19	11.15 ± 4.51	10.69 ± 2.32	10.91 ± 2.4
Nitrogen content (%)	2.21	ns	4.01	*	0.26	ns	2.08 ± 0.09	1.54 ± 0.18	2.18 ± 0.17	$1.87^{a} \pm 0.14$	$2.01^{b} \pm 0.14$	1.94 ± 0.1
Carbon content (%)	2.25	ns	0.08	ns	0.05	ns	32.82 ± 0.80	31.70 ± 1.29	28.94 ± 1.69	31.04 ± 1.20	31.35 ± 1.06	31.20 ± 0.7
$\delta^{15}N$	2.03	ns	11.26	**	2.55	ns	0.37 ± 0.12	0.55 ± 0.10	0.25 ± 0.15	$0.19^{a} \pm 0.09$	$0.59^{b} \pm 0.09$	0.39 ± 0.0
Pore water												
NH_4 (µmol L^{-1})	0.84	ns	12.80	**	0.79	ns	38.08 ± 29.26	14.56 ± 10.10	8.99 ± 1.56	$4.80^{a} \pm 0.83$	$36.29^{b} \pm 15.96$	20.54 ± 10.4
NO_3 (µmol L^{-1})	0.67	ns	1.89	ns	0.02	ns	24.16 ± 23.12	5.58 ± 5.06	3.26 ± 2.89	0.53 ± 0.24	21.47 ± 15.51	11.00 ± 7.8
o-PO_4 (µmol L^{-1})	1.06	ns	3.01	ns	0.34	ns	0.52 ± 0.12	0.33 ± 0.06	0.38 ± 0.06	0.35 ± 0.08	0.47 ± 0.06	0.41 ± 0.5

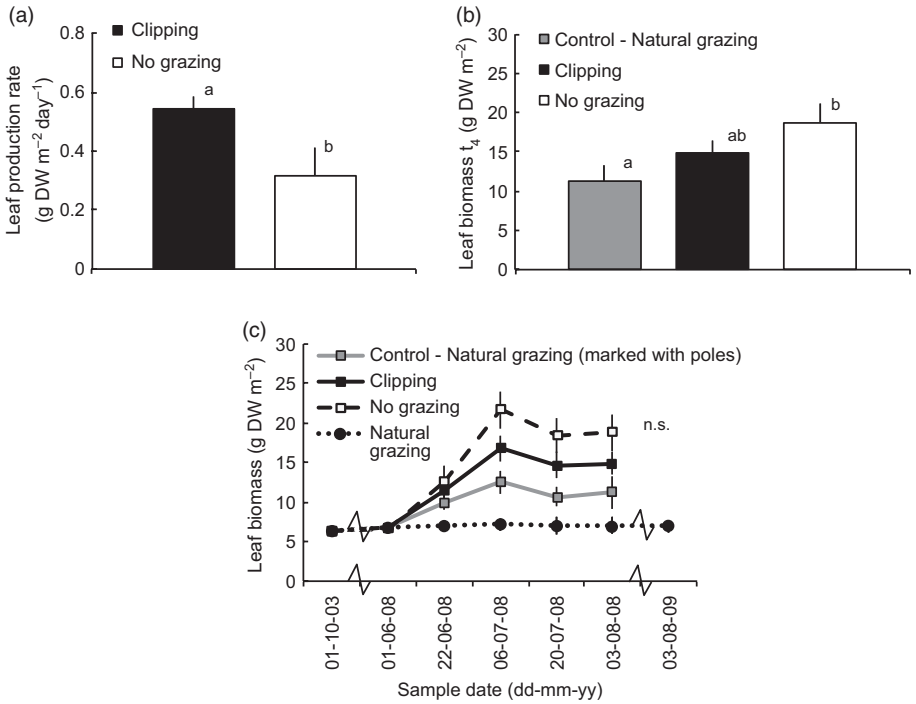

Fig. 2. Effects of mimicked turtle leaf grazing (clipping) and the absence of grazing on *Halodule uninervis* (mean ± SE); (a) leaf production, (b) standing leaf biomass after 63 days (t_4) and (c) biomass before, during and at the end of the experiment. Significant differences are shown by different letters in the legend, $*P < 0.05, n = 10$.

