Dynamics of environmental conditions during a decline of a *Cymodocea nodosa* meadow

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Abstract. The dynamics of the physicochemical and biological parameters were followed during the decline of a *Cymodocea nodosa* meadow in the northern Adriatic Sea from July 2017 to October 2018. During the regular growth of *C. nodosa* from July 2017 to March 2018, *C. nodosa* successfully adapted to the changes of environmental conditions and prevented H_2S accumulation by its re-oxidation, supplying the sediment with O_2 from the water column and/or leaf photosynthesis. The *C. nodosa* decline was most likely triggered in April 2018 when light availability to the plant was drastically reduced due to increased seawater turbidity that resulted from increased terrigenous input, indicated by a decrease in salinity accompanied with a substantial increase of particulate matter concentration, combined with resuspension of sediment and elevated autotrophic biomass. Light reduction impaired photosynthesis of *C. nodosa* and the oxidation capability of below-ground tissue. Simultaneously, a depletion of oxygen due to intense oxidation of H_2S occurred in the sediment, thus creating anoxic conditions in most of the rooted areas. These linked negative effects on the plant performance caused an accumulation of H_2S in the sediments of the *C. nodosa* meadow. During the decay of above- and below-ground tissues, culminating in August 2018, high concentrations of H_2S were reached and accumulated in the sediment as well as in bottom waters. The influx of oxygenated waters in September 2018 led to the re-establishment of H_2S oxidation in the sediment and remaining of the below-ground tissue. Our results indicate that if disturbance of environmental conditions, particularly those compromising the light availability, takes place during the recruitment phase of plant growth when metabolic needs are at maximum and stored reserves minimal, a sudden and drastic decline of the seagrass meadow occurs.

1 Introduction

Seagrasses are important ecosystem engineers constructing valuable coastal habitats which play a key role in the preservation of marine biodiversity and carbon sequestration (Duarte et al., 2013; Samper-Villareal et al., 2016). Seagrasses extend their active metabolic surfaces (i.e., leaves, rhizomes and roots) into the water column and in the sediment, where root activity might modify the chemical conditions (Marbà and Duarte, 2001). Their canopies and dense meadows are responsible for trapping substantial amounts of sediment particles and organic matter, enhancing water transparency and sediment stability with the dense network formed by the rhizome (Gacia and Duarte, 2001; Hendriks et al., 2008; Widdows et al., 2008). Seagrass rhizospheres store organic matter (Pedersen et al., 1997), promote sulfate reduction (Holmer and Nielsen, 1997), release oxygen (Pedersen et al., 1998) and alter sediment redox potential.

Seagrasses require some of the highest light levels of any plant worldwide to provide oxygen to roots and rhizomes and support a large amount of non-photosynthetic tissue (Orth et al., 2006). This make seagrasses sensitive to environmental changes, especially those that deteriorate light availability, such as sediment loading, eutrophication or epiphyte cover on seagrass leaves (Terrados et al., 1998; Halun et al., 2002; Brodersen et al., 2015; Costa et al., 2015). Seagrasses have adapted to a highly variable light environment providing tolerance to short-term periods of low light conditions by balancing carbon supply and respiratory requirements. In a healthy growing population this balance is achieved by increasing the photosynthetic activity, re-allocation of carbohydrate reserves from rhizomes and slowing down growth rates (Collier et al., 2009). Beside metabolic and physiological changes, stress responses under poor light conditions include shedding of leaves and shoots and production of new, altered tissue. At sub-lethal light levels, these changes may be permanent. Below these species-specific minimum light requirements seagrass populations are dying off (Collier et al., 2012). Membrane lipids, particularly polyunsaturated fatty acids (PUFA), as the most responsive constituents have a major role in the adaptation processes of primary producers to fluctuating environmental factors, such as temperature, irradiance or salinity (Viso et al., 1993; Lee et al., 2007; Schmid et al., 2014; Sousa et al., 2017; Beca-Carretero et al., 2018; Beca-Carretero et al., 2019). The changes in the unsaturation degree (UND) of membrane fatty acids affect the maintenance of membrane functions and its resistance to cold stress or

poor light conditions. UND depends mostly on the variation of α-linolenic (C18:3n-3, ALA) and linoleic (C18:2n-6, LA), the major unsaturated fatty acids in leaves, implicated in the evolution of oxygen during photosynthesis. LA and ALA are derived from oleic acid by desaturation in the chloroplast and this conversion considerably declines in the dark, being completely inhibited by anaerobiosis (Harris and James, 1965).

Sediments inhabited by seagrasses are usually anoxic, highly reduced and rich in sulfide (H_2S), a strong phytotoxin (Koch and Erskine, 2001) which has been implicated in several die-off events of seagrasses (Carlson et al., 1994; Borum et al., 2005; Krause-Jensen et al., 2011). H_2S is produced by sulfate-reducing bacteria that use sulfate as a terminal electron acceptor for the mineralization of organic matter (Jørgensen, 1977; Capone and Kiene, 1988, Canfield et al., 1993). High H_2S concentrations may occur as a consequence of enhanced mineralization due to increased temperature, organic loading or oxygen depletion (Moeslund et al., 1994; Pérez et al., 2007; Mascaró et al., 2009). Under these conditions, sulfides may intrude into plant. Re-oxidation of H_2S in the rhizosphere by incorporation of S^0 in the below-ground tissue has been recognized as a major survival strategy of seagrasses in sulfidic sediments (Pedersen et al., 2004; Holmer et al., 2005; Hasler-Sheetal and Holmer, 2015). Generally, the synergistic effect of oxygen depletion and other stresses, such as sulfide toxicity may shorten the survival of benthic communities and possibly accelerate mortality events (Vaquer-Sunyer and Duarte, 2010).

The seagrass *Cymodocea nodosa* (Ucria) Ascherson is widely distributed and common species throughout the Mediterranean (Terrados and Ros 1992; Pedersen et al., 1997; Cancemi et al., 2002; Agostini et al., 2003). For the northern Adriatic, however, only sparse data are available on the standing crop, seasonal dynamics or natural/anthropogenic pressures supporting the ecological or conservation status of *C. nodosa* meadows (Zavodnik et al., 1998; Orlando-Bonaca et al., 2015; 2016). Although *C. nodosa* show large phenotypic plasticity adapting to diverse natural and anthropogenic stressors by physiological and morphological adaptations, a severe decline has been reported during the last decades in coastal areas (Orth et al., 2006; Short et al., 2011; Tuya et al., 2002; 2014), including the northern Adriatic (Orlando-Bonaca et al., 2015; 2019). One of these declines was documented in our study performed from July 2017 to October 2018 in Saline Bay (northern Adriatic Sea). A series of monthly physicochemical and biological measurements were conducted in *C. nodosa* tissues, sediment underlying the *C. nodosa* meadow, non-vegetated sediments and

surrounding water to i) determine the link between ambient seawater and sediment environmental factors influencing the growth of *C. nodosa*, ii) document the response of *C. nodosa* to the changes in environmental conditions that led to the meadow decline and iii) evaluate the conditions leading to the decline of *C. nodosa*.

2 Materials and methods

2.1 Study site

Saline Bay is located 4 km northwest of Rovinj (Croatia) at the coast of the northern Adriatic Sea (45°7′5″N; 13°37′20″E, Fig. S1). The bay represents the terminal shallow part of an 800 m long inlet, open towards the northwest. The southeastern coast of Saline Bay is characterized by relatively pristine conditions, while the northwestern littoral part has been completely modified by the excavation of coastal mud and the addition of large amounts of gravel to create an artificial beach. Large amounts of silty red soil (*terra rossa*) can be found in the south eastern inner part of the bay in a large muddy flatland which is slowly being eroded by the sea and rain weathering. The main input of freshwater to the bay represents land drainage canals since the year 2017. Even though Saline Bay is protected from the prevailing winds (from the NE and SE) circulations from the northwestern quadrant can occasionally trigger bigger waves resuspending the surface sediments and giving the waters a muddy appearance. At the beginning of this study, the seafloor was covered with large *C. nodosa* meadows spreading from the southwestern coastal area (1.5 m depth) toward the central part of the bay (4 m depth), while at the end of the study only a few small patches persisted in tiny stripes along the shoreline.

2.2 Sampling

The sampling was performed for 15 months from July 2017 to October 2018. Seawater for analyses of nutrients, chlorophyll a (Chl *a*), particulate matter concentration and prokaryotic abundance was sampled using plastic containers (10 L). *C. nodosa* (3 – 4 m of depth) was collected together with rhizomes, roots and epiphytic macroalgae by divers using the quadrat sampling method. Three quadrats (20 x 20 cm) were randomly scattered in positions of maximum seagrass coverage (e.g. 100 %). Sediment samples were collected inside vegetated and non-vegetated sediment by divers using plastic core samplers (15 cm, 15.9 cm^2). For granulometric composition, organic matter, prokaryotic abundance, total lipids and fatty acid analyses, the cores were cut into 1 cm sections to a depth of 8 cm and lyophilized, except of

sections for prokaryotic abundance analysis, that were weighted (approx. 2 g) and fixed with formaldehyde (final conc. 4% v/v) immediately after slicing the sediment core.

2.3 Temperature (T) and salinity (S) measurements

T was measured continuously (in 30 min. intervals) using HOBO pendant temp/light Data Loggers (Onset, USA) which were replaced at each sampling. S was measured on sampling dates by a pIONneer 65 probe (Radiometer analytical, Copenhagen).

2.4 Inorganic nutrients, Chl *a* and particulate matter (PM) analysis

Seawater for all analysis was filtered through combusted Whatman GF/F filters. Nitrate (NO_3), nitrite (NO_2), ammonia (NH_4), phosphate (PO_4) and silicate (SiO_4) were analyzed spectrophotometrically according to Strickland and Parsons (1972). Chl *a* was determined on filters by the fluorometric procedure after extraction in 90 % acetone (Holm-Hansen et al., 1965). PM was determined gravimetrically after filtering up to 5L seawater on pre-weighed, filters which were dried (at 60°C) and reweighed.

