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Toxic effects of increased sediment nutrient and organic matter loading on the seagrass *Zostera noltii*

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ABSTRACT

As a result of anthropogenic disturbances and natural stressors, seagrass beds are often patchy and heterogeneous. The effects of high loads of nutrients and organic matter in patch development and expansion in heterogeneous seagrass beds have, however, poorly been studied. We experimentally assessed the in situ effects of sediment quality on seagrass (*Zostera noltii*) patch dynamics by studying patch (0.35 m diameter) development and expansion for 4 sediment treatments: control, nutrient addition (NPK), organic matter addition (OM) and a combination (NPK + OM). OM addition strongly increased porewater sulfide concentrations whereas NPK increased porewater ammonium, nitrate and phosphate concentrations. As high nitrate concentrations suppressed sulfide production in NPK + OM, this treatment was biogeochemically comparable to NPK. Sulfide and ammonium concentrations differed within treatments, but over a 77 days period, seagrass patch survival and expansion were impaired by all additions compared to the control treatment. Expansion decreased at porewater ammonium concentrations >2000 μmol L^{-1}. Mother patch biomass was not affected by high porewater ammonium concentrations as a result of its detoxification by higher seagrass densities. Sulfide concentrations >1000 μmol L^{-1} were toxic to both patch expansion and mother patch. We conclude that patch survival and expansion are constrained at high loads of nutrients or organic matter as a result of porewater ammonium or sulfide toxicity.

1. Introduction

Seagrass beds are among the most productive and biodiverse ecosystems in the world (Hemminga and Duarte, 2000; Orth et al., 2006). Unfortunately, they are severely threatened by increasing human activities in coastal areas (Orth et al., 2006; Waycott et al., 2009). The most imminent problems for seagrass meadows are related to the large-scale eutrophication of coastal waters (Burkholder et al., 2007; Duarte, 2002; Orth et al., 2006; Waycott et al., 2009). Eutrophication results in light limitation for submerged species, as it promotes the growth of epiphytes and phytoplankton, but may also directly affect seagrass vitality through ammonium toxicity (Brun et al., 2002; Van der Heide et al., 2008; van Katwijk et al., 1997). High ammonium levels are known to be able to disrupt the uptake of essential cations such as potassium by higher plants, affect their pH regulation, and decrease overall growth rates (Britto and Kronzucker, 2002).

In addition, eutrophication related to land use change (including deforestation) often increases the organic matter input into coastal sediments, thereby not only increasing ammonium levels even further, but also fueling the production of sulfide (Burkholder et al., 2007; Van der Heide, 2009). Sulfide is a strongly phytotoxic compound, as it blocks the activity of cytochrome oxidase and other metal containing enzymes (Lamers et al., 2013). It is formed in anaerobic sediments rich in organic matter, where sulfate is used as an alternative terminal electron acceptor during the microbial breakdown of organic matter (Gray and Elliott, 2009; Holmer and Nielsen, 1997). Sulfide toxicity, due to the build-up of sulfides in the sediment, may lead to massive seagrass die-offs (Borum et al., 2005; Carlson et al., 1994; Terrados et al., 1999).

The deterioration of habitat quality, including eutrophication and sulfide toxicity, has led to the complete disappearance of

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seagrass beds in some regions (Borum et al., 2005; Burkholder et al., 2007). In other areas, it has created heterogeneous, patchy seagrass landscapes (Duarte and Sand-Jensen, 1990; Frederiksen, 2004; Pasqualini et al., 1999) which reflect processes of recovery from disturbances (Brun et al., 2003a; Duarte and Sand-Jensen, 1990; Pickett and White, 1985). A patch, defined as "a surface area that differs from its surrounding in nature or appearance" (Turner et al., 2001), may result from both sexual and asexual colonization (Bostrom et al., 2006; Duarte and Sand-Jensen, 1990), and from habitat fragmentation of seagrass beds. Patches expand through shoot expansion, mainly through horizontal elongation of rhizomes at the patch edges (Duarte et al., 2006a; Marba and Duarte, 1998; Olesen and Sand-Jensen, 1994; Vermaat, 2009). Their shoot densities increase exponentially (self-accelerating) with increasing patch age and size (Bostrom et al., 2006; Sintes et al., 2005; Vermaat, 2009; Vidondo et al., 1997).

Most studies have tended to focus on large-scale processes (Bell et al., 1999) in seagrass beds, rather than on ubiquitous but small-scale dynamics. Studies describing seagrass patch dynamics (Almela et al., 2008; Brun et al., 2003b; Campbell and Paling, 2003; Di Carlo and Kenworthy, 2008; Duarte and Sand-Jensen, 1990) focused on plant physiology rather than on the impact of environmental characteristics including local sediment biogeochemistry. Eutrophication and sulfide toxicity, two environmental stressors of seagrass meadows that have rapidly become common at a global scale, may not only contribute to the formation of patches, but can also be expected to affect patch expansion itself. As patchy seagrass meadows are expected to be more vulnerable to stressors than homogeneous fields due to their lower regrowth capacities and the higher impact of hydrodynamics (Bos and van Katwijk, 2007; Duarte and Sand-Jensen, 1990; Olesen and Sand-Jensen, 1994), it is important to know the effects of both important, potentially phytotoxic stressors on seagrass patches. To our knowledge, experimental manipulation of sediment quality to study the effects on seagrass patch expansion has never been carried out before. We therefore assessed the possible toxic effects of changes in biogeochemical sediment quality related to eutrophication (increased levels of nutrients and organic matter) on small-scale seagrass patches, using the fast growing *Zostera noltii* as a model species.