Table 3. Synthesis of results. Calculations of seagrass leaf biomass, nutrient pools and fluxes in a simplified seagrass system with high green turtle densities. Letters correspond to those presented in Figure 5 (mean ± SE). Consumption = leaf intake and spillage during foraging. We assume that in this system, green turtles are net exporters of biomass and nutrients, and therefore, consumption equals export

	Mean ± SE	Fig. 5
Total biomass in grazed meadow (g DW m^{-2}) t_0	41.21 ± 5.46	
Leaf biomass in grazed meadow (g DW m^{-2}) t_0	6.81 ± 0.58	
Clipped leaf biomass (g DW m^{-2}) t_0	5.75 ± 0.65	
Leaf production after clipping = at least leaf consumption by green turtles (g DW m^{-2} day^{-1}) (Eqn. 2) t_1	0.55 ± 0.04	a
Green turtle density 2008 (turtle m^{-2}) (Christianen, unpublished results)	0.0015 ± 0.0002	
Consumption of leaf biomass per green turtle (g DW m^{-2} day^{-1} turtle^{-1})	364 ± 67	a
Epiphyte biomass = (10.94* o-PO_4 µmol L^{-1}) – 1.59 (if o-PO_4 > 0.4 µmol L^{-1})		c
Relative decrease (%) of below-ground biomass by N loading rates of 136 -181 mmol m^{-2} day^{-1} t_4	11.4 ± 0.22	d
Consumption of leaf biomass by green turtles, relative to standing biomass (%) (Eqn. 4)	8	
Consumption of leaf biomass by green turtles, relative to daily leaf production (%)	100	
Natural grazing interval = days needed for regrowth of consumed leaf biomass under current leaf grazing regime (day^{-1}) (Eqn. 1)	12	
% N	2.33 ± 0.08	
% P	0.26 ± 0.01	
N export (mg N m^{-2} day^{-1}) (Eqn. 5)	12.71 ± 3.77	b
P export (mg P m^{-2} day^{-1}) (Eqn. 6)	1.42 ± 0.29	b

experiment for phosphorus content of leaves and rhizomes, leaf biomass, phosphorus (o-PO_4) in pore water revealed no significant differences between the no fertilizer and fertilizer addition treatments (Fig. 3, Table 2). For the different variables roots, and tissue phosphorus, iron and carbon contents of all plant parts, no effect of nutrient addition was found.

Calcium carbonate sediments have a high affinity for phosphorus, making it less readily available for uptake. This could potentially explain the lack of seagrass response to P addition. To rule out this possibility, we tested different sources and methods of phosphorus addition in pilot experiments, including the addition of a high dose of small-granule hydrated tri-calcium phosphate and additional osmocote by spreading the beads evenly on top of the sediment. This had, however, no effect on phosphorus uptake nor on growth in any of the pilots (Appendix).