2.5 Determining prokaryotic abundance

For determining the prokaryotic abundance in seawater, 2 ml of formaldehyde (final conc. 4% v/v) fixed samples were stained with 4,6-diamidino-2-phenylindol (DAPI, 1 μg mL^{-1} final conc.) for 10 min (Porter and Feig, 1980). In sediment samples, prokaryotes were detached from the sediment particles by addition of Tween 80 (0.05 mL) and ultrasonicated for 15 min (Epstein and Rossel, 1995). After sonication, 1 mL of the supernatant was stained with DAPI (final conc. 5 μg/mL). DAPI stained samples were filtered onto black polycarbonate filters (Whatman, Nuclepore, 0.22 μm) and counted under an epifluorescence microscope (Zeiss Axio Imager Z1).

2.6 Biometry of *C. nodosa* and epiphytic macroalgae

The material from each quadrat was washed under running seawater to remove sediment. From each quadrat algae, leaves and rhizomes with roots were separated. The length of the longest leaf on each shoot was measured and the shoots were counted. Species of macroalgae were determined, and their coverage was estimated according to the Braun-Blanquet scale.

Separated samples were washed with filtered and autoclaved seawater, weighed, dried at 60 °C for 48 h and re-weighed. The dry mass was calculated per area (g m^{-2}).

2.7 Granulometric composition of the sediment and its organic matter content

For granulometric analysis of the sediment, each sample was wet sieved through a set of seven standard ASTM sieves (4-, 2-, 1-, 0.5-, 0.25-, 0.125-, 0.063-mm mesh size). The fraction that passed through the 0.063-mm sieve was collected and analyzed following the standard sedigraph procedure (Micromeritics, 2002). The material that was retained on the sieves was dried and weighted. The data obtained by both techniques were merged to obtain a continuous grain size range and analyzed with the statistic package Gradistat v 6.0. Sediments were classified according to Folk (1954). The sediment permeability was calculated based on median grain size (d_g) following the empirical relation by Gangi (1985). The organic matter content was determined as ignition loss after heating dried sediment sections at 450°C for 4 h in a muffle furnace.

2.8 Oxygen (O_2), hydrogen sulfide (H_2S) and redox potential (Eh) profiling

The microprofiles of O_2, H_2S and Eh were measured on intact cores immediately after sampling using a motorized micromanipulator (MMS9083) equipped with microsensors OX-100 and H_2S-200, redox microelectrode RD-200 coupled with reference electrode REF-RM (Unisense A/S, Denmark). Prior to the measurements, the OX-100 microsensor was calibrated using a two-point oxic – anoxic calibration; H_2S-200 was calibrated in fresh Na_2S solutions using eight-point calibration (1μM - 300 μM in a de-oxygenated calibration buffer (NaAc/HAc, pH <4); RD-200 with REF-RM was calibrated using two point calibration by simultaneous immersion of electrodes in quinhydrone redox buffers prepared in pH 4 and pH 7 buffers, all according to the manufacturer's recommendation. During measurements, sediment cores were placed in a pool filled with seawater from the sampling site to maintain *in situ* temperature. From July to October 2017 H_2S was measured spectrophotometrically in pore waters (Cline, 1969) squeezed out by centrifugation from each section (5 mm) of the sediment cores.

2.9 Total lipids, fatty acid composition and elemental sulfur (S^0)

Lyophilized samples of seagrass tissues, macroalgae, sediment or particulate matter were weighed and extracted into a solvent mixture of dichloromethane/methanol (DCM: MeOH, 2:1) in an ultrasonic bath at 35°C with three solvent mixture changes. The extracts were pooled and separated into layers by addition of 0.9% NaCl solution. Lower DCM layers (containing lipids) were released over Na_2SO_4 anhydride, collected in pre-weighed round bottom flasks and evaporated to dryness using rotavapor. After evaporation, flasks were re-weighed, and total lipid concentrations (TL, mg g^{-1} DW) were calculated from the difference in weight. For fatty acids determination, lipid extracts were saponified (1.2 M NaOH in methanol), acidified (6 M HCl), methylated (14% BF_3 in methanol) and extracted into DCM.

Fatty acid methyl esters (FAME) were analyzed by Agilent gas–liquid chromatography (GLC) 6890 N GC System equipped with a 5973 Network Mass Selective Detector, capillary column (30 m x 0.3 mm x 0.25 μm; cross-linked 5 % phenylmethylsiloxane) and ultra-high purity helium as the carrier gas. The GLC settings were as follows: programmed column temperature rise from 145°C by 4°C/min to 215°C, then by 1°C/min to 225°C and finally by 4°C/min to 270°C at constant column pressure of 2.17 kPa. Retention times, peak areas and mass spectra were recorded on the ChemStation Software. FAME were identified by mass spectral data and family plots of an equivalent chain length (ECL) for GC standards. Applied GC standards were: FAME mix C18–C20, PUFA1, PUFA3 standards (Supelco/Sigma-Aldrich, Bellefonte, PA, USA); C4–C24 FAME standard mix, cod liver oil and various individual pure standards (Sigma, Neustadt, Germany).

The following indices of fatty acid profiles were calculated: saturated fatty acids (SAT), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the unsaturation degree (UND). UND was employed to evaluate the degree of organic matter degradation due to more susceptibility of unsaturated, particularly polyunsaturated, components to degradation and calculated according to the formula [1*(% mono-)+2*(% di-)+3* (% tri-)+4*(% tetra-)+5*(% penta-)+6*(% hexa-enoic)]/% SAT (Pirini et al., 2007). To evaluate the input of terrestrial organic matter relative to that of marine origin in particulate matter, the terrestrial to aquatic acid ratio (TAR= C24+C26+C28 / C12+C14+C16) was used (Cranwell et al., 1987; Bourbonniere and Meyers, 1996).

In FAME chromatograms elemental sulfur (S^0), eluted as S_8 (m/z 256), was identified by comparison of retention time and characteristic fragment ions in samples and standard solutions. The concentration of S^0 was estimated on the base of the calibration curve prepared

for standard solution of S_8 (Aldrich, Germany) in cyclohexane (2-20 mg L^{-1}). The calibration curve was determined under the same GLC settings as FAME. Limit of detection (LoD) and limit of quantitation (LoQ) were calculated from the parameters of the calibration curve constructed on the basis of the 3 lowest concentrations in 3 replicates. LoD and LoQ (0.92 mg L^{-1} and 2.80 mg L^{-1}, respectively) were more than twice the values obtained by Rogowska et al. (2016) probably due to higher injector and column temperature used in this study than they proposed as optimal for S determination.

2.10 Data analyses

A multivariate analysis, hierarchical clustering and K-means methods (Systat 12) was applied to group *C. nodosa* above- and below-ground tissues according to the similarity of their fatty acid profiles and indices, i.e., physiological condition during the investigated period.

Sediment data were analyzed for two groups of sediment layers, the upper layer (0- 4 cm) where most of rhizomes and roots are located, and the lower layers (5-7 cm). Differences between vegetated and non-vegetated sediment samples in each sediment layer were tested by one-way ANOVA. Correlations among parameters were tested using the Pearson's correlation coefficient (r). The level of statistical significance was $p < 0.05$. A multivariate principal component analysis (PCA, Primer 6) was applied to identify the most important variables explaining differences between vegetated and non-vegetated sediments. Correlation matrices were constructed using variables: H_2S, Eh, O_2, S^0, PA, TL and UND. All variables were normalized due to their different scales. Only the principal components with eigenvalues >1 were considered.

3 Results

3.1 Water column

3.1.1 Environmental variables

During summer of 2017 daily means of sea-bottom temperature in *C. nodosa* meadow ranged between 26°C and 28°C. During autumn seawater temperatures decreased below 12°C until the end of December. The coldest period was recorded at the beginning of March lasting only for a few days (min. 8.62°C). From April to mid-July 2018, temperature increased with moderate fluctuations to the maximum of 29.26°C recorded in August 2018 (Fig. 1a).

Concentrations of inorganic nutrients and Chl *a* were generally low. The highest concentrations (DIN: 8.27 µM; PO_4: 0.18 µM; SiO_4: 9.82 µM; Chl *a*: 0.89 µg L^{-1}) associated with the lowest salinity (34.2) were found in September 2017 (Table S1). The abundance of prokaryotes (2.6-11.3 x 10^5 cell mL^{-1}) varied seasonally and significantly correlated to seawater temperatures ($r = 0.618$; $p < 0.05$). In contrast, salinity (S: 34.2 - 38.5) and concentrations of particulate matter (PM: 3.84 - 14.21 mg L^{-1}) showed irregular variations (Fig. 1b) and a significant opposite trend ($r = -0.630$; $p < 0.05$).

The particulate lipids exhibited the highest unsaturation degree (UND) during summer/early autumn 2017 and small increases of UND in April and September/October 2018 (Fig. 1c). UND was significantly correlated with Chl *a* ($r = 0.603$; $p < 0.05$). In contrast, terrestrial to aquatic ratio (TAR) considerably increased in April and was the highest in August 2018 (Fig. 1c). TAR was negatively correlated to UND ($r = -0.644$, $p < 0.05$) and positively to particulate matter ($r = 0.641$, $p < 0.05$). Although PUFA with 18 C atoms made the largest contribution to the total PUFA pool, C20 PUFA, mainly of phytoplankton origin, showed a similar trend as observed for UND (Fig. S2, Table S2).

3.2 *Cymodocea nodosa* meadow

3.2.1 Biometry

C. nodosa leaves and shoots reached the highest biomass (285.3 ± 57.4 g m^{-2}), length (102.4 ± 26.6 mm) and shoot density (3703±334 shoots m^{-2}) in October 2017 (Fig. 2a). After the appearance of the regular vegetation minimum in November 2017, biometric indices further decreased reflecting the decay of the meadow in summer 2018. In August 2018, only yellow to brownish leaves on sparse shoots were collected (4.5 ± 1.3 g m^{-2}, 5.4 ± 1.3 mm and 30 ± 35 shoots m^{-2}). In September and October 2018, no shoots or leaves were observed (Fig. 2a). The biomass of rhizomes and roots reached also its maximum in October 2017 (599.7 ± 36.8 g m^{-2}). In contrast to leaves and shoots, the below-ground biomass was stable until March 2018 when a decline was observed that continued until October 2018 (30.5 ± 6.8 g m^{-2}) (Fig. 2a).