2. Materials and methods

2.1. Study area

The experiment was conducted in an intertidal *Zostera noltii* bed on the mudflats of Viane (51°39′ N, 4°01′ E), the Oosterschelde, in the southwestern part of the Netherlands. The Oosterschelde delta has a surface area of 351 km^2 and a tidal amplitude of 2.5–3 m (Troost et al., 2009). Mean surface water temperature fluctuates between 0 and 22 °C annually. Freshwater input into the former estuary has become highly limited after the implementation of major water works in the past, and salinity is generally around 30 (Nienhuis and Smaal, 1994).

Zostera noltii meadows cover around 75 ha of the mudflats in the Oosterschelde, growing mainly in sheltered areas near dikes. At the experimental site, *Zostera noltii* was growing within 30 m of a dike, on a compact clay bank with a median grain size (D50) of 130 μm and % organic carbon of 0.72%. At the start of the experiment, the seagrass cover of the meadow was relatively homogeneous (2250 ± 124 shoots m^{-1}) and biogeochemical characteristics of the meadow were also determined (sulfide = 0.31 ± 0.22, NH_4 = 13.14 ± 2.09, NO_3 = 5.51 ± 1.44, PO_4 = 8.95 ± 0.84, μmol L^{-1}, n = 21). Experimental seagrass patches, as well as the natural bed, were located on the mudflat at an average height of 54 cm above Amsterdam Ordnance Datum (NAP), resulting in exposure to the atmosphere for on average 7 h during each low tide, depending on weather conditions. This intertidal mudflat was flooded and drained with each tidal cycle (semidiurnal).

2.2. Experimental design

Circular *Zostera noltii* sods of 35 cm in diameter and 7 cm depth were carefully collected from a homogeneously covered meadow, and transplanted to a bare stretch of the mudflat between the dike and the seagrass bed nearby. This area is known to be a suitable seagrass habitat, as seagrass was present and vital at this site until it was removed during dike reinforcement works in the previous growing season. The sods were transplanted into circular spots of 105 cm diameter and 7 cm depth. Before placing the sods, the different sediment treatments (details next section) were applied on the bare sediment. The sods, hereafter referred to as mother patches, were placed in the middle of the treated sediment spots and the remains were filled up with local sediment. Treatments were randomly assigned to the patches, using eight replicates (n = 8) per treatment. The experiment was carried out for 77 days, from June till September 2009.

2.3. Sediment treatments: nutrient and organic matter addition

To study the separate and interacting effects of sediment eutrophication, we applied four different biogeochemical treatments: a control treatment (Control), an organic matter treatment (OM), a nutrient treatment (NPK) and a combined treatment of both nutrients and organic matter (NPK + OM). We fertilized NPK and NPK + OM plots with 1.11 kg m^{-2} Osmocote® slow release fertilizer (g:g:g ratio N:P:K 18:9:10) with a longevity of 8–9 months. The fertilizer was distributed evenly beneath the seagrass sods and the surrounding, bare sediment. Loading rates were around 57 mmol N m^{-2} day^{-1} and 13 mmol P m^{-2} day^{-1} (Christianen et al., 2012; Vanlent et al., 1995).

For the organic matter treatments (OM and NPK + OM), we added 2 g L^{-1} of organic matter (1 g starch + 1 g cellulose L^{-1} sediment), which equaled a loading rate of about 157 mmol C m^{-2} day^{-1} (for 47 days), under the seagrass sods, which was expected to stimulate sulfide production (Peralta et al., 2003; Ruiz-Halpern et al., 2008). All treatments were applied only once, at the start of the experiment. However, the NPK and NPK + OM treatments appeared to generate biogeochemically similar results, as high nitrate levels suppressed sulfide production. As a result, this unforeseen effect of combined NPK and OM addition did not result in an expected interaction treatment.

2.4. Data collection in the field

Sediment samples were collected at the start of the experiment for chemical analyses to characterize the local habitat. Patch heights were measured using a RTK-DGPS (Real Time Kinematic Global Positioning System) (Van der Heide et al., 2010a), but did not differ significantly among treatments. During the experiment, sediment porewater samples were collected anaerobically at t = 0, 21, 49, 77 days, using 60 mL vacuumed syringes connected to ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), which were placed in the top 7 cm of the sediment. For each experimental patch, two porewater samples were taken, one inside the mother patch and one just outside the patch, but within the sediment treatment area (potential expansion area). Samples were pooled for analysis. Of each anaerobic sample, 10 mL was used for sulfide analysis on the same day (see below), and 40 mL was frozen until further chemical analyses. After porewater extraction, shoot numbers within the transplanted patches and shoot numbers of the patch outgrowth (patch expansion) were