Combined nutrient and macroalgae loads lead to response in seagrass indicator properties

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ABSTRACT

Excess nutrients are potential factors that drive phase shifts from seagrasses to macroalgae. We carried out a manipulative field experiment to study the effects of macroalgae *Ulva pertusa* loading and nutrient addition to the water column on the nitrogen (N) and carbon (C) contents (i.e., fast indicators) as well as on the morphology and structure (i.e., slow indicators) of *Zostera marina*. Our results showed rapid impact of increased macroalgae and nutrient load on *Z. marina* C/N ratios. Also, macroalgae addition resulted in a trend of decreasing belowground biomass of seagrasses, and nutrient load significantly decreased above to belowground biomass ratio. Although some morphological/structural variables showed relatively fast responses, the effects of short-term disturbance by macroalgae and nutrients were less often significant than on physiological variables. Monitoring of seagrass physiological indicators may allow for early detection of eutrophication, which may initiate timely management interventions to avert seagrass loss.

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1. Introduction

Seagrass meadows have been widely acknowledged as highly important coastal systems that support high biodiversity and productivity, and high trapping and storage of nutrients and carbon (Duarte et al., 2010; Hemminga and Duarte, 2000; Orth et al., 2006). Decline of seagrass meadows due to human activity has been reported in recent years throughout the world (Orth et al., 2006; Waycott et al., 2009). One of the most important threats to seagrasses is eutrophication, which could result from increased fertilizer use and marine cultivation (Burkholder et al., 2007; Orth et al., 2006). Nutrient enrichment can accelerate seagrass loss through direct effects, such as toxicity, increased system respiration and sediment anoxia (Burkholder et al., 2007; van Katwijk et al., 1997), but also through indirect effects via algal proliferation that could cause shading and/or smothering (Burkholder et al., 2007; Hauxwell and Valiela, 2004; van Katwijk et al., 2010).

Seagrass decline in temperate estuaries under high nutrient enrichment often coincides with high macroalgae biomass (Burkholder et al., 2007; Hauxwell et al., 2001; Short and Burdick, 1996; Thomsen et al., 2012; Valiela et al., 1997). The negative effects of macroalgal blooms on seagrasses have been documented in many areas of the world, such as Australia, Japan, America and Europe (e.g. Cummins et al., 2004; Huntington and Boyer, 2008; Martínez-Lüscher and Holmer, 2010; Sugimoto et al., 2007). Small-statured seagrass species are more impacted than larger species, and the effect is often proportional to the biomass of macroalgae (Thomsen et al., 2012). Shading by the macroalgae causes light reduction and is one of the most common mechanisms resulting in seagrass decline (Burkholder et al., 2007), which is indicated by reduction in the depth of the meadows, lowered shoot densities, poor recruitment, slower growth rates and decreased overall production of seagrasses (Krause-Jensen et al., 2000; McGlathery, 2001). Additionally, decomposition of macroalgae mats may decrease oxygen content in eutrophicated waters and further abate seagrass survival (McGlathery et al., 2007). Furthermore, high sulfide concentration due to the anoxia from macroalgae decomposition can decrease the photosynthetic rate of seagrasses, reducing growth and even resulting in mortality (Holmer and Nielsen, 2007; Koch et al., 2007; Pedersen et al., 2004; van der Heide et al., 2012). With average temperatures rising on a global scale, blooms of green algae such as *Ulva pertusa* may be expected to increase (Sousa-Dias and Melo, 2008), which could in turn further increase the competitive advantage of green algae over some seagrasses (Koch et al., 2013 and references therein).