3.2.2 Total lipid (TL) concentrations and fatty acid composition

TL in the *C. nodosa* above-ground tissue (6.7 - 25.3 ± 2.4 mg g^{-1} DW) increased until February 2018, when maximum TL concentrations were measured (Fig. 2b). Thereafter, TL concentrations decreased until August 2018. During this period, the below-ground TL

concentration (6.3 ± 1.9 – 15.9 ± 1.1 mg g^{-1} DW) was generally lower than the above-ground TL concentrations and the trend was similar to that of leaves. The minimum concentrations of TL were observed in September 2018, while in October 2018, concentrations similar to that measured in October 2017 were observed (Fig. 2b).

The major fatty acid components in *C. nodosa* tissues were palmitic (C16:0) amongst the saturated (SAT) and oleic (C18:1n-9) in monounsaturated fatty acids (MUFA). In the above-ground tissue, the main polyunsaturated fatty acids (PUFA) were α-linolenic (C18:3 n-3, ALA) and linoleic (C18:2 n-6, LA), while in the below-ground tissue LA was dominant (Fig. 2b). The dynamics of UND in the above-ground tissue was principally influenced by changes in ALA and LA. LA/ALA ratios were < 1 from July 2017 to March 2018, and > 1 from April to July 2018 (Fig. 2b). In August 2018, the LA/ALA ratio was infinite due to the absence of ALA (Fig. 2b). Elemental sulfur (S^0) was detected only in decaying leaves in August 2018 (0.21 mg g^{-1} DW). In the below-ground tissue, S^0 was detected in all samples (Fig. 2b). Higher concentrations were measured during summer 2017 (up to 0.39 ± 0.06 mg g^{-1} DW). S^0 increased from minimum concentrations in April (0.02 ± 0.01 mg g^{-1} DW) until September 2018 reaching 1.42 mg g^{-1} DW (Fig. 2b).

According to the fatty acid profiles, *C. nodosa* leaves were classified in three groups, except for the leaves collected in August 2018 (Fig. 3). The most distinguishing features specifying physiological differences between Group 1 (July - October 2017 and February - March 2018), Group 2 (November - December 2017 and April - May 2018) and Group 3 (June and July 2018) were decreasing mean values of PUFA, UND, ALA and LA and increasing means of SAT and the proportion of long-chain saturated fatty acids ($C \geq 24$). In the ungrouped leaves from August 2018 ALA was not found, PUFA and UND were at a minimum, while SAT and $C \geq 24$ at a maximum (Table S3). Three groups of rhizomes and roots (Group 1: July - October 2017 and February - March 2018; Group 2: November - December 2017 and April - May 2018 and Group 3: (June - October 2018) showed similar characteristics to the groups 1, 2 and 3 of related leaves (Table S4).

3.2.3 Epiphytic macroalgae

From July 2017 to February 2018 different taxa of macroalgae belonging to the three phyla Chlorophyta (*Halimeda tuna*, *Dasycladus vermicularis*, *Cladophora prolifera*, *Udotea petiolata*), Rhodophyta (*Rytiphlaea tinctoria*, *Peyssonnelia* spp, *Gelidium* sp.) and

Ochrophyta (*Dictyota dichotoma*) were covering the meadow in varying proportions and abundances (Fig. 4). After March 2018, when only few individuals of *Peyssonnelia* sp. were found, macroalgae were no longer present in the *C. nodosa* meadow.

Although the fatty acid profiles of macroalgal communities were highly variable, the contribution of 18- and 20 PUFA to the total PUFA pool generally depended on the prevailing phyla and their characteristic PUFA pattern. The algae belonging to Rhodophyta and Ochrophyta are richer in 20 PUFA (C20:5n-3, C20:4n-6), while Chlorophyta are generally showing prevalence of 18 PUFA (C18:3n-3, C18:2n-6) (Schmid et al., 2014, Gao et al., 2018). Furthermore, their contribution to biomass varied due to large differences in morphology, which most likely also contributed to the variability of fatty acid profiles. 18 PUFA and 20 PUFA showed the highest contribution to the total PUFA pool during the dominance of Chlorophyta and Rhodophyta in the macroalgal community, respectively. In most samples, the lowest contribution to the total PUFA pool was observed for 16 PUFA and 22 PUFA (Fig. S3).

3.3 Sediment

3.3.1 Granulometric composition

According to the granulometric composition, median grain sizes (d_g) and permeability (k) the vegetated and non-vegetated sediments were classified as slightly gravelly sandy mud (g)sM, fine grained ($d_g < 165$ µm) and low permeable to impermeable sediment ($k < 2 \cdot 10^{-11}$ m^2). In general, the *C. nodosa* sediment consisted of a significantly higher proportion of sand (Sa), and lower proportion of silt (Si) and clay (C) (Sa, 41.11 ± 4.34 %; Si, 46.44 ± 2.86 %; C, 9.63 ± 2.76 %) in comparison to non-vegetated sediment (Sa, 20.53 ± 10.49 %; Si, 53.24 ± 6.76 %; C, 23.29 ± 4.86 %). The median grain size and permeability in *C. nodosa* sediment (d_g, 37.51 ± 17.97 µm, k, $1.22 \cdot 10^{-12} \pm 1.13 \cdot 10^{-12}$ m^2) were significantly higher than in non-vegetated sediment (d_g, 10.86 ± 5.34 µm; k, $1.04 \cdot 10^{-13} \pm 1.02 \cdot 10^{-13}$ m^2). The upper layers of both cores (0 - 4 cm) had larger particles, while the lower layers (5 - 8 cm) showed a uniform distribution of smaller grain sizes (Fig. 5).

3.3.2 O_2, E_h, H_2S and S^0

Oxygen concentrations (O_2) in the bottom water of the *C. nodosa* meadow varied in a wide range (0 µM - 171.4 ± 17.6 µM) and generally followed the O_2 saturation trend (Fig. 6a).

From May to June 2018, O_2 decreased below 62.5 µM, considered as severe hypoxia (Vaquer-Sunyer and Duarte 2008) and was completely depleted in July 2018 (Fig. 6a). From August to October 2018, O_2 increased again. The variations of O_2 in the bottom water of the non-vegetated sediment were similar to those in the *C. nodosa* meadow albeit generally higher (79.4 ± 10.4 µM – 212.2 ± 33.4 µM) than in the vegetated sediment except for September and October 2018 (Fig. 6a).

In general, O_2 penetration depth in the vegetated and non-vegetated sediment co-varied with the O_2 concentration in the bottom layer, penetrating deeper when its concentration in the bottom water was higher (Fig. 6b). In the vegetated sediment, O_2 was mainly depleted down to 1 cm of depth. In the non-vegetated sediment, the oxygen penetration depth was up to 4 times higher than in vegetated sediments, except for the period from August 2018 to October 2018 when the penetration depths were similar (Fig. 6b).

The thickness of the oxic (Eh > 150 mV) and suboxic (150 mV > Eh > 0 mV) layers in the vegetated sediment increased from July 2017 (~ 0.5 cm) to March 2018 (~ 4 cm), and decreased progressively from April (~ 0.8 cm) towards the surface in July 2018, when the entire sediment core was anoxic (Eh < 0). From August (~ 1 cm) to October 2018 (~ 2.5 cm) the oxic and suboxic layer thickness increased again (Fig. 7). Oxic conditions (Eh > 0) generally reflected O_2 concentrations in the bottom waters. The dynamics of Eh in non-vegetated sediment were similar to those in the vegetated sediment. However, the thickness of the oxic layer was considerably larger than in the vegetated sediment. Reducing conditions (Eh < 0) were only recorded in July and August 2017 (Fig. 7).

Concentrations of free H_2S in the pore water of the vegetated sediment generally increased with depth creating an accumulation zone mainly within the upper sediment layers (1 - 4 cm) (Fig. 7). From July to November 2017, H_2S concentrations increased up to 120 µM (at 4 - 5 cm). In December 2017, H_2S was low and uniformly distributed throughout the core (< 5 µM). H_2S concentrations increased and the accumulation layer was ascending from March (up to 34.2 ± 12.8 µM; 5 - 7 cm) to April 2018 (up to 177.2 ± 125.1 µM; 3.5 - 4.5 cm). During May 2018 (up to 107.8 ± 75.9 µM; 2.5 - 4 cm), June (up to199.0 ± 6.3 µM; 1.5 - 6 cm) and July (up to 210.1 ± 138.9 µM; bottom water - 6 cm) a propagation of the accumulation zone was observed in addition to an increase in H_2S (Fig. 7). In August 2018 (up to 1164.1 ± 702.1 µM; bottom water - 7 cm) extremely high concentrations over the entire sediment core were recorded. In September and October 2018, H_2S concentrations decreased (down to 140.0 ±

25.3 and 72.7 ± 52.7 μM; bottom water - 7 cm and 1 - 7 cm, respectively). In the non-vegetated sediment, H_2S depth profiles were similar to those in vegetated sediments, but the concentrations were generally lower, except for the summer of 2017 when the concentrations were comparable but the accumulation zones deeper (Fig. 7).

S^0 mainly occurred in oxic (Eh > 150 mV) and suboxic (150 mV > Eh > 0 mV) layers of both, vegetated and non-vegetated sediments (Fig. 7). Generally, the ranges of approximated S^0 concentrations in vegetated sediment ($8.5 \cdot 10^{-5}$ - 0.39 mg·g^{-1} DW ~ $2.6 \cdot 10^{-3}$ - 12.1 μmol·g^{-1} DW), except for the extreme value in April 2018 (0.99 mg·g^{-1} DW ~ 30.8 μmol·g^{-1} DW), were similar to those found at the non-vegetated sites ($2.9 \cdot 10^{-4}$ – 0.28 mg·g^{-1} DW ~ $9.2 \cdot 10^{-3}$ – 8.9 μmol·g^{-1} DW).

3.3.3 Prokaryotic abundance

Prokaryotic abundance varied largely in vegetated (2.1 - 39.9· 10^7 cells g^{-1} fresh weight, FW) and non-vegetated sediments (3.7 - 24.1·10^7 cells g^{-1} FW). Prokaryotic abundance was significantly higher in the upper than the lower layers of vegetated ($F = 40.553$, $p < 0.05$) and non-vegetated ($F = 52.531$, $p < 0.05$) sediments (Fig. 8). Prokaryotic abundance showed significant monthly changes in the upper ($F = 3.053$, $p < 0.05$) and lower layer ($F = 5.035$, $p < 0.05$) of vegetated sediments, in contrast to both layers of non-vegetated sediments ($p > 0.05$). Prokaryotic abundances were significantly higher in the upper layers ($F = 44.577$, $p < 0.05$) and significantly lower in the lower layers ($F = 5.986$, $p < 0.05$) of vegetated than in the respective layers of non-vegetated sediments (Fig. 8). In the upper sediment layer, prokaryotic abundances were significantly higher in the vegetated than in the non-vegetated sediments from July to October 2017 and from June to August 2018 (Fig. 8). In the lower layers of vegetated sediments, prokaryotic abundance was significantly higher than in the non-vegetated sediments in October 2017 and in August and September 2018 (Fig. 8).

