



Early warning indicators for river nutrient and sediment loads in tropical seagrass beds: A benchmark from a near-pristine archipelago in Indonesia

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ABSTRACT

In remote, tropical areas human influences increase, potentially threatening pristine seagrass systems. We aim (i) to provide a bench-mark for a near-pristine seagrass system in an archipelago in East Kalimantan, by quantifying a large spectrum of abiotic and biotic properties in seagrass meadows and (ii) to identify early warning indicators for river sediment and nutrient loading, by comparing the seagrass meadow properties over a gradient with varying river influence. Abiotic properties of water column, pore water and sediment were less suitable indicators for increased sediment and nutrient loading than seagrass properties. Seagrass meadows strongly responded to higher sediment and nutrient loads and proximity to the coast by decreasing seagrass cover, standing stock, number of seagrass species, changing species composition and shifts in tissue contents. Our study confirms that nutrient loads are more important than water nutrient concentrations. We identify seagrass system variables that are suitable indicators for sediment and nutrient loading, also in rapid survey scenarios with once-only measurements.

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1. Introduction

Seagrass losses accelerate worldwide, whilst recovery is absent or slow and restoration success is low (e.g. Godet et al., 2008; Paling et al., 2009; van Katwijk et al., 2009; Waycott et al., 2009). As to date, most of the still pristine meadows including those in tropical regions are increasingly threatened by rapidly growing economic activities (e.g. Kamp-Nielsen et al., 2002; Freeman et al., 2008), with the combination of deforestation, fish and shrimp culturing and increasing inhabitation generating increasing nutrient and sediment loads in coastal waters. As recent studies indicate that seagrass collapse may follow alternative stable state theory, implying the risk of unexpected fast collapse (e.g. Munkes, 2005; van der Heide et al., 2007, 2010a), it is important to obtain good reference values based on relatively pristine meadows now that they still are present, and identify early warning indicators.

In oligotrophic waters, increased total nutrient loads may initially lead to non-significant, or small increases in water and sediment nutrient concentrations, because the extra nutrients are rapidly incorporated in algae, seagrasses and microorganisms

(e.g. Tomasko et al., 1996; van Tussenbroek et al., 1996; Morris and Virnstein, 2004; Burkholder et al., 2007). When regarding nutrient loading, it is important to include the loads of organically bound nutrients, as they are rapidly available to seagrass beds and macro algae, either after mineralisation or directly (Evrard et al., 2005; Barrón et al., 2006; Vonk and Stapel, 2008). With increasing nutrient loading, the seagrass system will at some point shift towards an algae dominated system. Due to feedback processes, such transition between seagrass dominance and bare sediments with algal dominance will often not be linear (Duarte, 1995; Terrados et al., 1998; Morris and Virnstein, 2004; Munkes, 2005). The presence of strong positive and negative feedbacks make seagrass systems liable to sudden and irreversible state transitions in line with alternative stable states theory (e.g. Duarte, 1995; Munkes, 2005; van der Heide et al., 2007, 2010a). Accordingly, recovery may take decades to millennia (Rollon et al., 1998; Moore et al., 2000; Frederiksen et al., 2004; González-Correa et al., 2005; Bernard et al., 2007; van der Heide et al., 2007; Godet et al., 2008). The non-linearity of seagrass declines, the fact that natural recovery is slow or absent and restoration difficult (e.g. Paling et al., 2009; van Katwijk et al., 2009, 2010) stresses the importance of the recognition of early warning indicators.

Before the increasing sediment and nutrient loads will cause a total shift of the system towards algal dominance (Duarte, 1995; Havens et al., 2001; Munkes, 2005), seagrass systems can respond

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at different levels (reviews in Burkholder et al. (2007) and Leoni et al. (2008)). At the individual plant level, physiological changes occur, like tissue nutrient levels, free amino acid composition, productivity and fluorescence parameters (as an estimate of vitality) (Erftemeijer, 1994; Agawin et al., 1996; Udy and Dennison, 1997; van Katwijk et al., 1997, 1999; Armitage et al., 2005; Olive et al., 2009). At the community level, shifts in seagrass species composition occur (e.g. Terrados et al., 1998; Agawin et al., 1996; Uku and Bjork, 2001; Kamermans et al., 2002; Herbert and Fourqurean, 2008), along with increased epiphyte loads (Bohrer et al., 1995; Uku and Bjork, 2001) and shifts in epiphyte species composition (van Tussenbroek et al., 1996; Uku and Bjork, 2001, but see Fourqurean et al. (2010)). As a result of these changes and/or increased organic loads, the species composition of seagrass-associated benthic and pelagic fauna will change (McClelland and Valiela, 1998; Valentine and Heck, 2001).

