

## ***Interactive comment on “Dynamics of environmental conditions during a decline of a *Cymodocea nodosa* meadow” by Mirjana Najdek et al.***

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We greatly appreciate all the reviewers' comments and suggestions. Please find our response letter below. Interactive comment on “Dynamics of environmental conditions during a decline of a *Cymodocea nodosa* meadow” by Mirjana Najdek et al. Anonymous Referee #2 Received and published: 5 March 2020 The paper entitled “dynamics of environmental conditions during a decline of cymodocean nodosa meadow” reported biomass changes of the seagrass along with environmental changes in both seawater and sediments during one year period of 2017 to 2018. The results showed that *C. nodosa* successfully adapted to the changes of environmental conditions and prevented

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H<sub>2</sub>S accumulation by its re-oxidation, supplying the sediment with O<sub>2</sub> from the water column and/or leaf photosynthesis, implying that the *C. nodosa* die-off would be most likely caused in April 2018 by a reduction of light availability. Unfortunately, solar irradiances changes either at the surface of water column or in water were not supplied. Especially, the light levels during investigation periods were not provided. Attenuation of light in water during different seasons with different amounts of PM can be directly link to photosynthetic performance of the seagrass. While the authors showed that in the sediment, depletion of oxygen due to intense oxidation of H<sub>2</sub>S led to anoxic conditions in most of the rooted areas. This could negatively affect respiration of the plant root, therefore, its growth. With reduced growth capacity, high concentrations of H<sub>2</sub>S were observed in the sediment cores and bottom waters. This is an interesting result indicating the relationship of H<sub>2</sub>S levels with photosynthetic O<sub>2</sub> evolution. Generally speaking, the paper has scientific significance, and is suitable to be accepted to BG after revision. Specific comments: 1. Daily sunlight doses should be in parallel with water temperature, and should be provided, which can be easily obtained from local meteorological stations if the authors did not measure. Authors response: Light intensity in the water column was not measured. The temperature of natural water bodies varies in response to diurnal and seasonal changes in solar radiation. Our temperature data showed no unusual diurnal and/or seasonal pattern. Therefore, it could be reasonably assumed that during the study period there was no big difference in the amount of solar radiation received by the sea. On the other hand, the penetration of light in the water column, which regulates photosynthesis, is strongly influenced by the transparency of the water. The attenuation of light in the water column and therefore, the reduced availability of light required for *C. nodosa* photosynthesis in April 2018 was attributed to the measured increased concentration of particulate matter in the water column due to the resuspension of sediment and influx from the land, as well as simultaneous sediment redeposition on the leaves. A sentence linking the reduction of available light to the meadow with increased water turbidity in April 2018 has been added to Abstract. It now reads: The *C. nodosa* decline was most likely triggered in

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April 2018 when light availability to the plant was drastically reduced. Such conditions resulted from increased seawater turbidity due to terrigenous input, sediment resuspension and elevated autotrophic biomass.

2. Decline of the seagrass meadow can hardly be attributed to light availability, grazing pressure or others. It must be a result of multiple drivers impact. Therefore, the discussion should be re-sorted and holistically analyzed. Authors response: We agree that the decline of the seagrass meadow in Saline Bay was the result of multiple drivers' impact. It all began with the increased water turbidity which weakened the plant and made it susceptible to other stressors such as lack of oxygen and H<sub>2</sub>S penetration within the plant tissue. Marks of visible grazing were only observed in July and August 2017 and not in April 2018 when the die-off was triggered. For this reason we did not include grazing as one of the main drivers in the decline of *C. nodosa* meadow. The discussion has been re-arranged accordingly. The discussion now reads: Saline bay is a shallow, highly dynamic coastal area characterized by frequent turbid waters due to the combined effect of land run-off and wind-driven resuspension of the fine sediment. Nutrients and Chl a (as a proxy for autotrophic biomass) varied in the ranges characteristic for the oligotrophic coastal waters off Rovinj (Ivančić et al., 2018). The increases in particulate matter concentration were associated with freshwater input, while their enrichment with unsaturated fatty acids, deriving from phytoplankton, was observed during the increases of autotrophic biomass. Only in September 2017, this increase was supported by nutrients from the water column, while all other less pronounced increases were most likely supplied with nutrients through sediment resuspension. In temperate Mediterranean coastal waters *C. nodosa* meadows show a clear unimodal annual growth cycle, reaching maximum development in summer, minima during winter and a particularly active phase in spring (Terrados and Ross, 1992; Zavodnik et al., 1998; Agostini et al., 2003). In Saline Bay, the maximum growth was shifted towards early autumn. This shift was most likely due to an intense grazing activities (Cebrian et al., 1996; Valentine and Duffy, 2006) suggested by a prevalence of visibly grazed leaves during July and August 2017. A minimum growth occurred during late autumn/winter,