3.3.4 Organic matter, total lipids and fatty acid composition

The concentrations of organic matter (OM) and total lipids (TL) were highly correlated in vegetated (OM: 37.6 - 231.1 mg/g DW, TL: 0.15 - 2.75 mg/g DW; $F = 214.172$, $p < 0.05$) as well as in non-vegetated sediments (OM: 56.7 - 160.3 mg/g DW, TL: 0.33 - 2.39 mg/g DW; $F = 45.569$, $p < 0.05$). OM and TL generally decreased with depth and exhibited similar

418 changes throughout the investigated period with significantly higher concentrations in upper
419 than in lower sediment layers ($p < 0.05$) (Fig. 9).
420 In the vegetated sediment, TL showed significant monthly changes in the upper ($F =$
421 11.418, $p < 0.05$) and lower sediment layers ($F = 3.186$, $p < 0.05$), in contrast to both layers of
422 non-vegetated sediment ($p > 0.05$). From July to October 2017, in the upper layer of vegetated
423 sediments, TL was significantly higher than in non-vegetated sediments (Fig. 9). From
424 November 2017 onwards, TL decreased slightly until April 2018, reaching similar
425 concentrations as TL in non-vegetated sediments (Fig. 9). TL concentrations decreased
426 markedly in May and continued until August 2018. During that period, TL in vegetated
427 sediments was significantly lower than in non-vegetated sediments. In September and October
428 2018, TL concentrations in vegetated sediments were similar to those in non-vegetated
429 sediment (Fig. 9).
430 The fatty acid composition of vegetated and non-vegetated sediments was similar and in
431 both layers characterized by the prevalence of SAT (vegetated upper: 71.2 - 90.4%, lower:
432 75.9-89.1%; non-vegetated upper: 71.2-80.7%, lower: 78.2-82.5%) over MUFA (vegetated
433 upper: 7.6-22.9%, lower: 9.0-19.9%; non-vegetated upper: 17.8-24.1%, lower: 15.3-18.2%)
434 and PUFA (vegetated upper: 1.9-6.9%, lower: 1.9-5.1%; non-vegetated upper: 1.7-4.8%,
435 lower: 1.7-3.9%). The trends of the monthly changes in UND were similar in both layers of
436 both sediment types. Those variations were less pronounced in the non-vegetated sediment
437 where UND varied in narrower ranges in both layers (upper: 0.26-0.51, lower: 0.23-0.33) than
438 in vegetated sediment (upper: 0.13-0.57, lower: 0.14-0.37). From July to October 2017 and in
439 April 2018, UND was higher in the upper layers of vegetated sediment than in non-vegetated
440 one, while from November 2017 to March 2018, UNDs of both sediments were lower than in
441 previous period (Fig. 9). From June to August 2018, UND decreased considerably in
442 vegetated sediment, being lower than in non-vegetated sediments. During September and
443 October 2018, an increase of UND was observed in both sediments. In the lower layers,
444 UNDs were similar, except for July and August 2018 when a considerable decrease of UND
445 was observed in vegetated sediments (Fig. 9).
446 The proportions of PUFAs with chain lengths of 16, 18, 20, and 22 C atoms within the
447 PUFA pool were similar between the respective layers of both sediments. Throughout the
448 study period, the highest contribution of 18PUFA originated from *C. nodosa* detritus and
449 Chlorophyta was observed (Fig. S4, Table S2). From July to October 2017, April to May

2018 and September to October 2018, a contribution of 20PUFA attributed to phytoplankton and Rhodophyta was also detected. 16PUFA and 22PUFA accounted for the smallest contribution to the PUFA pool and were found in seston and macroalgae (Fig. S4, Table S2). The similarities between the sediments were also observed in the contribution of the main SAT components to the SAT pool from July 2017 to March 2018 and from September to October 2018 (Fig. S4, Table S2). From April to August 2018, an increase of the long-chain ($C \geq 24$) and common (C16:0 + C18:0) fatty acids followed by the decrease of bacterial fatty acids (BACT) contribution to the SAT pool was observed in both layers of the vegetated sediment. In contrast, the contribution of these components to the SAT pool was fairly invariable in non-vegetated sediments during the same period (Fig. S4, Table S2).

3.3.5 Relationship between different physicochemical parameters

The relationships between H_2S, O_2, TL, S^0, PA, Eh and UND in vegetated and non-vegetated sediment are shown in the principal component analysis, where PC1 explained 42.5 % and PC2 14.4 % of variability (Fig. 10). The loadings for positive relationships were obtained for H_2S (0.298) on PC1 and Eh (0.541) and O_2 (0.327) on PC2. For the negative relationships, the loadings were for TL (-0.534), UND (-0.494), S^0 (-0.388), Eh (-0.327), PA (-0.296) and O_2 (-0.191) on PC1, and H_2S (-0.536), S^0 (-0.485), TL (-0.165) and UND (-0.221) on PC2.

PC1 separated most of the upper sediment layers (July 2017 - May 2018, September - October 2018) according to the higher concentrations of TL and S^0, higher UND and more positive Eh from the most of the lower layers and upper layers of vegetated sediments (June - August 2018) with increased H_2S concentrations. On PC2, the vegetated was separated from the non-vegetated sediment due to higher concentrations of H_2S, S^0 and more negative Eh, which characterized vegetated sediments during almost the entire study period. The extreme concentrations of S^0 and H_2S found in the upper layer in April and the lower layer in August 2018, respectively, were responsible for the considerable separation of these layers from all other vegetated layers (Fig. 10).

4 Discussion

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 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 dynamics of particulate matter was associated with freshwater input. The higher contribution from autochthonous sources was observed during the increases of autotrophic biomass. However, only in September 2017, this increase was supported by nutrients from the water column, while all other increases were most likely connected to bottom waters where phytoplankton could have been supplied with nutrients through sediment resuspension. The considerable increase in the particulate matter of terrigenous origin from April to August 2018 suggested the enhanced land run-off in that period.

In temperate Mediterranean coastal waters *C. nodosa* meadows show a clear unimodal annual growth cycle, reaching maximum development in summer, and minima during winter and a particularly active growth phase in spring (Terrados and Ross, 1992; Zavodnik et al., 1998; Agostini et al., 2003). In Saline Bay, the maximum biomass was measured in October 2017. This shift from summer to early autumn 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 2018, phenological parameters continued to decrease in spite of 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 above-ground tissue in August 2018. The below-ground tissue followed a similar trend, but with less expressed changes. Still, their recognizable remnants were found after the loss of the 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 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 sites indicates an important contribution of detritus imported from the meadow as a source of organic matter for prokaryotes in non-vegetated sediments. This close coupling could be expected due to site 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 within the meadow (Gacia and Duarte, 2001). Such

514 easily utilizable organic matter, including dissolved monomeric carbohydrates, leaching out
515 during decomposition of *C. nodosa* leaves stimulated prokaryotic growth as previously
516 observed (Peduzzi and Herndl, 1991).
517 From July 2017 to March 2018, an adaptation of *C. nodosa* leaves to the decreasing light
518 and temperature occurred. Until October 2017, the temperature of the water column was still
519 optimal for elongation of the leaves and biomass increase, while the ambient light intensities
520 were continuously decreasing. An additional reduction of available light might occur from the
521 self-shading effect due to high canopy biomass, and/or shading due to epiphytic macroalgae
522 growth. Desaturation of low and fairly invariable lipids during the most active growth phase
523 suggested an increase in the membrane fluidity to optimize photosynthetic activity under low
524 light conditions. Such physiological adaptation was found in seagrasses living along a depth
525 gradient (Beca-Carretero et al., 2019) and macroalgae in contrasting seasons (Schmid et al.,
526 2014). In late autumn 2017 g 2018, the decrease in desaturation indicated a reduced fluidity
527 and activity of photosynthetically active membranes (Quigg et al., 2006; Wacker et al., 2016).
528 This was associated with a decreased abundance of shoots and above-ground biomass. By
529 shedding leaves and shoots the plant further balances metabolic requirements and mobilize
530 energy from the carbohydrate reserves stored in the below-ground tissue (Alcoverro et al.,
531 2001; Lee et al., 2007). During the winter, due to a sharp and continuous decrease in water
532 temperature, rapid desaturation of increasing lipids provided a cold resistance, as regularly
533 observed in algae and plants (Terrados and Lopezjimenez, 1996; Iveša et al., 2004; Upchurch,
534 2008).
535 In a healthy seagrass meadow, the oxygen generated by seagrass photosynthesis is
536 transported to below-ground tissues to maintain an oxic microsphere around roots and
537 rhizomes, re-oxidize sulfide to non-toxic S^0, thus preventing an invasion of H_2S into the plant
538 (Pedersen et al., 1998; Holmer et al, 2005). S^0 was found in the *C. nodosa* below-ground
539 tissue during the entire investigation period, as already observed in seagrasses living in
540 sulfidic sediments (Holmer and Hasler-Sheetal, 2014; Hasler-Sheetal and Holmer, 2015). The
541 relatively low accumulation of H_2S (< 30 μM) during the summer and early autumn 2017
542 indicated that H_2S was apparently rapidly recycled within the rooted area via re-oxidation by
543 O_2 to S^0 and/or removal by precipitation with iron compounds. Most of S^0 was found in oxic
544 layers or suboxic/anoxic boundaries, being in ranges typical for sulfidic coastal sediments
545 (Troelsen and Jørgensen, 1982; Panutrakul et al., 2001; Pjevac et al., 2014). The oxidation of