In this study, we aim to (i) provide a bench-mark for a near-pristine seagrass system in an archipelago in East Kalimantan, by quantifying a large spectrum of abiotic and biotic properties in seagrass meadows and (ii) identify early warning indicators for river sediment and nutrient loading, by comparing the seagrass meadow properties over a gradient with varying river influence. Measured properties were all static (in contrast to dynamic variables like growth and production), because potential indicators that require repeated measuring are less suitable for surveys in remote areas with limited accessibility and facilities. Potential indicators for the health of seagrass beds were evaluated at three levels: individual level (plant physiology and morphology), community level (seagrass species composition and benthic macrofaunal biomass) and landscape level (seagrass cover). Potential abiotic indicators for nutrient and terrestrial sediment loading are measured in water, pore water and sediments. As a proxy for the temporal scale required for early warning indicators for increased riverine nutrient loads, we used a spatial scale, i.e. zones of increasing distance to the river mouth. Note that these zones also vary with the distance to the coast, so coastal run-off and exposure to water dynamics vary accordingly, additionally contributing to nutrient availability.

The research was performed in the Berau archipelago at East Kalimantan, Indonesia, which is a particularly suitable study area as it represents a relatively pristine situation, where eutrophication and increased sediment loads are still limited, although currently increasing (e.g. de Voogd et al., 2009). Deforestation of the catchment for timber logging and coal mining activities has only started during the last one or two decades and 80–85% of the rain forests is still present.

2. Materials and methods

2.1. Study area

The Berau archipelago (East Kalimantan, Indonesia), consists of 31 islands in front of the Berau river mouth (Fig. 1). In 2005, the area was declared a Marine Protected Area covering an area of 12,700 km² (IUCN-WCPA, 2008). Upstream land based activities as logging, coal mining, or estate crops increased deforestation, from an area of 2530 (1990), to 4781 km² (2005) (Permana, submitted for publication). In addition, the area for oil palm, rubber and timber plantations have increased from 405 (1990) to 1099 (2005) km², and the swamp forest is logged at a high rate for shrimp farming (Permana, submitted for publication), which all may contribute to the eutrophication and sedimentation in the Berau archipelago. The seagrasses inhabit inter- and subtidal reef flats (maximal tidal range 2.1 m) surrounding the islands, with reefs on the outer edge of the reef flat. The islands of Derawan, Panjang, Samama and Maratua are an important feeding ground

for high densities of green turtles *Chelonia mydas* (15 turtles/ha Derawan Island, Christianen, unpublished data), and occasionally of dugong *Dugong Dugon*. Coral reef geography and reef related biodiversity in the Berau archipelago are extensively documented in Renema (2006) and de Voogd et al. (2009), as are the surrounding water quality and flow patterns (Buschman et al., 2009; Tarya et al., 2010; Ambarwulan et al., 2011).

In October 2003, at 15 seagrass sites spread over 7 islands and 1 mainland location in the Berau archipelago (Fig. 1, 2°05'–2°21'N–118°37'–118°04'E), covering an area of ca. 200 km², we estimated plant characteristics and sampled and analysed plants, macrofauna, sediments, sediment pore water and overlying water. Three zones were distinguished, the outer reef, intermediate zone and coastal zone based upon their distance to the river (Fig. 1). The delta-front barrier reef system (intermediate zone) may block the river discharge to outer, offshore reefs (Tomascik et al., 1997).

2.2. Rationale for the potential indicators measured in this study

We expect tissue nutrient contents to reflect nutrient availability and thus be a suitable indicator for increased nutrient loading (e.g. Duarte, 1990; Erftemeijer, 1994; Güsewell et al., 2003; Johnson et al., 2006; van Katwijk et al., 1997; Burkholder et al., 2007). In addition we measured several other tissue elements to test whether low salinity influence of the river water would be reflected in the elemental composition and in this way provide a useful indicator for river influence. We expect the percentage of low C/N amino acids to increase under nitrogen loading (Udy and Dennison, 1997; Burkholder et al., 2007; van der Heide et al., 2008; Christianen et al., 2011). Photosynthetic yield (PAM fluorometry) may serve as an indicator for plant health, and is known to respond to light availability (e.g. due to sediment loading or phytoplankton blooms) (e.g. Campbell et al., 2007; Silva et al., 2009).

Seagrass cover, aboveground biomass, leaf length and width can be affected by nutrient loading and shading; in general an increase is recorded during the initial phases of eutrophication, followed by a decrease when eutrophication is more severe (e.g. Erftemeijer, 1994; Agawin et al., 1996; Terrados et al., 1998; Lee and Dunton, 2000; Burkholder et al., 2007; Shi et al., 2010). Shifts in seagrass species composition are expected along nutrient or silt gradients (e.g. Bach et al., 1998; Terrados et al., 1998; Agawin et al., 1996; Uku and Bjork, 2001; Kamermans et al., 2002; Herbert and Fourqurean, 2008), along with increased epiphyte loads (Bohrer et al., 1995; Uku and Bjork, 2001). In our study, the epiphyte biomass could not be measured as epiphytes were very sparse at all locations, and when incidentally present they were ephemeral and loosely attached. As a result of increased nutrient availability, the carrying capacity of macrobenthos is expected to increase and species composition may change (McClelland and Valiela, 1998; Valentine and Heck, 2001).