as commonly observed. However, during the spring of 2018, phenological parameters continued to decrease despite established favorable environmental conditions for growth, i.e. increase in water temperature, intensity and period of solar radiation. This decrease continued until the complete extinction of the aboveground tissue in August 2018. The belowground tissue followed a similar trend, but with less expressed changes. Still, their recognizable remnants were found after the loss of above-ground tissues. Organic matter and closely correlated total lipids in the sediment of *C. nodosa* rooted area changed significantly throughout the investigated period, in contrast to the organic matter in non-vegetated sediment. Nevertheless, considerable similarity in the quality and degradation of lipid matter at both, the vegetated and the non-vegetated areas indicates an important contribution of detritus from the meadow as a source of organic matter for prokaryotes in non-vegetated sediments. This close coupling could be expected due to sites proximity and lower organic content of the non-vegetated sediment, which should enhance the dependence of prokaryotes on the imports of seagrass detritus from the adjacent meadows (Holmer et al., 2004). Significant enrichment of *C. nodosa* sediment with unsaturated, more labile components only during abundant growth of meadow could be explained by more efficient entrapment of seston material from the water column within the meadow (Gacia and Duarte, 2001). Such easily utilizable organic matter, including dissolved monomeric carbohydrates, leaching out during decomposition of *C. nodosa* leaves stimulates prokaryotic growth (Peduzzi and Herndl, 1991). This effect could be observed, as a higher prokaryotic abundance in *C. nodosa* sediment during this period. From July 2017 to March 2018, an adaptation of *C. nodosa* leaves to the decreasing light and temperature occurred. Until October 2017, the temperature of the water column was optimal for elongation of the leaves and biomass increase, while the ambient light intensities were continuously decreasing. An additional reduction of available light might occur from self-shading effect due to high canopy biomass and epiphytic macroalgae growth. Desaturation of low and relatively invariable lipids during the most active growth suggested the increase in the membrane fluidity to optimize photosynthetic activity under low light condition. Such physiological

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adaptation was found in seagrasses along a depth gradient (Beca-Carretero et al., 2019) and macroalgae in contrasting seasons (Schmid et al., 2014). In late autumn the decrease in desaturation indicated a reduced fluidity and activity of photosynthetically active membranes. The lower fluidity reduces proton leakage through the thylakoid membranes and energy consumption for their maintenance (Quigg et al., 2006; Wacker et al., 2016). The reduced photosynthetic activity was associated with a decreased density of shoots and aboveground biomass. By shedding leaves and shoots the plant further balances metabolic requirements and mobilize energy from the carbohydrate reserves stored in the below-ground tissue (Alcoverro et al., 2001; Lee et al., 2007). During the winter, the rapid desaturation of increasing lipids could be attributed primarily to a sharp and continuous decrease in water temperature. The desaturation provides a mechanism for the thermo-adaptive regulation of membrane fluidity and cold resistance in algae and plants (Terrados and Lopezjimenez 1996; Iveša et al. 2004; Upchurch, 2008). In a healthy seagrass meadow, the oxygen generated by seagrass photosynthesis is transported to below-ground tissues to maintain an oxic microsphere around roots and rhizomes, where it oxidizes sulfide to non-toxic  $S_0$ , preventing the intrusion of  $H_2S$  into the plant and the anoxia of the roots (Pedersen et al., 1998; Holmer et al, 2005).  $S_0$  was found in the *C. nodosa* below-ground tissue during the entire investigated period, as regularly observed in seagrasses living in sulfidic sediments (Holmer et al. 2006; Holmer and Hasler-Sheetal, 2014; Hasler-Sheetal and Holmer, 2015). The relatively low accumulation of  $H_2S$  ( $< 30 \mu M$ ) during summer and early autumn 2017 indicated that  $H_2S$  was apparently rapidly recycled within the rooted area via re-oxidation by  $O_2$  to  $S_0$  and/or removal by precipitation with iron compounds. Most of  $S_0$  was found in oxic layers or suboxic/anoxic boundaries with concentrations typical for sulfidic coastal sediments (Troelsen and Jørgensen, 1982; Panutrakul et al., 2001; Pjevac et al., 2014). The oxidation of  $H_2S$  could occur spontaneously by chemical reaction with free oxygen or mediated by sulfide-oxidizing bacteria surrounding or being attached to seagrass roots (Jørgensen, 1977; Cucio et al., 2016; Ugarelli et al., 2017; Fahimipour et al., 2017). In November, due to the degradation of organic mat-