546 H_2S could occur spontaneously by chemical reaction with free oxygen or mediated by sulfide-
547 oxidizing bacteria surrounding or being attached to seagrass roots (Jørgensen, 1977; Cucio et
548 al., 2016; Ugarelli et al., 2017; Fahimipour et al., 2017). In November, due to the degradation
549 of organic matter and reduced oxygen production and leakage in the rooted zone caused by *C.*
550 *nodosa* senescence, the re-oxidation capacity of the sediment was greatly decreased. This
551 resulted in considerable accumulation of H_2S (> 100 μM) which extended up to the sediment
552 surface. During winter and early spring, H_2S production generally decreased, likely due to the
553 reduced activity of sulfate reducing prokaryotes at lower temperatures, and the sediment
554 gradually shifted towards a more oxidized state. H_2S detected even in within the oxic
555 sediment and in the rooted area in February 2018 could be attributed to the sediment
556 heterogeneity and the presence of reducing micro-niches where anaerobic metabolism could
557 occur regardless of surrounding redox conditions (Jørgensen, 1977; Frederiksen and Glud,
558 2006).
559 In April 2018, *C. nodosa* had been most probably exposed to increased siltation, due to an
560 intensification of terrigenous input as indicated by a decrease in salinity (Δ 1.5 with respect to
561 March) and a substantial increase in particulate matter concentration (up to 3 times than in
562 March, Fig. 1b) combined with resuspension of sediment, provoking an elevated autotrophic
563 growth. The intensive siltation is associated with the increased light attenuation, both through
564 the direct shading effect of suspended sediments and through the promotion of phytoplankton
565 and epiphyte growth by the associated increase in nutrients (Terrados et al., 1998; Halun et
566 al., 2002; Brodersen et al., 2015). Therefore, the increase in seawater turbidity and
567 considerable sediment re-deposition on the leaves might have severely impaired the light
568 availability and slowed down the plant's photosynthetic activity as indicates LA/ALA > 1 in
569 the above-ground tissue resulting from decreased conversion of LA to ALA (Harris and
570 James, 1965). When the minimum light requirements (~14% of incidence light) are not met,
571 *C. nodosa* intensely sheds leaves and shoots (Collier et al., 2012). Such light condition
572 apparently persisted until May 2018 and most likely prevented the re-establishment of
573 photosynthesis and *C. nodosa* continued to shed shoots and leaves. The reduced
574 photosynthesis and therefore O_2 transport from the leaves to the rhizome-root system
575 probably minimized root respiration. The maintenance of the oxic rhizosphere and the internal
576 O_2 partial pressure in the lacunae further depended mainly on the diffusion of O_2 from the
577 water column. From April to June 2018, O_2 in the bottom water drastically decreased.

Although in such conditions of limited light and O_2 the seagrass might be capable for rapid modulation of metabolic pathways and enhance its photosynthetic rate, as shown for *Zostera muelleri* (Kim et al., 2018), it appeared that O_2 content of the *C. nodosa* below-ground tissue was still too low to maintain the internal pressure and therefore, the plant tissues became potentially accessible to sulfide intrusion (Pedersen et al., 2004).

At the same time, the sediment was enriched with fresh organic matter derived from increased autotrophic biomass in bottom waters. In addition to the induction of the bloom, strong sediment resuspension, most likely by aeration, stimulated the intense oxidation of H_2S that started to produce in the rooted zone (up to 180 μM), due to increased activity of sulfate reducing prokaryotes possibly triggered by the increase in temperature. An increase in S^0 concentration that reached its maximum in the same layer suggests a simultaneous oxidation of the produced H_2S. 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^0, detected only in the suboxic layer, considerably decreased possibly by disproportionation or respiration by members of the sulfate reducing bacteria (Pjevac et al., 2014).

During June and July 2018, a sudden and significant deterioration of *C. nodosa* physiological condition was indicated by the further 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 and therefore oxygen production, including the transport of oxygen to below-ground tissue (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 a permanent breakdown of photosynthesis leading to the final decay of the above-ground biomass and considerable loss of below-ground biomass. As the bottom waters were completely depleted in O_2 the whole plant was exposed to sulfides. H_2S inhibit cytochrome c oxidase by binding to regulatory sites on the enzyme, reducing the rate of cellular respiration and leading to the chemical asphyxiation (Nichols et al., 2013).

From June to August 2018, the decomposition of organic matter, encompassing the entire sediment core, was intensified and accompanied by a large increase in H_2S concentrations (up to 1200 μM). The degradation process involved rhizomes and roots, as suggested by the

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 the leachate mass (Vichkovitten and Holmer, 2004) most probably supported the increase in prokaryotic abundance observed in June and July 2018. However, the significant decrease in prokaryotic abundance that coincided with a maximum degradation of organic matter and H_2S 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, H_2S concentrations drastically decreased, and the sediment was gradually enriched in fresh organic matter. Due to the combined effect of freshened oxygenated water inflow and resuspension which gradually deepened the oxic layer, re-oxidation of H_2S increased. Biogeochemical studies suggest that most sulfides (80 – 90 %) are eventually re-oxidized; 10 – 20 % are ultimately buried as complexes with iron (i.e. FeS, FeS_2) or with organic matter after sulfurization (Jørgensen, 1977; 1982). H_2S scavenging with iron and formation of iron sulfides might be more important 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_2S concentrations or presence of vegetation.

5 Conclusions

Our results provide insights into the interaction of multiple stressors that have led to the meadow decay, triggered in the sensitive recruitment phase of meadow growth. Even after the improvement of the sediment conditions by the end of the summer 2018, *C. nodosa* was not able to recolonize its previously occupied areas. This finding combined with a visible alteration of the water column and sediment indicates a considerable loss of the *C. nodosa* habitat. Further research is needed to examine the fate of Saline Bay meadows and an eventual recolonization of the area.

Beyond seagrass itself, this loss had extensive consequences as it has endangered many species that depend on seagrass for food, shelter and nursery. Given the lack of data on the ecological and conservation status of the still numerous seagrass meadows along the northern

Adriatic coast, the identification and monitoring of the main pressures acting on them are needed to protect such valuable habitats from degradation and extinction.

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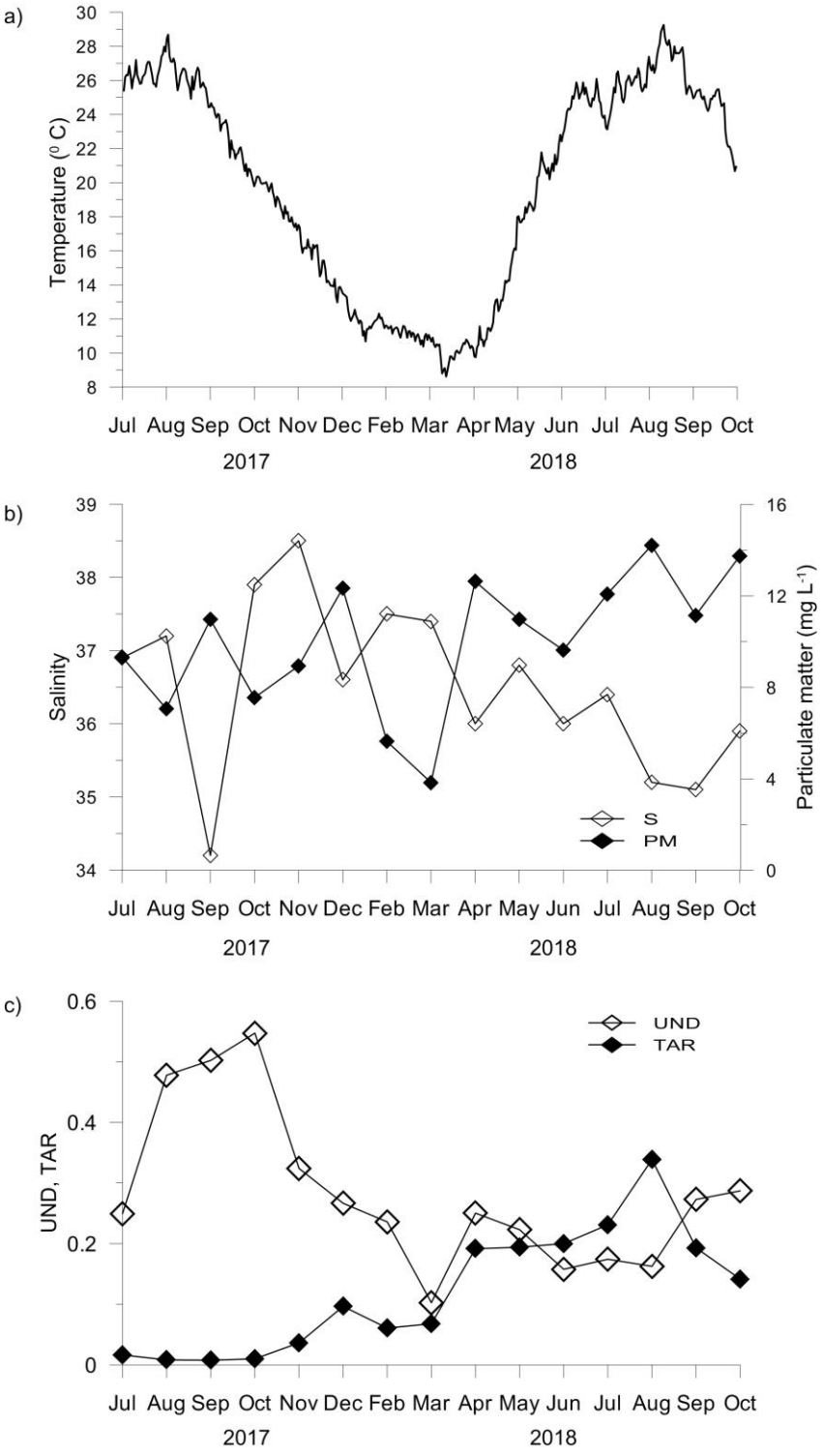


Figure 1. Temperature (a); salinity (b), particulate matter concentration (b); unsaturation degree (UND) and terrestrial to aquatic ratio (TAR) of the particulate lipid matter (c) in seawater.