Sediment and pore water nutrients may be increased by nutrient and sediment loading, whereas water column nutrients are expected hardly to be influenced because of rapid uptake by plankton, seagrass or algal communities (e.g. Erftemeijer, 1994; Tomasko et al., 1996). However, in order to verify this, we included all: sediment, pore water and water column measurements. Sediment grain size is expected to decrease and organic matter content to increase towards the land (e.g. Erftemeijer, 1994; Terrados et al., 1998). Sediment iron, silicon, aluminium and manganese may serve as a marker of terrestrial origin, whereas, Ca, Mg, S and Zn contents in the sediment may serve as a proxy for marine sources (Kamp-Nielsen et al., 2002). Salinity and ions related to salinity (Na, K, Cl) were measured to assess to what extent the river water would reach the sampling stations. Redox potential was measured as it may serve as an indicator for organic matter loading, or eutrophication in general (e.g. van der Heide et al., 2009).

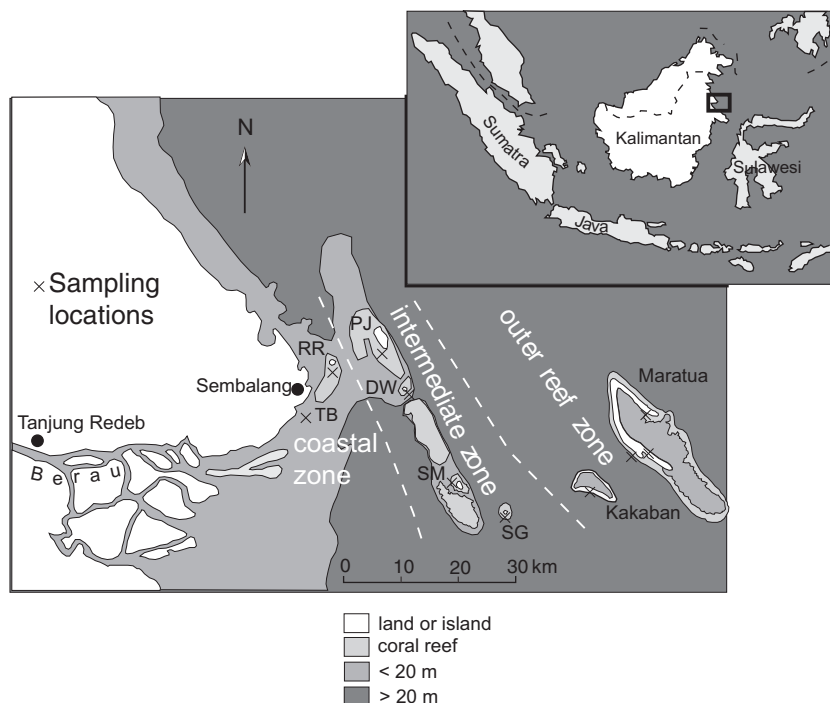


Fig. 1. Study area in the Berau archipelago, East Kalimantan, Indonesia, showing the sampling locations. Three zones were distinguished with diminishing river influence: the coastal, intermediate and outer zone. TB, Tanjung Batu; RR, Rabu Rabu; PJ, Pulau Panjang; DW, Derawan; SM, Samama; SG, Sangalaki. At the island of Derawan, five stations were investigated.

2.3. Sampling

Seagrass cover and species composition were estimated by visual inspection by snorkelling an area of 500×50 m, located in the low intertidal zone (exposed only at spring low tides). Cover was estimated on a 0–100% scale when submersed (Short and Coles, 2001). Within this 500×50 m area, plants were collected for free amino acid analyses and vitality measurements (fluorescence measurements by PAM). Fresh tissue samples were immediately frozen for amino acid analysis and kept frozen until analysis. Also, five vegetated and five unvegetated plots were selected within the area and sampled for pore water, sediment, macrofauna and plant properties. Seagrass plants and macrofauna were sampled to a sediment depth of 20 cm once per plot, using a core of 16.5 cm diameter. The cores were washed through a 0.5 mm mesh sieve using a wash box and gentle flow of seawater to ensure gentle fragmentation of the sediment. Seagrass plants in the sample of vegetated plots were taken to the laboratory for further measurements. The remainder of the residue of the sieving was immediately fixed in 10% formaldehyde, buffered with sodium tetraborate decahydrate. Fresh weight of the macrofauna was determined upon return

in the lab, and they were sorted into six groups: polychaetes, crustaceans, molluscs, echinoderms, fish and miscellaneous. Morphological parameters (leaf length and width) of the seagrass plants were measured and the sample was subsequently dried until constant weight at 60–80 °C, weighed and used for tissue analyses.