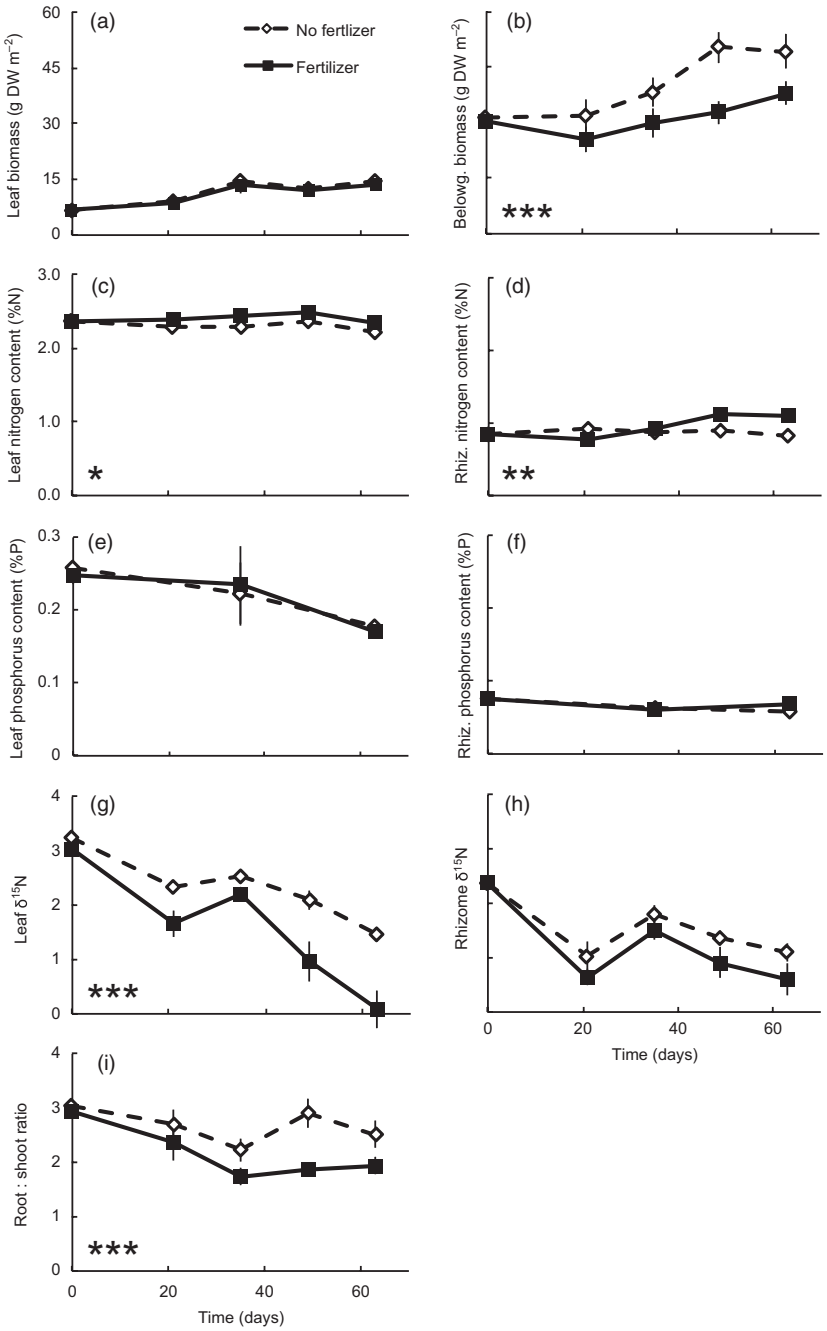

Fig. 3. Effects of nutrient addition on *Halodule uninervis* (mean ± SE) with significance levels of the repeated measures ANOVA ($n = 5$) for (a) leaf biomass, (b) below-ground biomass (= root + rhizome), (c) leaf nitrogen content, (d) rhizome nitrogen content, (e) leaf phosphorus content, (f) rhizome phosphorus content, (g) leaf $\delta^{15}N$, (h) rhizome $\delta^{15}N$, (i) root-to-shoot ratio. Because grazing had no effect in time on these variables, values for different grazing treatments have been pooled to 'no fertilizer' and 'fertilizer', $n = 15$. *$0.01 \leq P \leq 0.05$, **$0.001 \leq P \leq 0.01$, ***$P < 0.001$.

NUTRIENT LEVELS IN THE WATER COLUMN AND PORE WATER

Ammonium (NH_4) and nitrate (NO_3) in pore water were significantly increased after nutrient addition in time ($P < 0.05$, repeated measures ANOVA), and at the end (t_4) of the experiment NH_4 in the pore water was still increased from 4.8 ± 0.8 μmol L^{-1} (no fertilizer) to 36.3 ± 15.9 μmol L^{-1} (fertilizer) ($p = 0.002$), in contrast to NO_3 concentrations that averaged 11.0 ± 7.7 μmol L^{-1} for both treatments (2-way ANOVA, Table 1). Pore water NO_3 concentrations ranged between 0.3 and 21.5 μmol L^{-1}, and NH_4 concentrations ranged between 4.3 and 71.4 μmol L^{-1}. NO_3 and NH_4 concentrations in the water column were unaffected by nutrient addition (results not shown). Water column NO_3 concentrations ranged between 0.3 and 10.3 μmol L^{-1}, and NH_4 concentrations ranged between 1.2 and 28.9 μmol L^{-1}.