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ter, reduced oxygen production and leakage in the rooted zone caused by *C. nodosa* senescence, the re-oxidation capacity of the sediment was greatly decreased. This resulted in considerable accumulation of H<sub>2</sub>S (> 100 μM) which extended up to the sediment surface. In winter and early spring, H<sub>2</sub>S production generally decreased, likely due to reduced activity of the sulfate-reducing bacteria at lower temperatures, and the sediment gradually shifted to a more oxidized state. H<sub>2</sub>S detected even in the oxic sediment and in the rooted area could be attributed to the sediment heterogeneity and the presence of reducing micro-niches where anaerobic metabolism could occur regardless of surrounding redox conditions (Jørgensen 1977; Frederiksen and Glud, 2006). In April 2018, *C. nodosa* had been most probably exposed to increased siltation, due to an increase in terrigenous input combined with resuspension of sediment provoking elevated autotrophic growth. The intensive siltation is associated with the increased light attenuation, both through the direct shading effect of suspended sediments and through the promotion of phytoplankton and epiphyte growth by the associated increase in nutrients (Terrados et al., 1998; Halun et al., 2002; Brodersen et al., 2015). Therefore, the increase in seawater turbidity and considerable sediment redeposition on the leaves might have been severely impaired the light availability and photosynthetic activity. The reduction of light below minimum requirements for *C. nodosa* survival was indicated by LA/ALA > 1 in *C. nodosa* above-ground tissue due to a decrease in the conversion of LA to ALA (Harris and James, 1965). When minimum light requirements are not met (~14% of incidence light) the seagrass intensely sheds its leaves and shoots, while at a decrease to < 1% die-off is inevitable (Collier et al., 2012). This reduced light condition apparently persisted until May 2018 and most likely prevented the re-establishment of photosynthesis and *C. nodosa* continued to shed shoots and leaves. The reduced photosynthesis and therefore O<sub>2</sub> transport from the leaves to the rhizome-root system probably minimized root respiration. The maintenance of the oxic rhizosphere and the internal O<sub>2</sub> partial pressure in the lacunae further depended mainly on the diffusion of O<sub>2</sub> from the water column (Pedersen et al., 1998; Greve et al., 2003; Sand-Jensen et al., 2005). From April to June 2018, O<sub>2</sub> in the

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bottom water drastically decreased. Due to poor supply, O<sub>2</sub> content of below-ground tissue was too low to maintain an oxic microenvironment and therefore, the plant tissues became potentially accessible to sulfide intrusion (Pedersen et al., 2004). At the same time, the sediment was enriched with new organic matter derived from increased autotrophic biomass in bottom waters. In addition to the induction of the benthic bloom, strong sediment resuspension, most likely by aeration, stimulated the intense oxidation of H<sub>2</sub>S that started to produce in the rooted zone (up to 180  $\mu$ M, Fig. 7). An increase in S<sub>0</sub> concentration that reached its maximum in the same layer suggests a simultaneous oxidation of the produced H<sub>2</sub>S. The sulfide oxidation probably caused oxygen depletion in the rooted zone and anoxic zone extension up to the sediment subsurface. In May 2018, the excess of organic matter accumulated in April 2018 was degraded. The concentrations of S<sub>0</sub>, detected only in the suboxic layer, considerably decreased possibly by disproportionation or respiration by members of the sulfate-reducing bacteria. S<sub>0</sub>-disproportionating Desulfobulbaceae and S<sub>0</sub>-respiring Desulfuromonadales are frequently detected in anoxic coastal sediment (Pjevac et al., 2014). During June and July 2018, a sudden and significant deterioration of *C. nodosa* physiological condition was indicated by the increase in LA/ALA ratio in the leaves and overall saturation of decreasing lipids in above- and below-ground tissues. Additionally, the loss of leaf tissue negatively impacted the photosynthetic carbon fixation (Lee and Dunton, 1997; Lee et al., 2007). The below-ground tissue that was not supported by photosynthetically derived oxygen became anoxic. Thus induced anaerobiosis most likely caused a complete inhibition of the fatty acid desaturation chain (Harris and James, 1965) and permanent breakdown of photosynthesis. In July, the bottom waters were completely depleted in O<sub>2</sub> and the whole plant exposed to sulfides. To reach the leaves, sulfide has to exceed below-ground tissue oxidation capacity, invading the meristems where sulfide toxicity can have drastic effects on shoot growth and survival (Greve et al., 2003; Frederiksen et al., 2008). H<sub>2</sub>S inhibit cytochrome c oxidase by binding to regulatory sites on the enzyme, reducing the rate of cellular respiration and leading to the chemical asphyxiation of cells (Nichols et al., 2013). From June to August 2018, the