In each area, samples of the surface water were collected in 500 mL polyethylene bottles. In each of the 10 plots plot, sediment pore water was collected anaerobically using ceramic cups of 5 cm length (Eijkelkamp, Agrisearch, Giesbeek, The Netherlands) that were installed in the upper 7 cm of the sediment. The pore water was collected by connecting the cups to vacuum syringes (50 mL). After pH and redox measurements, see below, the pore water and surface water samples were treated with mercury chloride to conserve nutrients and prevent microbial activity. A sample from the upper sediment layer (0–10 cm) was collected with a PVC tube (diameter 2 cm) in each plot.

2.4. Analyses

Right after sampling, the pH and redox potential were measured in the collected water samples. The pH of the surface water and

Table 1
Water column and pore water properties in the Berau archipelago. No significant differences between the three zones were detected. Mean and (SD) are presented. Number of stations per zone is presented, for pore water samples per station five samples were pooled.

| | Salinity PSU | pH | NH ₄ (μM) | oPO ₄ (μM) | K (μM) | Redox (mV) | Sulphide (μM) |
|----------------------|--------------|-----------|----------------------|-----------------------|-------------|------------|---------------|
| Water column | | | | | | | |
| Coastal zone (n = 2) | 31.7 (2.4) | 8.0 (0.2) | 4.9 (1.2) | 0.13 (0.15) | 7723 (110) | | |
| Intermediate (n = 8) | 33.0 (0.5) | 8.2 (0.1) | 6.8 (6.5) | 0.12 (0.08) | 7710 (2380) | | |
| Outer reef (n = 4) | 32.5 (1.3) | 7.9 (0.5) | 8.9 (6.6) | 0.08 (0.06) | 7971 (877) | | |
| Pore water | | | | | | | |
| Coastal zone (n = 2) | 32.7 (1.2) | 7.5 (0.3) | 10.5 (7.7) | 0.87 (0.54) | 8140 (80) | −19 (119) | 0.8 (1.0) |
| Intermediate (n = 8) | 33.2 (0.8) | 7.8 (0.1) | 13.0 (6.0) | 0.96 (0.58) | 8650 (250) | −26 (73) | 1.8 (2.0) |
| Outer reef (n = 4) | 33.2 (0.9) | 7.5 (0.2) | 6.4 (2.5) | 0.62 (0.28) | 8400 (310) | 26 (26) | 1.6 (1.4) |

pore water samples were measured with a Cyberscan pH 300 series pH meter (Eutech, Singapore) with a double Ag/AgCl reference pH electrode (Orion 9156BNWP, Thermo Scientific, USA). The redox-potential (Eh in mV) of the pore water was measured with a multimeter (p901, Consort, Belgium), a platinum electrode and an Ag/AgCl reference electrode (Metrohm, Switzerland). The measured values were corrected for the used reference electrode. The concentration of free S^{2-} was determined in a 10 mL subsample, fixed immediately after collection with S^{2-} antioxidant buffer containing sodium hydroxide, sodium EDTA and ascorbic acid (Van Gernerden, 1984). A S^{2-} ion-specific Ag electrode and a double junction calomel reference electrode were used. The surface water and sediment pore water NH_4^+ and PO_4^{3-} concentrations were analysed by the use of an Auto Analyser system (model III, Bran and Luebbe, Nordstedt, Germany) using a salicylate method for NH_4^+ and an ammonium molybdate method for PO_4^{3-} . For the total elemental composition (P, Ca, K, S, Al, Fe, Mg, Mn, Si and Zn) ICP-OES (model IRIS Intrepid II XDL, Thermo Fisher Scientific, Waltham, MA, USA) was used.

Dried aboveground plant material was ground in liquid nitrogen. Ground plant (200 mg) and sediment (200 mg) material was digested in 4 mL HNO_3 (65%) and 1 mL H_2O_2 (35%), using an Ethos D microwave (Milestone, Italy) (Kingston and Haswell, 1997). Total elemental compositions of plant and sediment digestives were measured by ICP-OES as described above. Carbon and nitrogen of dry plant material in ultra light-weight tin capsules were analysed by a CNS elemental analyser (EA 1110, Carlo Erba, Thermo Fisher Scientific, USA). Organic matter contents were analysed by determining the weight loss of a dry weight sample on ignition at 550 °C. Grain size classes of the sediments were divided by sieving.