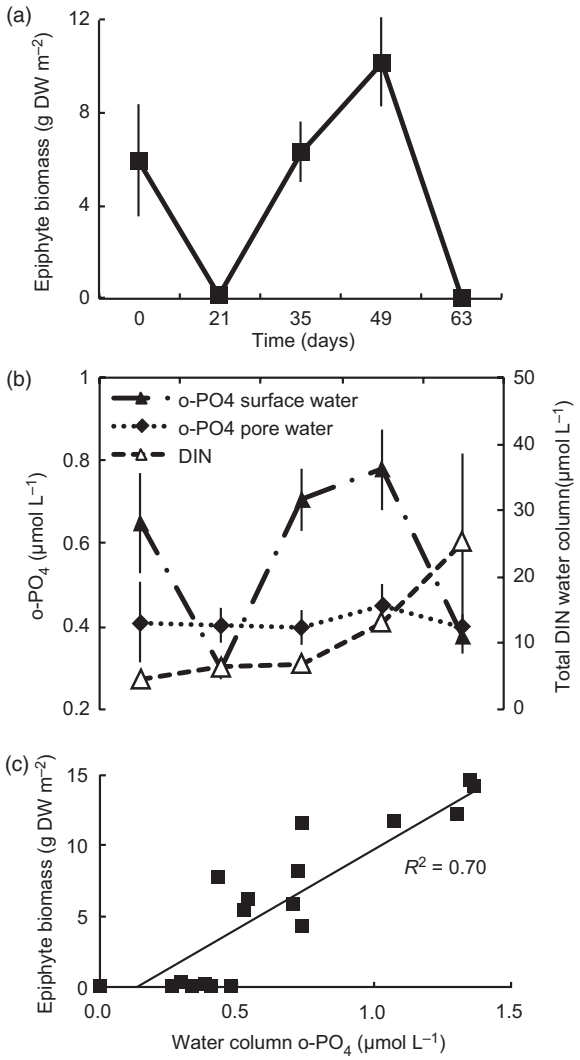

Fig. 4. (a) Biomass of epiphytes, (b) ortho-phosphate (o-PO_4) and dissolved inorganic nitrogen (DIN) in the water column (mean ± SE) and (c) correlation between epiphyte biomass and ortho-phosphate with its correlation coefficient ($P = 0.01$), $n = 30$.

Ortho-phosphate (o-PO_4) levels ranged between 0.30 and 0.78 µmol L^{-1} in the pore water and between 0.23 and 0.85 µmol L^{-1} in the water column (Table 2). Concentrations in the water column were more variable in time (Fig. 4b), in contrast to pore water concentrations. Concentrations of o-PO_4, salinity, pH and $\%O_2$ of the pore water and water column samples did, however, not show differences between treatments.

EPIPHYTES AND PHOSPHATE

Epiphyte biomass attached to *H. uninervis* strongly fluctuated in time (Fig. 4a). Epiphyte biomass (g DW m^{-2}) was highly correlated with the fluctuating o-PO_4 levels in the water column (Fig. 4, $R^2 = 0.65$, $P = 0.01$). During our experiment, we only observed epiphyte biomass when the o-PO_4 concentration exceeded ±0.4 µmol L^{-1} in the water column, which may suggest a threshold for epiphyte blooms. The epiphyte community was dominated by long filamentous algae, which detached when the clumps of epiphytes grew so large that entrapped oxygen bubbles (from photosynthesis) made them float to the surface. Although nutrient addition led to significant depletion of $\delta^{15}N$ values and increased nitrogen contents of the seagrass epiphytes from 1.9 ± 0.1 to 2.0 ± 0.1%, we did not find a correlation between epiphyte biomass and nitrogen content, ($P = 0.001$, Table 2). Dissolved inorganic nitrogen (DIN) or pore water o-PO_4 concentrations did not show a correlation with the fluctuation in epiphyte biomass (Fig. 4b). As the o-PO_4 concentrations were not correlated with the nutrient addition treatment, its source cannot be the slow-release fertilizer applied.

NITROGEN, PHOSPHORUS AND BIOMASS EXPORT

The sediment samples collected by the corer showed that a detrital layer was almost absent; total organic matter content of the sediment was as low as 1.2 ± 0.1%. Assuming that the daily clipped leaf biomass inside exclosures equals the minimal daily intake and spills by natural turtle grazing and that turtle excrements are exported from the meadow, the green turtles exported at least 0.6 ± 0.1 g DW m^{-2} day^{-1} (eqn 2). This indicates that per day on average 8.0% of the total standing leaf biomass is removed (eqn 4, Table 3). By multiplying the daily exported biomass by the nutrient content, we calculated that green turtle leaf grazing led to an export of 12.7 ± 3.8 mg nitrogen m^{-2} day^{-1} and 1.4 ± 0.3 mg phosphorus m^{-2} day^{-1} (Table 3). The yearly biomass production at our study site was 197.1 g m^{-2} $year^{-1}$.