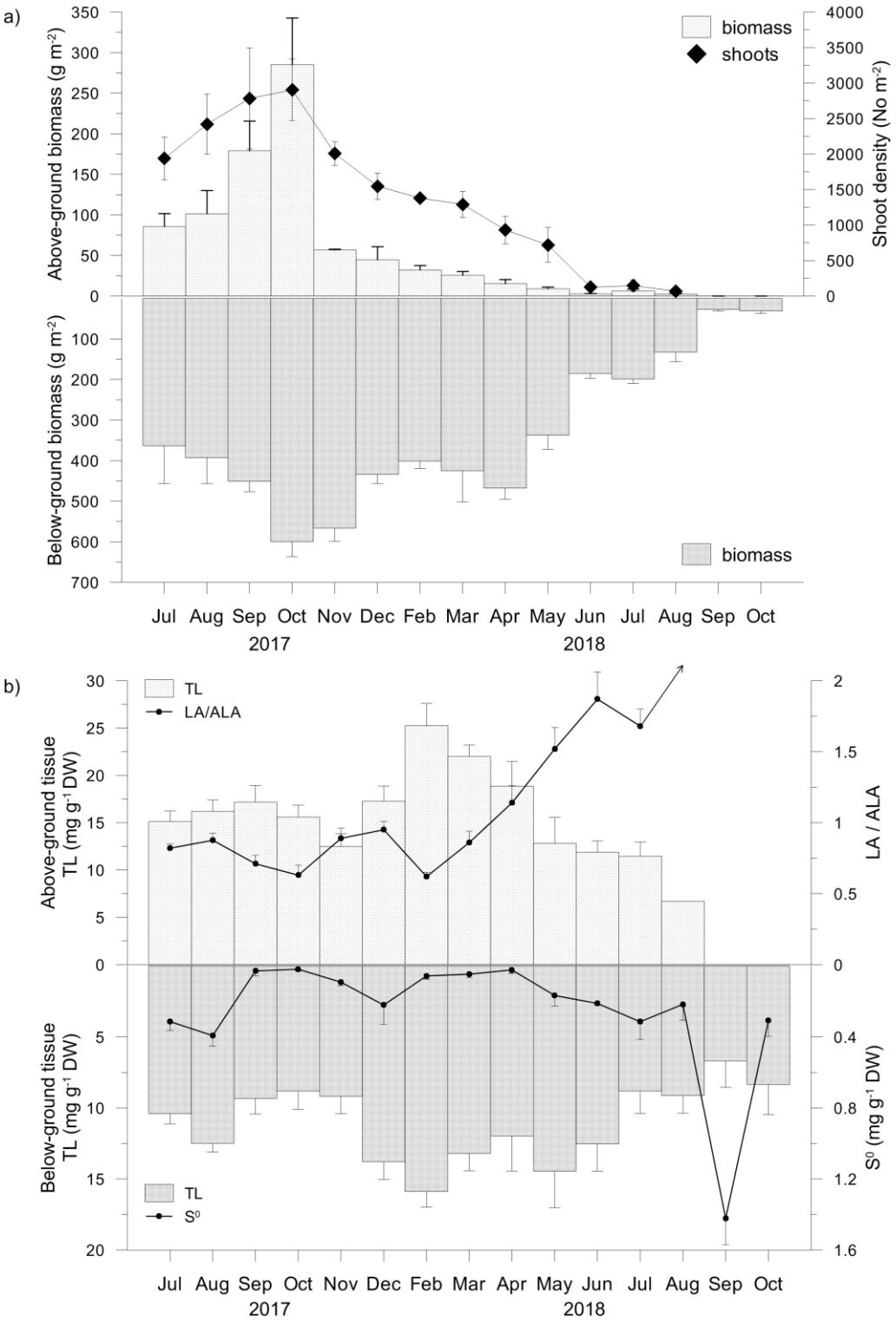
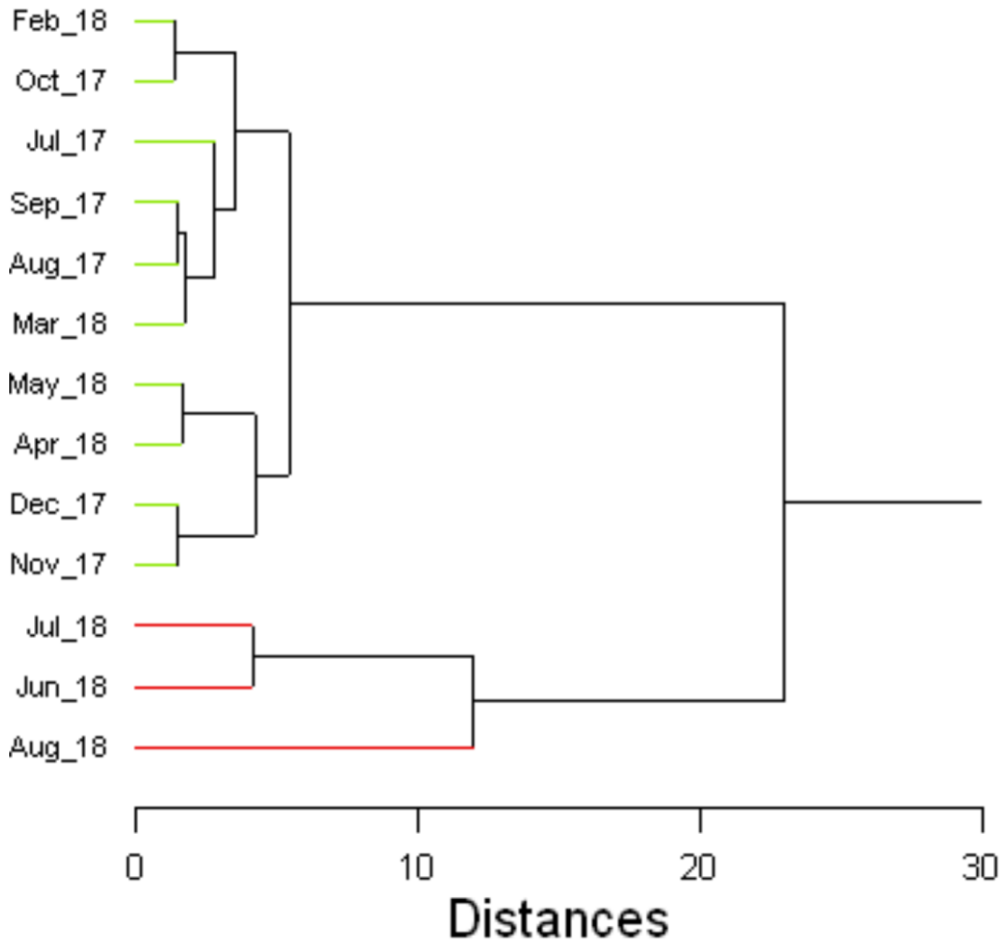


Figure 2. Above- and below-ground tissue biomasses and shoot density (a), total lipid concentrations (TL) and linoleic to α-linolenic fatty acids ratios (LA/ALA, an arrow indicates an infinite value) in above-ground tissue and TL and approximated concentrations of elemental sulfur (S^0) in below-ground tissue (b).



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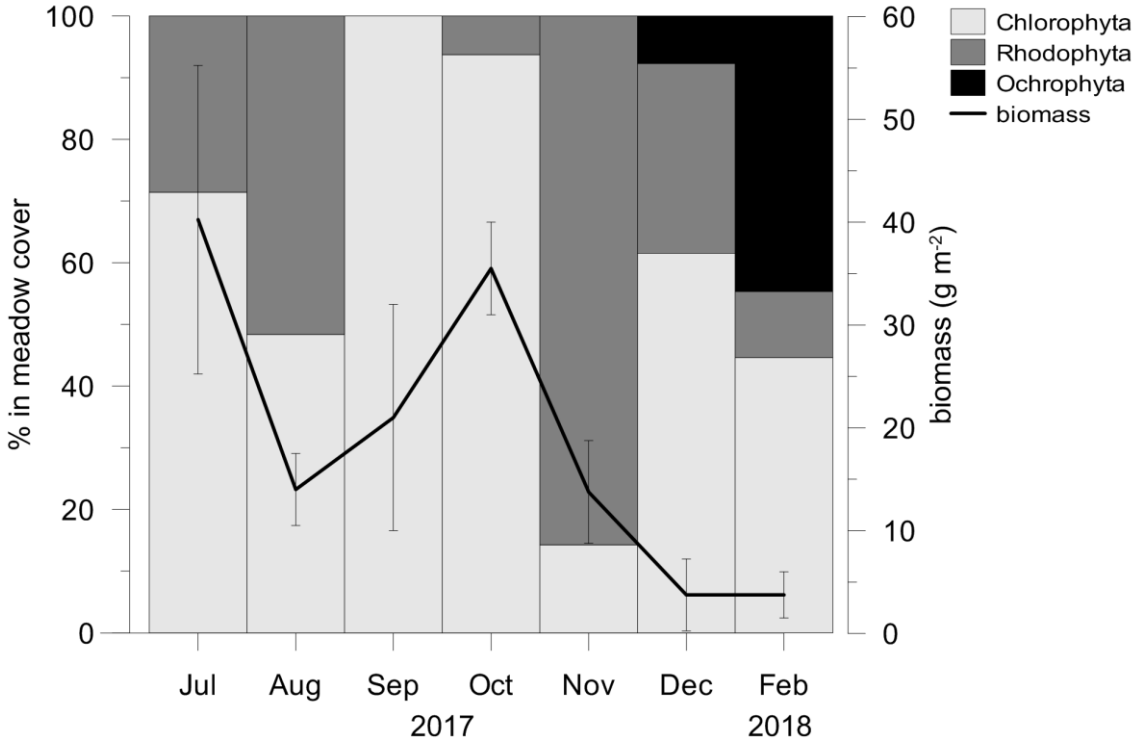
908 Figure 3. Cluster analysis dendrogram of fatty acid composition of *C. nodosa* leaves.

909 Summary statistics is given in Table S3.



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911 Figure 4. The contribution of macroalgal phyla in a meadow cover and total macroalgal
912 biomass. After February 2018 macroalgae were no longer present in the *C. nodosa* meadow.