The photosynthetic yield of *Halodule uninervis* was determined by Pulse Amplitude Modulation (PAM) fluorometry (Diving PAM, Heinz Walz GmbH, Effeltrich, Germany). The photosynthetic yield was calculated from the fluorescence after the leaves had been kept in the dark for at least 15 min (F_0), and the maximum fluorescence after a strong light pulse (F_m), which is saturating for photosynthesis (Yield = $F_0 - F_m/F_m$). Free amino acids were extracted according to van Dijk and Roelofs (1988). After precolumn derivatation with 9-fluorenylmethyl-chloroformate (FMOC-Cl), fluorescence was measured with HPLC (Star 9050 variable wavelength UV-vis and Star 9070 fluorescence detector; Varian Liquid Chromatography, Palo Alto, USA). Norleucine was used as the internal standard.

2.5. Statistical analyses

Differences in abiotic variables between vegetated and bare sediments were not statistically different (analysis of variance, $p > 0.1$), therefore, we pooled them. Statistical units were the stations in three zones: the outer reef (number of stations = 4), intermediate zone ($n = 8$) and coastal zone ($n = 2$). As sediment patterns in the coastal zone are complex and temporally variable (e.g. Buschman et al., 2009; Tarya et al., 2010) actual distance to the river mouth would represent a pseudo-accuracy and zones were tested instead. To ordinally test the effect of river influence as an explanatory variable we assigned values of 1, 4 and 9 (rather than 1, 2 and 3) to these zones, respectively, to reflect radial dispersal. All variables except plant cover and pH were lognormally distributed and were, therefore, logtransformed. Ratio's were not logtransformed. Tests were performed using ANOVA for unequal sample sizes. Effects of species and river influence on plant properties were tested in a nested ANOVA for unequal sample sizes. Only dominant seagrass species were included in this analysis, i.e. *H. uninervis*, *Halophila ovalis*, *Thalassia hemprichi* and *Cymodocea rotundata*.

3. Results

3.1. Abiotic parameters

None of the measured variables in the water column and pore water (Table 1) showed a relationship with river influence. Column water salinity gradually increased going downstream the river (results not shown), but in the seagrass meadows in the coastal zone, the salinity was already high (31.7 PSU), and did not significantly increase further in the direction of the outer reef (Table 1). In con-

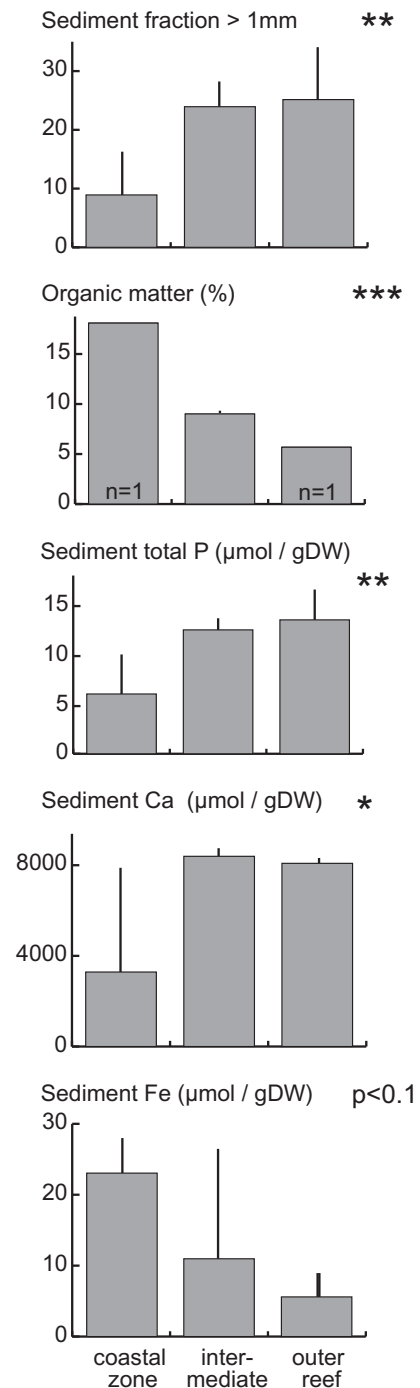


Fig. 2. Sediment characteristics varying between the coastal zone ($n = 2$), intermediate zone ($n = 8$) and outer zone ($n = 4$). Means and standard error are presented. River influence was numerically tested assigning values 9, 4 and 1 to the zones, respectively; ** $p < 0.01$, *** $p < 0.001$ (ANOVA).

trast to the water samples, sediment samples showed significant differences. Total concentrations of phosphorus and calcium and the coarse sediment fraction (>1 mm) increased from the coastal zone to the outer reef (Fig. 2), as did magnesium (147–457 $\mu\text{mol/g}$ DW sediment, $p < 0.05$), sulphur (67–115 $\mu\text{mol/g}$ DW sediment, $p < 0.05$) and zinc (4.0–8.3 $\mu\text{mol/g}$ DW sediment, $p < 0.05$). In all these instances the pattern was roughly similar; only the coastal zone substantially differed from the other two zones (Fig. 2). Sediment total iron concentrations as well as the organic matter fraction tended to decrease towards the outer reef ($p < 0.1$, and $p < 0.001$, respectively, Fig. 2). Total manganese, silicon and aluminium concentrations in the sediment were not related to river influence, nor were the sediment fractions other than the coarse fraction mentioned above (results not shown).