Discussion

The ongoing global seagrass decline is generally suggested to be the indirect result of increased nutrient loads, causing seagrasses to be overgrown by algae and epiphytes (Hauxwell, Cebrian & Valiela 2003; Orth *et al.* 2006; Burkholder, Tomasko & Touchette 2007; Waycott *et al.* 2009; van Katwijk *et al.* 2010). At the same time, most seagrass stands nowadays grow in the absence of megaherbivores such as green turtles and serenians, which were present during the past 100 million years (Larkum & den Hartog 1989; Jackson 2001). This raises the question whether the large-scale loss of grazing may have intensified the impact of nutrient loading. To our knowledge, the present study is the first that experimentally tested the role of large marine herbivores in the functioning of seagrass ecosystems while exposed to high nutrient loads and to quantify the nutrient and biomass export by green turtle grazing. We found that grazing almost doubled leaf biomass production rates and resulted in a substantial daily export of biomass (8%) and its incorporated nutrients. Nutrient addition resulted in decreased rhizome biomass. In addition, epiphyte growth was strongly correlated with high water column o-PO_4 concentrations. Top–down control by megaherbivore grazing, rather than bottom–up control by nutrient limitation, appeared to be the key factor determining seagrass growth. The present results

show the pivotal (now mostly historical) role of megaherbivores in coastal systems.

EFFECTS OF GRAZING ON BIOMASS PRODUCTION

Megaherbivore (green turtle) leaf grazing was shown to have a strong effect at multiple levels in the seagrass ecosystem; it doubled seagrass production and increased exports (see next paragraph). The increase in seagrass leaf production enhances the seagrass biomass that is available for green turtle grazing, resulting in a positive feedback. A similar increase in productivity after grazing was reported for both terrestrial grasses and seagrasses (McNaughton 1985; Valentine *et al.* 1997; Valentine 2000; Moran & Bjorndal 2005) suggesting compensation for biomass loss by herbivory, at least for a short period. Our study showed increased productivity even after exposure to intensive grazing of at least 6 years, as shown by standing seagrass biomass comparisons over time.

The low standing leaf biomass remained unchanged over a period of 6 years, and together with the finding that leaves were young and leaf tips were predominantly (82%) absent, this suggests that the total daily leaf production is harvested when green turtles consume or spill the leaves during foraging, throughout the year. This is further supported by the natural grazing frequency (12 days), which is almost equal to the plastochron interval, the interval between the initiation of successive leaves, of Indo-Pacific *H. uninervis* (9.6 days: Vermaat *et al.* 1995; 10.5 days: Uy 2001). Green turtles did not maintain particular grazing plots at our sites, unlike what was observed for green turtles grazing on *Thalassia testudinum* in the Atlantic Ocean (Bjorndal 1980; Ogden, Tighe & Miller 1980; Williams 1988), but they instead grazed the whole meadow down to an average grazed leaf length of 28 mm, which was shorter than the average bite size of green turtles which was 50 mm (Bjorndal 1980; Williams 1988). While it is known that aquatic herbivores are able to temporarily graze 100% of the daily primary production (Cebrian 2004), previous researchers have not described a constant high grazing pressure on seagrass meadows as found in this study, indicated by the continuously low standing stock of seagrass (2003–2010) and by incidental turtle density measurements (2008–2010; 15 turtles ha^{-1} in 2008; Christianen, unpublished data).

The high grazer densities of the marine coastal system we studied support the resource availability hypothesis (Coley, Bryant & Chapin 1985; Endara & Coley 2011). The seagrass plants in our study have adapted to an environment that is relatively rich in resources and show high inherent growth rates and short leaf lifetimes. The amounts of constitutive defences are low due to carbon allocation trade-off, which maximizes the realized growth even though this supports higher herbivory rates.