Fewer studies have investigated the effect of nutrients on the interaction between seagrasses and macroalgae beyond the effect of light reduction (but see Burkholder et al., 2007; Vonk et al., 2008). In general, when nutrient concentrations are low in seawater, seagrasses are dominant over macroalgae (Fourqurean et al., 1995), due to the competitive advantage conferred by roots and rhizomes in absorbing nutrients from

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sediment porewater (Hemminga, 1998; Vonk et al., 2008). In contrast, the competitive interaction is reversed under high nutrient concentrations in seawater because macroalgae can absorb nutrients more effectively through the thallus than seagrasses can through their leaves, almost irrespective of the form of nitrogen (N) (Burkholder et al., 2007; Vonk et al., 2008). In many developing countries, high levels of nutrients are discharged into coastal areas due to fertilizer use and untreated sewage or waste loading, either within catchment areas or directly into the seawater during mariculture (de Lacerda et al., 2006; Edinger et al., 1998; Lin et al., 2005).

The accelerated seagrass loss due to nutrient enrichment, either via direct effects or indirect effects, may eventually result in a phase shift from seagrass-towards macroalgae-dominated systems (Hauxwell et al., 2001; Montefalcone et al., 2007; Orfanidis et al., 2003). Most studies about such phase shifts from seagrasses to macroalgae have been conducted in highly eutrophic systems, where the shift to macroalgal dominance had already occurred (Cardoso et al., 2004; Montefalcone et al., 2007; Short and Burdick, 1996). However, few studies have directly assessed the combined effects of macroalgae cover and high nutrients on seagrass performance during macroalgae blooms before the phase shift from seagrasses to macroalgae, i.e. in systems where seagrasses and macroalgae still co-occur. Examining this stage of the interaction is highly relevant, especially for rapidly developing countries, where nutrient run-off towards seagrass meadows is rapidly increasing.

Human activities and the rapid economic development in China have already resulted in increasing nutrient loads on the surrounding seas. For example, dissolved inorganic nitrogen in the Yellow Sea has been increasing since 1976 (Lin et al., 2005), and has exceeded 14 μmol/L in more than 50% of the areas sampled since 2003 (State Oceanic Administration, 2008-2012). The world's largest macroalgae blooms during the period 2008–2012 occurred in the Yellow Sea, and over one million tons of wet macroalgae were removed from the coast in 2008 (Liu et al., 2013). Eutrophication by release of nutrients from wastewater, agriculture, and aquaculture has fostered macroalgae blooms in this area (Liu et al., 2013). In recent years, seagrasses have rapidly declined in China (Han et al., 2007; Huang et al., 2006). In some areas of the north coast of China, seagrass meadows have disappeared and been replaced by macroalgae mats (personal observations from Qiuying Han). At other areas, macroalgae mats cover seagrass *Zostera marina* meadows during extended algae blooms every summer from June to July. However, few studies have investigated the causes of seagrass degradation in these areas.

We aim to quantify experimentally in the field how both algae cover and nutrients interactively affect the remaining seagrasses, which has been often suggested but not empirically tested, in a factorial experiment. In order to evaluate the response of seagrasses to macroalgae cover, nutrients, and their combination, two types of indicators differing in their response time (fast vs. slow) were used: physiological (fast), morphological/structural (slow).

Physiological variables, such as nitrogen (N) and carbon (C) contents of seagrass tissues, are normally used as fast indicators (days to weeks) of nutrient availability and light reduction (Burkholder et al., 2007; Fourqurean et al., 1992, 1997; van Katwijk et al., 2011). Seagrass morphological and structural characteristics (e.g. number of leaves per shoot, blade width, leaf length, biomass and above to belowground biomass ratio) are slow indicators of variations in nutrient enrichment compared to the nitrogen and carbon contents of seagrass tissues (Burkholder et al., 2007; Lee et al., 2004; Roca et al., 2016). To evaluate the responses of both (fast and slow) types of indicators, we conducted a manipulative field experiment to investigate macroalgae *U. pertusa* cover and nutrient effects on the tissue nitrogen and carbon contents, biomass, and morphology of the seagrass *Z. marina* during a 6-week experiment. A period of 6 weeks was chosen in order to mimic the time scale of typical algae blooms encountered in the *Z. marina* meadows in this area, which are usually short and sudden. Our results will contribute to the knowledge base that may assist in the monitoring and conservation of seagrasses under situations of macroalgae blooms and/or nutrient enrichment worldwide.