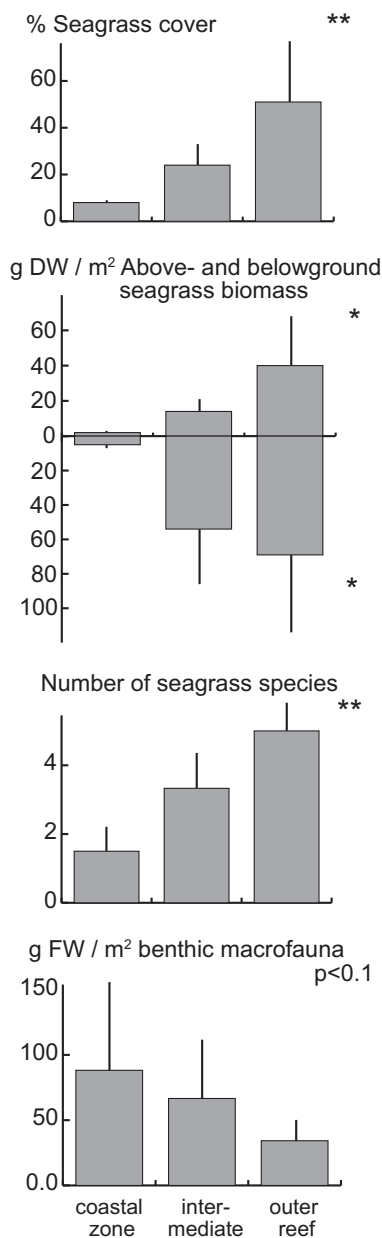


Fig. 3. General biological characteristics: seagrass cover, number of seagrass species and g FW benthic macrofauna per m^2 . River influence was numerically tested assigning values 9, 4 and 1 to the coastal zone, intermediate zone and outer reef, respectively, ** $p < 0.01$ (ANOVA).

3.2. Biotic parameters

At a *landscape level*, the seagrass meadows responded with an increasing seagrass cover and above- and belowground biomass going to the outer reef (Figs. 3, 4 and Table 2). At an *ecosystem level*, the number of seagrass species increased towards the outer reef (Fig. 3). Moreover, the species composition changed; though *H. uninervis*, *H. ovalis* and *T. hemprichii* occur in all three zones (the coastal zone, the intermediate zone and the outer reef zone), *Syringodium isoetifolium* and *C. rotundata* occur only in the latter two more pristine zones, and *Enhalus acoroides* was only found in sheltered bays in the outer reef zone (Table 3). The macrofauna biomass within the seagrass beds showed a tendency ($p < 0.1$) to decrease towards the outer reef (Fig. 3), coinciding with a (non-significant) decreasing mollusc biomass. At an *individual level*, leaf length and number of leaves per shoot of particularly *C. rotundata* and *T. hemprichii* increased towards the outer reef (Fig. 4 and Table 2). Seagrass tissue contents %P, %N, Fe decreased, and C/N ratio increased towards the outer reef (Fig. 5). Also, other tissue