EFFECTS OF GRAZING ON NUTRIENT EXPORT

Green turtle grazing results in a substantial biomass export (here: 8% of standing biomass day^{-1}), which is equal to 100% of the daily primary production, and this corresponds to nutrient export rates of 13 mg N m^{-2} day^{-1} and 1.4 mg P m^{-2} day^{-1}. In contrast to most recent seagrass ecosystems, which are detritus-based (Valentine & Duffy 2006), the leaves in these meadows were grazed down to such a length that they could hardly trap detritus, and consequently, the total organic matter content was as low as 1.2% and any form of detrital layer was absent. This suggests that the majority of the primary production is either converted into secondary production for green turtle biomass, metabolism and reproduction, or exported after turtle excretion or as spill during outgoing tide (personal observation). As a result, the seagrass detrital cycle is partly opened up because the nutrient-rich faecal seagrass material is being exported from seagrass the ecosystem, to more distant systems.

The exported seagrass may therefore be an important link that connects food webs of surrounding habitats. Green turtle faeces and spill floats, and faeces and urine are excreted while turtles graze in the seagrass meadow or while they rest on the fringing coral reef between foraging shifts during low tide (Bjorndal 1980; Balazs, Fujioka & Fujioka 1993; Heck *et al.* 2008). We assume that the excess of organic matter was exported to this reef zone (max 500 m distance) or across the reef to the open ocean's deep trenches, where seagrass detritus may support deep-sea food webs (Suchanek *et al.* 1985; Vetter 1998) and help to sequester carbon (e.g. Duarte, Middelburg & Caraco 2005). This immense trophic transfer of seagrass material by megaherbivores may, in oligotrophic areas, also strongly enhance growth rates of coral reefs and other surrounding habitats, as studies on fish grazing have shown previously (Meyer & Schultz 1985; Heck *et al.* 2008). This suggests a strong impact of megaherbivore density on production rates in coastal areas including coral reefs and deeper ocean ecosystems.

EFFECTS OF GRAZING ON PLANT NUTRIENTS

Continuous intense green turtle grazing is generally assumed to deplete seagrass reserves (e.g. nutrients) especially given the higher production that has to be maintained in grazed plots. However, the simulated leaf grazing did not decrease seagrass nutrient content compared to ungrazed seagrass, which is in contrast to other studies that described less intense grazing (Moran & Bjorndal 2005, 2007; Aragones *et al.* 2006; Heck *et al.* 2006; Fourqurean, Muth & Boyer 2010). This unexpected response may be explained by (i) the long history of grazing on Derawan Island, which has resulted in a shift towards an early successional species and (ii) low standing biomass (6.5 g DW m^{-2}), which may be up to eighteen times lower than in an average oligotrophic meadow that is not grazed by megaherbivores (e.g. compared with 118 g DW m^{-2} measured in Sulawesi, Vonk, Christianen & Stapel 2010) or 3) nutrient input from the nearby river (located at a distance of 16 km from river mouth (van Katwijk *et al.* 2011). Like small invertebrate grazers are able to buffer effects of moderate nitrogen enrichment on algae in the Baltic Sea (Worm, Lotze & Sommer 2000), we now show

that green turtles are able to buffer the effect of nitrogen loads at a larger scale.

The dominance of the early successional species *Halodule uninervis* as a result of grazing has also been observed under high grazing pressure by dugongs (Preen 1995). This seagrass species is more tolerant to grazing due to its high productivity and strong recolonization ability. Increased green turtle densities in other foraging areas in the Indian Ocean have also caused a shift towards an early successional species and a reduction of structure complexity (Lal *et al*. 2010). However, as seen in other coastal ecosystems, the shift towards a species with a less complex canopy could also have changed the response of the plant to resource loading (Eriksson, Rubach & Hillebrand 2006). In addition, the change to a more productive system might have increased the effect of herbivores on species diversity (Worm *et al*. 2002).