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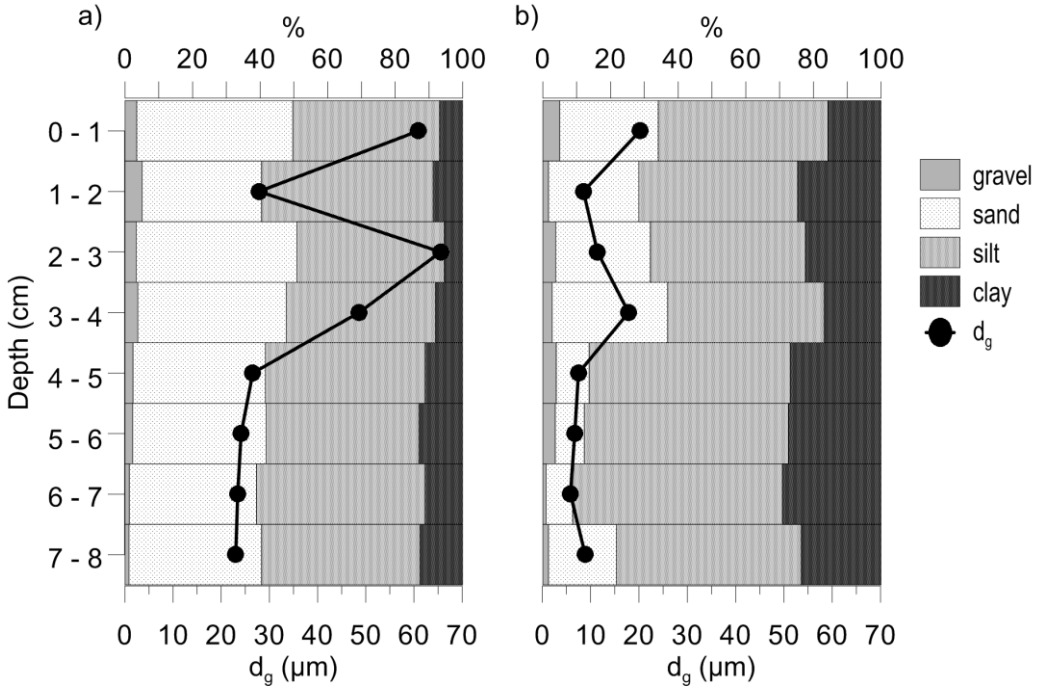


914

915 Figure 5. Granulometric composition and median grain size (d_g) of vegetated (a) and non-
916 vegetated sediment (b).

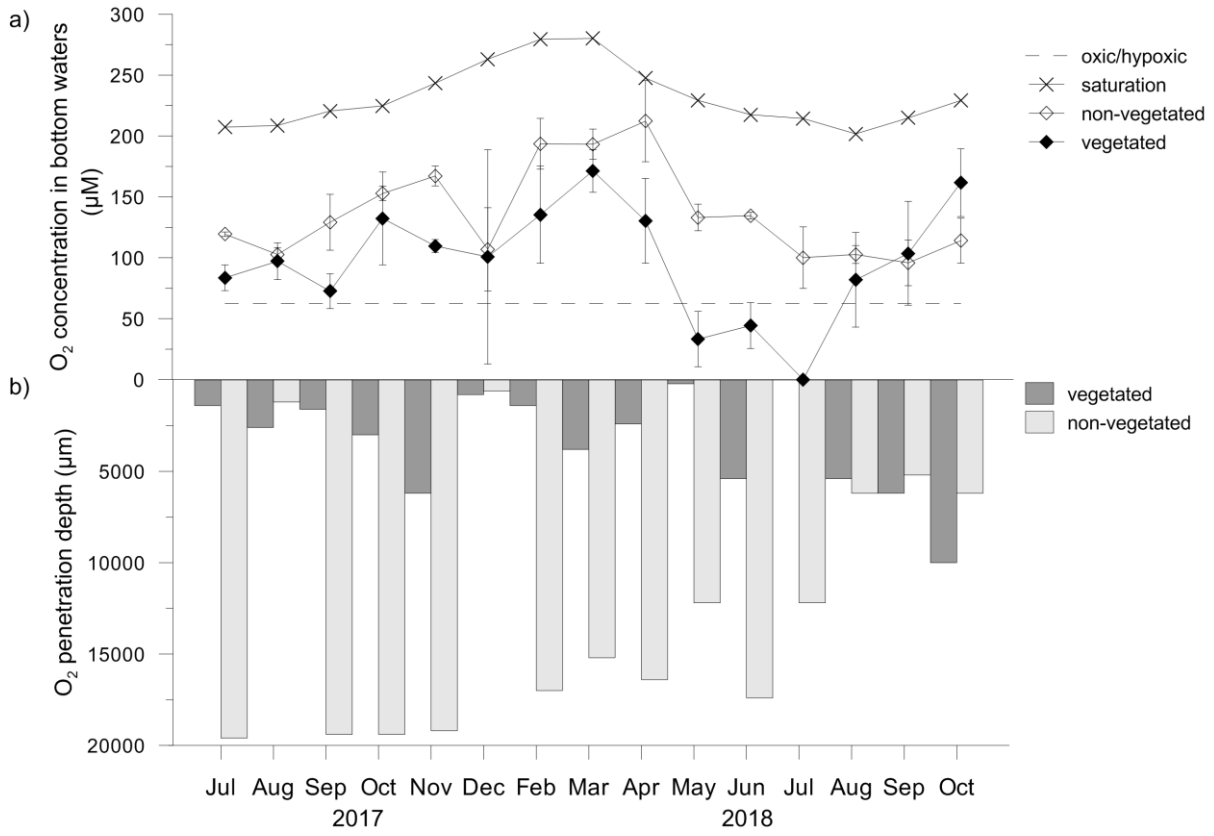


Figure 6. Oxygen concentrations (O_2) in bottom waters (a) and O_2 penetration depths (b) above and in vegetated and non-vegetated sediment, respectively. O_2 at the saturation level was calculated according to the temperature and salinity measured in seawater at the sampling dates; O_2 at the hypoxic frontier (~ 62.5 µM) was taken from Vaquer-Sanyer and Duarte (2008).

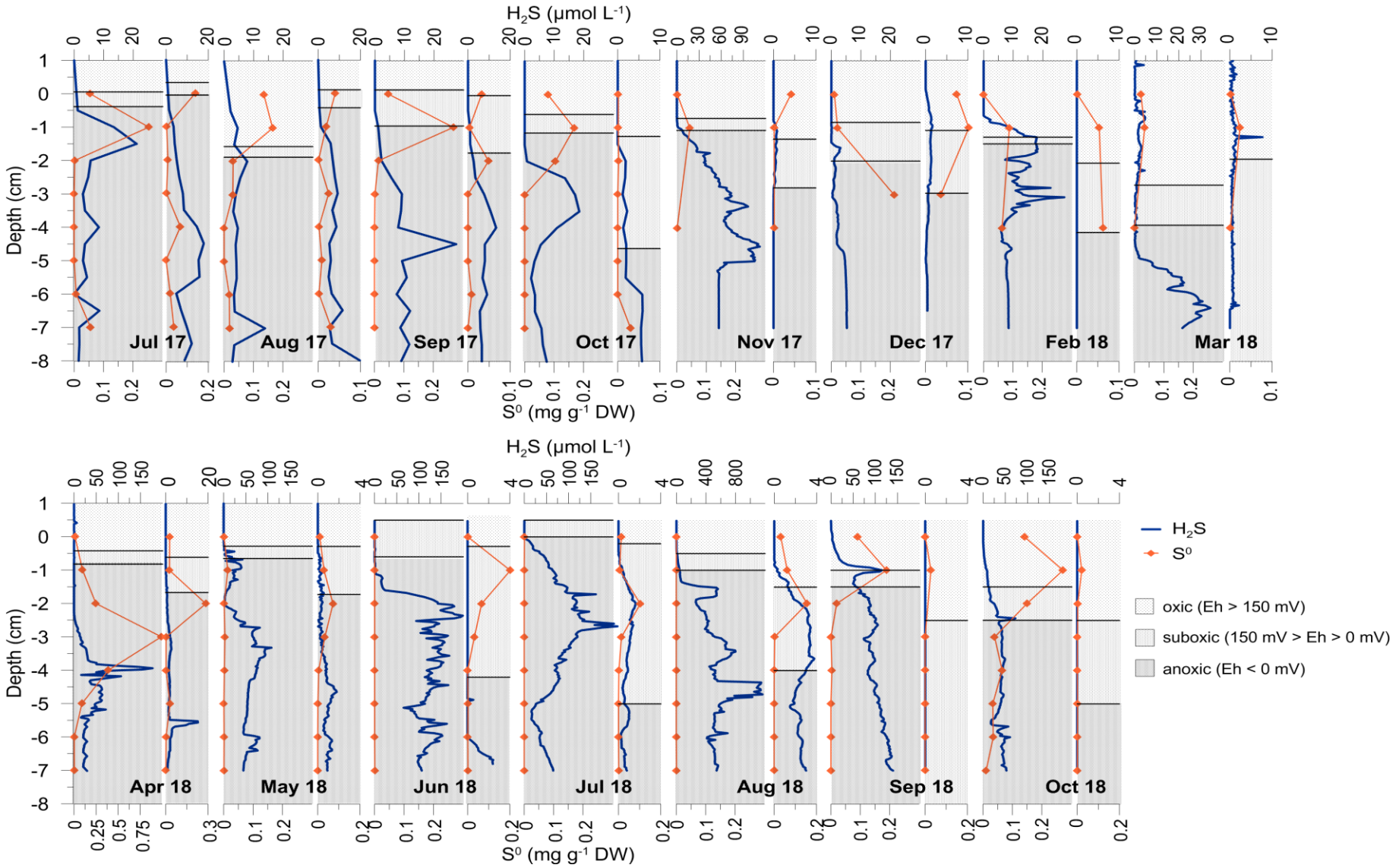
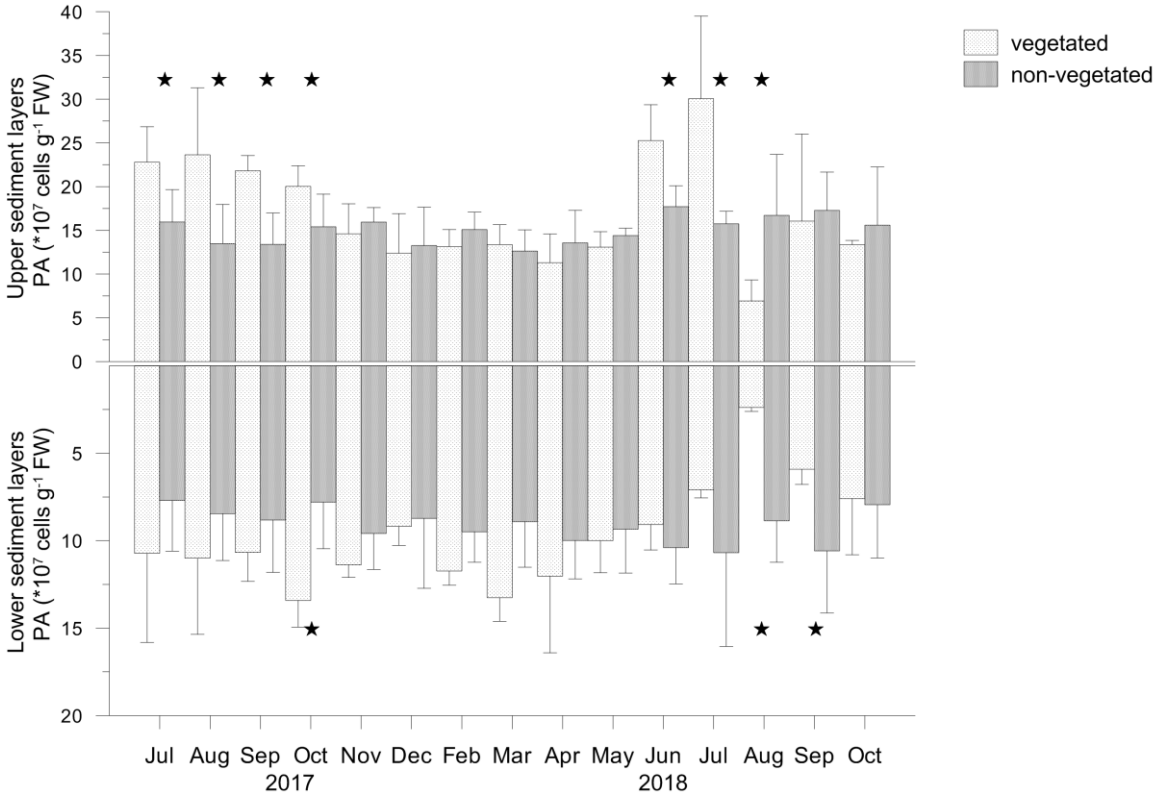