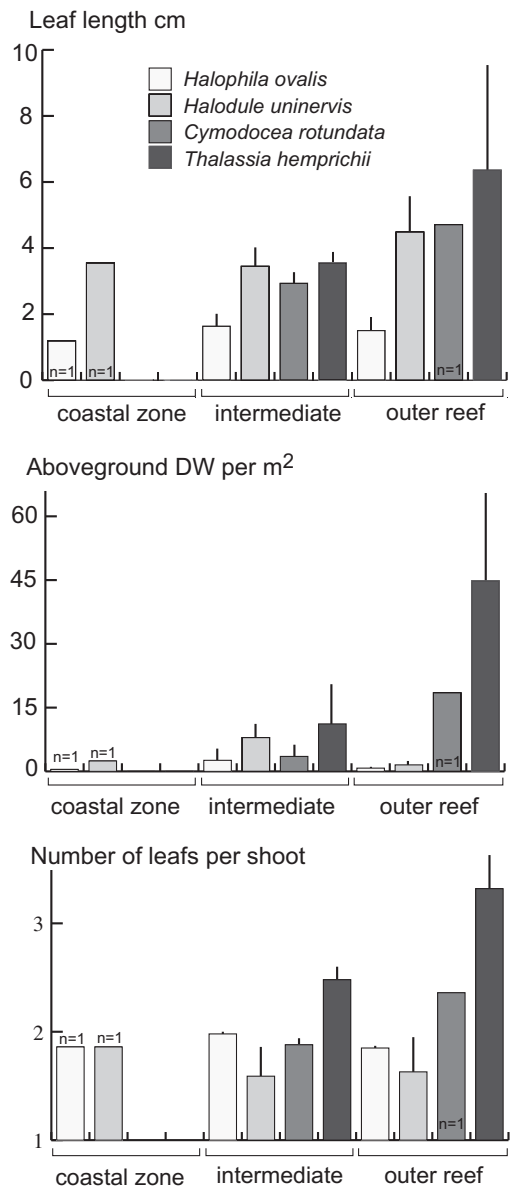


Fig. 4. Morphological characteristics of the seagrass plants under river influence, per species. Significances see Table 2.

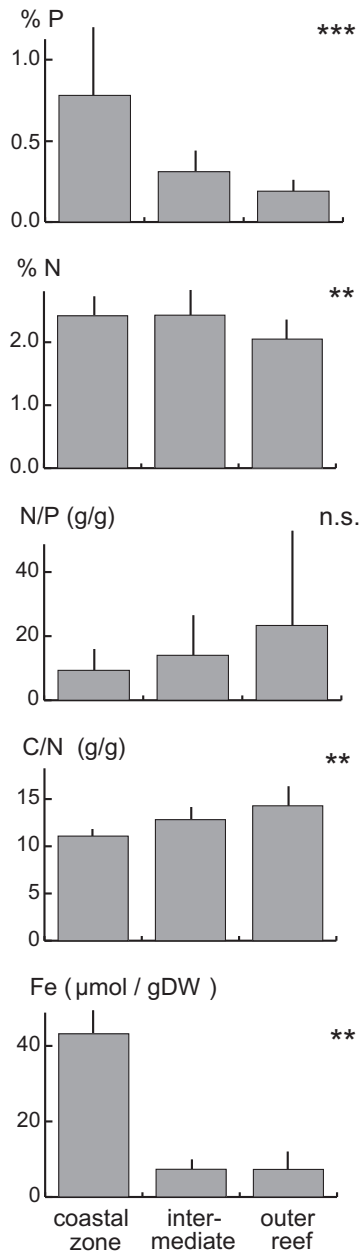


Fig. 5. Tissue contents of aboveground dominant seagrasses. The variables did not vary between species (ANOVA, $p > 0.05$) and were therefore pooled. River influence was numerically tested assigning values 9, 4 and 1 to the coastal zone, intermediate zone and outer reef, respectively; ** $p < 0.01$, *** $p < 0.001$ (ANOVA).

Duarte (1995) who found that interspecific differences in elemental tissue contents were smaller than the environmentally induced differences (but see Campbell and Fourqurean, 2009). Leaf mass (mg/cm^2) was not a good indicator in our area (namely highly var-

iable), contrasting to results found in three US Bays (Lee et al., 2004; Burkholder et al., 2007). The measurement of photosynthetic yield by fluorescence, a parameter widely used, did not differ between the regions and the values indicate photosynthetically vital plants in all zones.

From our results we can also assess to what extent nutrients are limiting or in excess in this relatively pristine archipelago. Our results show that, at least near the coast, nitrogen is more limiting than phosphorus or iron: the seagrass tissue nitrogen concentration is only mildly positively related to river influence in comparison to the P and Fe tissue contents (Fig. 5). This was tentatively confirmed by the N/P ratios which ranged from 9 (g/g corresponding to a molar N/P ratio of 20) in the coastal zone to 14 (=31 M N/P ratio) in the intermediate zone to 23 (=51 M N/P ratio) in the outer zone. N/P ratios of 20–25 (g/g) indicate a balanced nutrient supply (Duarte, 1990; Güsewell et al., 2003). However, N/P ratios are notably variable (Duarte, 1990; Johnson et al., 2006), also in our case, and the relationship with river influence is not significant. In absolute sense, nutrients are not severely limiting in the Berau archipelago: tissue nitrogen contents are equal or above the threshold for limitation (1.8%N, Duarte, 1990); tissue contents of P are slightly below the threshold for limitation only in the outer zone (0.19%P recorded, threshold is 0.20%, Duarte, 1990) and tissue contents of Fe are well above the threshold for limitation 1.7 $\mu\text{mol}/\text{g}$ DW (Duarte, 1995), namely 7.3 $\mu\text{mol}/\text{g}$ DW, both in the outer reef zone as in the intermediate zone, and in the coastal zone even higher. Therefore, though nitrogen is relatively more limiting near the river mouth, the seagrasses are relatively depleted in P farther from the coast, showing possible P limitation, despite the high P contents in the sediment, which are largely bound to the carbonate sediments and, therefore, not readily available to the seagrass (e.g. Burkholder et al., 2007).