EFFECTS OF NITROGEN LOADING ON PLANTS

The only bottom–up effect that we found on the pristine *H. uninervis* meadow was that nitrogen addition resulted in a 23% decline of the below-ground biomass, while above-ground biomass was not affected. The strong decrease in leaf $\delta^{15}N$ levels during and after 63 days of nutrient addition showed that plants discriminated more strongly against the ^{15}N isotope and thus that nitrogen was in surplus by the fertilizer addition. Seagrasses can extract nitrogen both from sediment pore water (mostly NH_4^+)and from the overlying water column (especially NH_3^-) (Stapel *et al*. 1996; Lee & Dunton 1999; Touchette & Burkholder 2000;. Elevated nutrient levels in the water column may have decreased the need to take up nitrogen by the roots and favoured leaf uptake, which has a higher uptake affinity than root tissue (e.g. Pedersen, Paling & Walker 1997; Lee & Dunton 1999; Burkholder, Tomasko & Touchette 2007). This may explain the increased tissue nitrogen concentrations in the above-ground parts and the decreased below-ground biomass. A decreased root-to-shoot ratio has been found previously. Here, nitrogen enrichment simultaneously reduced below-ground biomass and increased above-ground biomass, not only in the species that we studied *H. uninervis* (Udy & Dennison 1997; Lee & Dunton 1999) but also in experiments on terrestrial species (Aerts, Boot & van der Aart 1991). However, never before was a decreased root-to-shoot ratio found to be the result of a decreased below-ground biomass alone. If root stabilization decreases under increasing nutrient loads, one of the important ecosystem services of seagrass meadows, the stabilization of shorelines, may decline. During extreme storm events, high wave energy can more easily uproot the seagrass plants, leading to seagrass erosion and loss, with slow and sometimes minimal recovery (Fonseca 1983; Kirkman & Kirkman 2000). The response of *H. uninervis* in our study was contrasted with the responses of *H. uninervis* in a previous experiment on nutrient addition (Udy & Dennison 1997), indicating that the responses to nutrient addition can also depend on the nutritional status of the specific seagrass meadow.

EFFECTS OF PHOSPHATE LOADING ON PLANTS

Because neither phosphorus concentrations nor leaf biomass changed after fertilizer addition, we cannot conclude that the added phosphorus was available to the seagrasses or whether there was phosphorus limitation. However, the high natural fluctuations in ortho-phosphate concentrations, measured in the water column, did not result in increased seagrass growth. Based on a combination of pilot experiments with other phosphorus sources and a correlative study in the Berau archipelago (van Katwijk *et al*. 2011), we suggest that seagrass growth was unlikely to be phosphorus limited, in view of the phosphorus levels we found. The limited effect of phosphorus addition on seagrass was also found previously in studies where phosphorus had been added as slow-release granules (Neckles, Wetzel & Orth 1993; McGlathery 1995; Udy *et al*. 1999; Heck *et al*. 2000, 2006).

EFFECTS OF PHOSPHATE LOADING ON EPIPHYTES

An important outcome of our study was that epiphyte biomass correlated positively with pulses of high ortho-phosphate levels in the water column. To our knowledge, such a strong correlation with epiphyte growth has not been found before in the field. In our system, epiphytes, mainly filamentous algae, only started to grow above a threshold o-PO_4 concentration of 0.4 μmol L^{-1}, which is higher than the levels found in Florida Bay where epiphyte load was described as a poor indicator of phosphorus availability (Fourqurean, Muth & Boyer 2010). The o-PO_4 and epiphyte level varied strongly over time, as was found in previous studies (Fourqurean, Muth & Boyer 2010). Fluctuating phosphorus concentrations may also have followed local plankton blooms, nutrient upwelling or periods of heavy rain above the mainland, causing increased nutrients from river discharge from the nearby Berau River. It has well been established that cultural eutrophication can stimulate algal overgrowth, which reduces the available light, flow, nutrient, oxygen and carbon availability for seagrasses and promotes their decline (see reviews by Duarte 1995; Burkholder, Tomasko & Touchette 2007; Michael *et al*. 2008). In our experiment, seagrass biomass was not shown to be negatively affected by epiphytal overgrowth, probably because the epiphyte bloom lasted only for a maximum of 20 days and epiphytes started to float away after some time.

EFFECTS OF GRAZING ON EPIPHYTES

The reduction of seagrass overgrowth by selective epiphyte grazing, as found for mesoherbivores (Short, Burdick & Kaldy 1995; Gacia, Littler & Littler 1999; Heck *et al*. 2000; Boyer *et al*. 2004; Hays 2005), is of minor importance at our study site, as megaherbivores cannot selectively remove epiphytes from leaves. However, high green turtle densities may reduce epiphyte overgrowth of seagrass by (i) indirectly decreasing nutrient contents in the meadow by exporting seagrass, (ii) shortening the leaves and reducing the proportion of old leaves, which are the preferred substrate for epiphytes (Heijs