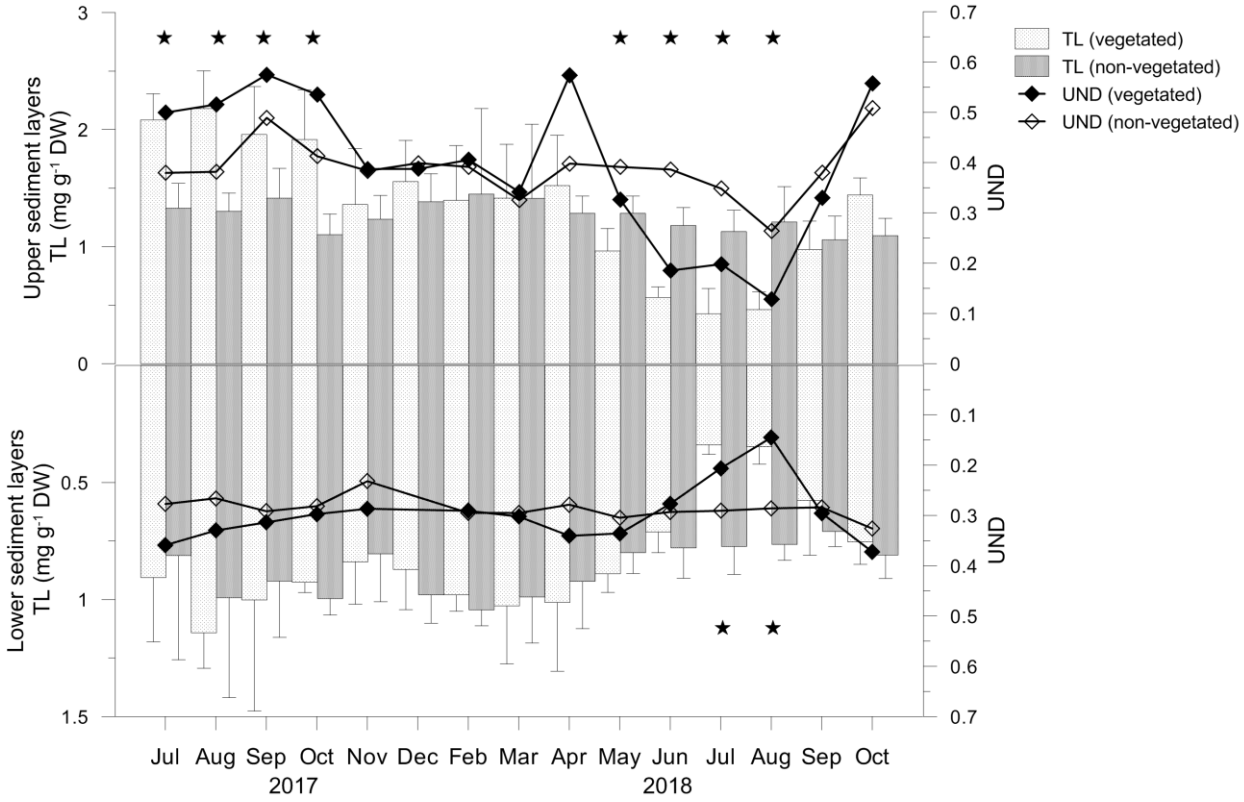
Figure 7. Depth profiles of H_2S and S^0 concentrations in vegetated and non-vegetated sediment (adjacent narrow graphs). The redox potential (Eh) in both sediments is shown as areas corresponding to oxic (Eh > 150 mV), suboxic (150 > Eh > 0 mV) and anoxic (Eh < 0 mV) conditions.



926

927 Figure 8. Prokaryotic abundance (PA) in the upper (0 - 4 cm) and lower (5 - 8 cm) layers of
928 vegetated and non-vegetated sediments; significant differences in PA between the sediments
929 are indicated by asterisks.

930



931

932 Figure 9. Total lipid concentrations (TL) and unsaturation degree (UND) in the upper (0 - 4
933 cm) and lower (5 - 8 cm) layers of vegetated and non-vegetated sediments. Significant
934 differences in TL between the sediments are indicated by asterisks.

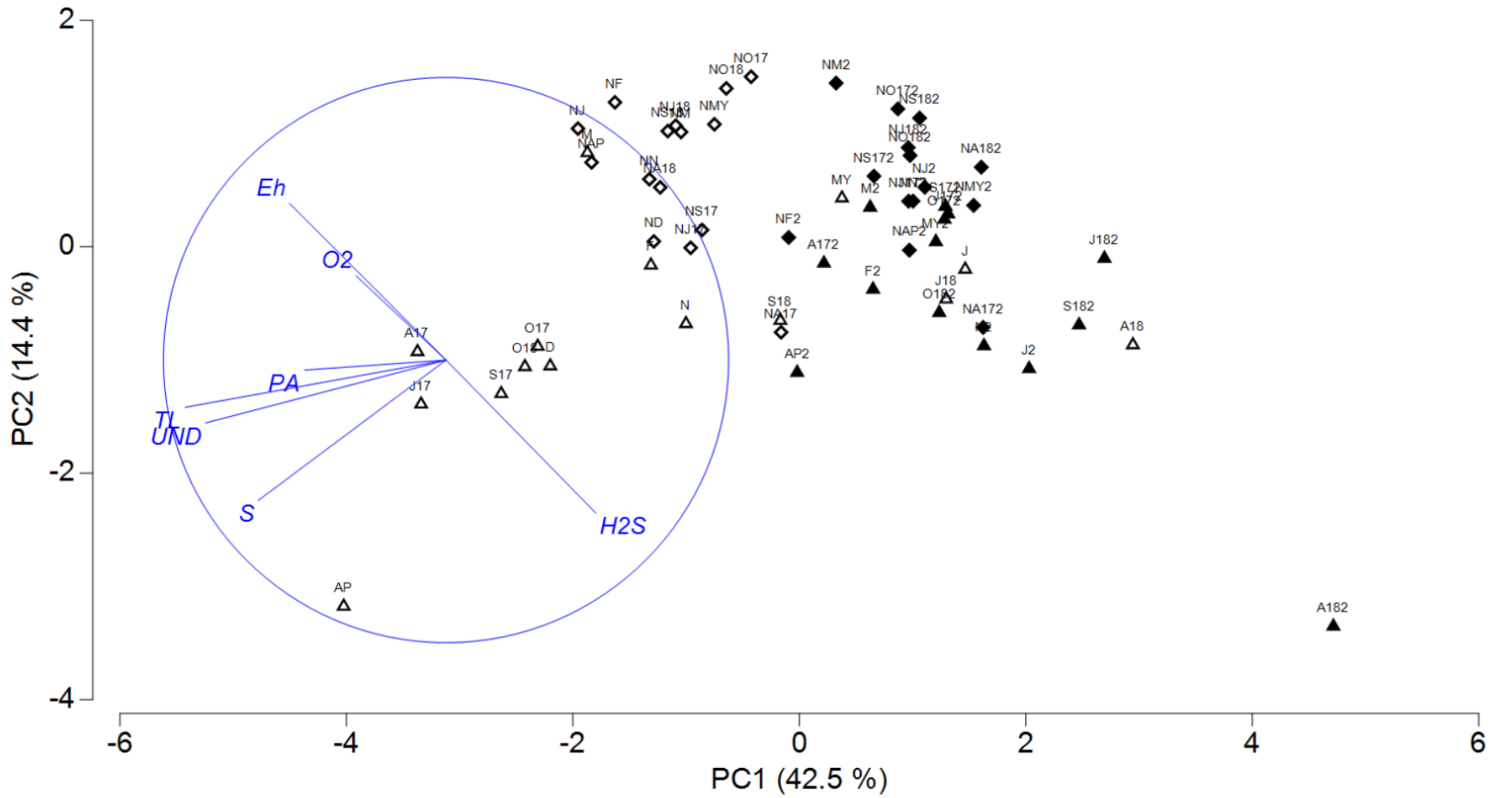


Figure 10. PCA plot of redox potential (Eh), oxygen (O_2), hydrogen sulfide (H_2S), sulfur (S), total lipids (TL) and prokaryotes (PA) concentrations and unsaturation degree (UND) in the upper (0 – 4 cm; △, ◇) and lower (5 – 7 cm; ▲, ◆) layers of vegetated and non-vegetated sediments, respectively. Projections of variables are given in circle