The seagrass tissue nitrogen concentrations did not show an absolute nitrogen limitation, but neither an excess. We can conclude this from the amino acid composition, which showed no relationship with river influence. With an overload of nitrogen, seagrasses tend to increase the percentage of amino acids that have low C/N ratios like glutamine and asparagine (Udy and Dennison, 1997; Burkholder et al., 2007; van der Heide et al., 2008; Christensen et al., 2011). It is likely that the organic input into the coastal area from the river is relatively depleted in nitrogen due to denitrification processes that take place in the river and its fringing wetlands and riparian buffers that are high in organic compounds (e.g. Mitsch et al., 2001; Seitzinger et al., 2006; Valiela and Cole, 2002; Heffernan et al., 2010). As a result, the outflow to the coastal area will be relatively poor in nitrogen as compared to phosphorus and iron.

The concentration of Na in the seagrass tissues is highly increased near the river mouth, and decreased towards the outer reef, as well as the other measured elements Ca, Mg, K, S and Mn. This has never been reported before in the literature. We do not have a clear explanation for this, but may speculate that reduced growth near the river mouth (which might be due to turbidity, sediment dynamics, salinity fluctuations, Fe toxicity, etc.)

Table 4

Aboveground seagrass tissue contents ($\mu\text{mol}/\text{g}$ DW) in addition to Fig. 5. Means, standard deviations and significant correlation with river influence (ANOVA) are presented. Species were not significantly different with respect to the presented plant tissue contents, except for tissue Ca that had higher concentrations in *Halophila ovalis* and *Thalassia hemprichii* as compared to *Halodule uninervis* (ANOVA, $p < 0.05$). Only dominant seagrass species were included in the analysis, i.e. *Halodule uninervis*, *Halophila ovalis*, *Thalassia hemprichii* and *Cymodocea rotundata*.

| Significance | Mg*** | | K*** | | Na*** | | S*** | | Mn*** | | Ca*** | |
|----------------------|-------|-------|------|-------|-------|--------|------|-------|-------|--------|-------|-------|
| | Mean | (SD) | Mean | (SD) | Mean | (SD) | Mean | (SD) | Mean | (SD) | Mean | (SD) |
| Coastal zone (n = 2) | 1541 | (516) | 1421 | (592) | 8099 | (2292) | 941 | (277) | 3.32 | (1.22) | 1110 | (704) |
| Intermediate (n = 8) | 708 | (262) | 536 | (155) | 4563 | (2241) | 414 | (143) | 0.54 | (0.18) | 820 | (383) |
| Outer reef (n = 4) | 388 | (92) | 307 | (64) | 2725 | (858) | 237 | (58) | 0.45 | (0.28) | 484 | (197) |

*** $p < 0.001$.

results in accumulation of elements, just like growth results in dilution. In that case we can assume that Na, being an inert element, is a proxy for this accumulation. If N and P were not limiting in the coastal zone they would accumulate accordingly. Our results showed that this was the case for P, but not for N (which was lower – did not accumulate – in the coastal zone), confirming the relative N limitation in the coastal zone.

5. Conclusions

Our study demonstrates that (i) water and pore water parameters are no indicators in early eutrophication stages, (ii) sediment composition is an indicator of river influence, namely sediment loading and to a lesser extent Fe loading, but not P and N loading, (iii) total P in the sediments was inversely related to the river influence, probably related to the increasing calcium carbonate content and (iv) seagrass systems, respond at three levels to river influence, namely landscape level, ecosystem level and individual level. They are, therefore, suitable as early warning indicators in the trajectory towards a critical transition to a potential collapse (e.g. van der Heide et al., 2007, 2010a; Scheffer et al., 2009). Present results serve as a benchmark for characterising relatively pristine seagrass systems under influence of a river. Such systems are relatively rare, difficult to access and rapidly disappearing.

The static indicators identified in this study will allow for a rapid assessment of the effects of increased sediment and nutrient loads on the coastal system. Factors like seagrass cover, species composition, morphological characteristics and epiphyte cover are already included in the seagrass-watch manuals (www.seagrasswatch.org), and provide an indicator of siltation and/or eutrophication stress (see also Terrados et al., 1998). In addition, tissue content analyses could provide a valuable addition as an early warning indicator of eutrophication and allowing the distinction between nitrogen versus phosphorus limitation. In the seagrass-watch manuals it could be added that seagrass samples be dried (e.g. in the sun or in rice warmers), and sent to laboratories which should unite efforts to frequently analyse samples from seagrass watch participants in areas that are threatened by eutrophication.

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