2. Materials and methods

2.1. Experimental field location

An in situ manipulative experiment was conducted in Swan Lake (N36°43′-37°27′, E122°09′-122°42′), located in the eastern part of the Shandong peninsula on the north coast of the Yellow Sea of China (Fig. 1). Swan Lake is a 4.8 km^2 lagoon, connected via an inlet to the north Yellow Sea. The seaweed *Laminaria japonica* is cultured on a mass scale in an area of about 6600 ha around Swan Lake and about 200 t of the bivalve *Ruditapes philippinarum* are also harvested each year. Two seagrass species can be found in mostly separate continuous assemblages in the lagoon, *Z. marina*, which is the dominant species in extended meadows, and *Zostera japonica*, which become mixed only at the edge of each seagrass meadow. More than 10,000 swans inhabit the lake, particularly in winter, and feed on *Z. marina* (Dong et al., 2007). In the past 10 years, *Z. marina* seagrass biomass has decreased, reportedly through the process of eutrophication (Dong et al., 2007). *U. pertusa* is the dominant macroalgae species in Swan Lake and blooms of this species often occurs during summer, especially in June (Zhang et al., 2014).

At the beginning of this study, seawater samples were collected from Swan Lake, put into refrigeration boxes in the field, and transferred to the laboratory for nutrient analysis. Samples were then filtered through cellulose acetate membranes (Whatman, 0.45 μmol/L). Nutrient analysis was conducted using a Flow Injection Analysis system (AA3, Bran + Luebbe, Norderstedt Germany) to measure the following parameters: ammonium, nitrite, nitrate and soluble reactive phosphorus (SRP). Nutrient analysis was executed according to the WOCE Methods Manual (Gordon et al., 1993). Dissolved inorganic nitrogen (DIN) was calculated as the total of ammonium + nitrite + nitrate. Mean values (±SE) for soluble reactive phosphate (SRP), nitrate, ammonium and DIN concentration of natural seawater were found to be 0.28 ± 0.19, 3.74 ± 1.02, 5.22 ± 1.57 and 9.41 ± 2.60 μmol/L, respectively. Using a YSI 30 portable meter, the following environmental parameters were directly measured in the field: seawater temperature, pH, dissolved oxygen concentration and salinity. Mean water column temperature, pH, dissolved oxygen concentration and salinity were 21.85 ± 3.79 °C, 6.61 ± 0.43, 5.6 ± 0.69 mg/L and 30.8 ± 0.53, respectively at the beginning of the field experiment.

2.2. Experimental design

We tested the combined effects of macroalgae in cages ("HA" = high algae: 1800 g/m^2 fresh macroalgae biomass was applied at the beginning of the experiment to mimic a relatively high macroalgae loading, "NA" = no algae: algae were removed at the beginning of the experiment) and nutrients ("HN" = high nutrient addition: 565 g osmocote slow-release fertilizer each week (g/g ratio N:P:K; 26:11:11), "NN" = no nutrient added) addition on *Z. marina* meadows in the field. The high algae treatment resembles the ambient loads to some extent. The initially applied amount of algae is approximately four times higher than the ambient fresh mean biomass of *U. pertusa* macroalgae in June (490 ± 170 g/m^2, n = 10), as it decomposes quickly 23 ± 17 g/m^2 of fresh biomass per day (n = 30), without new input in the cages. Plots assigned to the "High nutrients" treatment were fertilized with osmocote slow-release fertilizer each week. The osmocote fertilizer was inserted into nylon stockings hung above the sediment using bamboo poles in the middle of the cage, which enabled the nutrients to discharge continuously into both the water column and sediment porewater. Average loading rates of osmocote fertilizer were 204 mmol N m^{-2} day^{-1} and 92 mmol P m^{-2} day^{-1}. A total of four treatments, i.e. HA–HN, NA–HN, HA–NN, and NA–NN (control), with 5