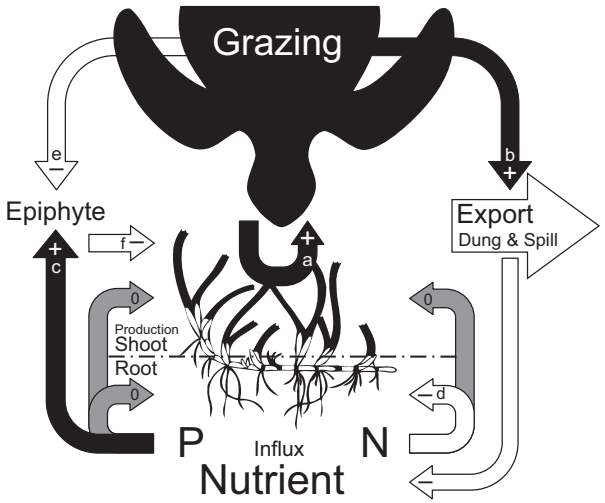

Fig. 5. A conceptual model of seagrass functioning under megaherbivore leaf grazing and relatively high nutrient loads. Leaf grazing by green turtles increases seagrass production (arrow a) and also increases net export of the daily primary production (arrow b). Thus, under megaherbivore grazing, the export of nutrients from the seagrass system increases, which indirectly increases the tolerance of the system to nutrients overloading. When extra nutrients are added, rhizome biomass decreases (arrow d). This may result in a destabilization of the sediment, increasing the vulnerability to storms. Additionally, from correlative evidence in our study and from literature, it is known that nutrients will increase epiphyte loads (arrow c), but grazing will decrease epiphyte overgrowth (arrow e), which will directly improve conditions for seagrass (arrow f). Plus, minus and zero signs indicate positive, negative and absent effects of one flux or turnover rate on another. Letters a–d correspond to this study (Table 3), (e) Short, Burdick & Kaldy 1995; (f) Valentine & Duffy 2006. *N* = effect of increased nitrogen, P = effect of increased phosphorus. Note the positive feedback of grazing on leaf production, providing more food for turtles.

1985; Borum 1987; Fourqurean, Muth & Boyer 2010), (iii) eating epiphytes together with seagrass and (iv) removing the large filamentous algae by their flipper movements while feeding in the seagrass meadow, thereby preventing long lasting epiphyte blooms (personal observation).

MEGAHERBIVORES INCREASE SEAGRASS TOLERANCE UNDER HIGH NUTRIENT LOADS

We propose a conceptual model of seagrass functioning under megaherbivore leaf grazing and eutrophication by combining present results with literature data (Fig. 5). In this study, we showed that (i) grazing increased seagrass production by a factor of 1.7 (arrow a in Fig. 5), thereby increasing the food availability for green turtles and (ii) the amount of seagrass biomass and nutrients exported by the turtles was 8% of the standing biomass per day, equalling 13 (N) and 1.4 (P) mg m^{-2} day^{-1} (arrow b in Fig. 5). This export by megaherbivores is probably the most important controlling factor for seagrass under grazing and high nutrient loads. By decreasing the nutrient pool in the meadow, the export indirectly reduces epiphyte overgrowth of seagrass (arrow c in Fig. 5) and prevents rhizome biomass decrease (arrow d in Fig. 5). When nutrients are increased, grazing thereby directly improves conditions for seagrass (arrow f in Fig. 5) and inhibits destabilization of the sediment, resulting in an increased tolerance of the seagrass to disturbance by storms. This, however, also implies that, under increasing nutrient loads, the system's ability to absorb shocks, resist phase shifts and regenerate after natural and human-induced disturbances will be more and more dependent on the continuation of green turtle grazing and the subsequent nutrient export. The loss of green turtles, e.g. by illegal harvesting of eggs and adults, is therefore very likely to render the system more vulnerable to the detrimental effects of eutrophication. The conceptual model that we propose here (Fig. 5) needs further empirical testing, but this will be difficult because pristine seagrass areas with high green turtle densities are increasingly scarce. In this paper, we provide experimental evidence that offers a better explanation of the pivotal, but unfortunately mainly historical role of megaherbivores in seagrass ecosystem functioning, which is critical for effective management of these important natural resources.

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Supporting Informaiton

Additional Supporting Information may be found in the online version of this article.

Video S1. http://www.youtube.com/watch?v = RwPEhSLq23Q.

Video S2. http://www.youtube.com/watch?v = EiUVCHEsLc4.

Appendix A. Pilot experiment phosporus addition.

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