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decomposition of organic matter, encompassing the entire sediment core, was intensified and accompanied by a large increase in H<sub>2</sub>S concentrations (up to 1200  $\mu$ M). The degradation process involved rhizomes and roots, as suggested by an apparent loss of below-ground biomass. Such loss typically occurs in the first stage of plant decay, the leaching phase (Trevathan-Tackett et al., 2017). Readily available, soluble carbohydrates that largely contribute to leachate mass (Vichkovitten and Holmer, 2004) most probably supported the increase in prokaryotic abundance observed in June and July 2018, and also high rates of sulfate reduction. However, the significant decreases in PA that coincided with a maximum degradation of organic matter and H<sub>2</sub>S production in August 2018 might indicate that remaining compounds were not degradable by the sulfate reduction pathway (Arndt et al., 2013) and needed the presence of prokaryotes specialized in the anaerobic degradation of refractory compounds, including cellulose and lignin. During September and October 2018, due to the combined effect of freshened oxygenated water input and resuspension which gradually deepen the oxic layer, H<sub>2</sub>S concentrations drastically decreased due to re-oxidation. Biogeochemical studies suggest most sulfide (80–90%) is eventually re-oxidized, while only 10–20% is ultimately buried as complexes with iron (i.e. FeS, FeS<sub>2</sub>) or with organic matter after sulfurization (Jørgensen, 1977; 1982). H<sub>2</sub>S scavenging with iron and formation of iron sulfides might be more critical in Saline Bay since terrestrial waters are washing out terra rossa, rich in Fe-oxides and oxyhydroxides (Durn, 2003). For this reason, sediment cores were most likely always black with sulfuric odor, irrespective of H<sub>2</sub>S concentrations or presence of vegetation.

3. Decreased root respiration may also contribute to the dying off Authors response: We agree that the decreased root respiration contributed to *C. nodosa* dying off. We believe that reduced O<sub>2</sub> transport from the leaves to the rhizome-root system minimized or even stopped root respiration. Moreover, the lack of sediment oxygenation compromised the metabolic activities of aerobic bacteria around the roots and their oxidation of H<sub>2</sub>S. A commentary regarding root respiration was added within the re-arranged Discussion.

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4. Changes of unsaturated fatty acids could be attributed to many sources, since phytoplankton or microalgae are the main producers of these compounds, it is hard to guess. Authors response: We are aware that changes in unsaturated fatty acids could be attributed to many sources. Particularly in the sediment their changes depend on the dynamic interactions between primary producers and their consumers within the food web. In this paper, the intention was to compare vegetated and control (non-vegetated) sediment using different markers and indices which together provided an indicative interpretation of the predictable sources. More complex analysis of the sources exceeds the scope of this paper and will be the topic of further investigation.

5. While grazing rate might be responsible for the changes in seasonal change of stand crop of the seagrass, the authors did not provide any record that grazing rate is higher in the Saline Bay. Authors response: In July and August 2017 the leaves were very short with visible signs of bites, so we assumed that significantly lower biomass in these two months was the result of grazing activity of herbivores. In addition, in a nearby bay where we performed biometric measurements in *C. nodosa* meadow simultaneously, we did not notice such intense leaves damage and the vast majority of leaves had intact apexes.

6. During summer period, high light and temperature may synergistically reduce the biomass of the seagrass due to higher respiration and higher photoinhibition. Authors response: We are aware of this synergistic effect of high light and high temperature on the reduction of biomass related to seagrasses growing near or within the intertidal zone where they may be exposed to high light stress which may then result in down-regulation of photosynthetic apparatus or if irradiance is too high by photoinhibition. As the sampling depth in Saline Bay (3 - 4 m) was not in the intertidal region and tidal oscillation does not exceed 50 cm, we believe that this mechanism did not contribute to the reduction of *C. nodosa* biomass either in summer 2017 or in summer 2018. The biomass reduction and meadow die-off started when the temperature were still moderate. When the temperature reached its maximum (August 2018) the meadow had

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already died and therefore, the effect of the high temperature could not be displayed.

Technical corrections: 1. Repeated wordings should be avoided in a sentence or paragraph. Authors response: This was checked and corrected accordingly.

2. Unit of silicate should be double checked, might be mistaken Authors response: The unit of silicate was double checked. Silicates (orthosilicates or reactive silicates) were determined spectrophotometrically by molybdenum blue method. Calibration was performed in the range 0.5 – 20  $\mu\text{mol L}^{-1}$  of sodium silicofluoride,  $\text{Na}_2\text{SiF}_6$ . Accordingly, the results were presented in  $\mu\text{mol L}^{-1}$  ( $\mu\text{M}$ ).

Line 83 change shorter to shorten. . . changed

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