Seagrass excretes sugars to their rhizosphere making them the sweet spots in the sea

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Summary

Seagrasses are one of the most efficient natural sinks of carbon dioxide (CO_2) on Earth[1]. Despite covering less than 0.1 % of coastal regions, they have the capacity to bury up to 10 % of marine organic matter[1] and can bury the same amount of carbon 35 times faster than tropical rainforests[2]. On land, the soil's ability to sequestrate carbon is intimately linked to microbial metabolism[3]. Despite the growing attention to the link between plant production, microbial communities, and the carbon cycle in terrestrial ecosystems[4-6], these processes remain enigmatic in the sea. Here, we show that seagrasses excrete organic sugars, namely in the form of sucrose, into their rhizospheres. Surprisingly, the microbial communities living underneath meadows do not fully use this sugar stock in their metabolism. Instead, sucrose piles up in the sediments to mM concentrations underneath multiple types of seagrass meadows. Sediment incubation experiments show that microbial communities living underneath a meadow use sucrose at low metabolic rates. Our metagenomic analyses revealed that the distinct community of microorganisms occurring underneath meadows is limited in their ability to degrade simple sugars, which allows these compounds to persist in the environment over relatively long periods

of time. Our findings reveal how seagrasses form blue carbon stocks despite the relatively small area they occupy. Unfortunately, anthropogenic disturbances are threatening the long-term persistence of seagrass meadows. Given that these sediments contain a large stock of sugars that heterotopic bacteria can degrade, it is even more important to protect these ecosystems from degradation.

Main

Seagrasses are marine angiosperms that form the foundation of coastal regions on 7 of the 8 continents, excluding Antarctica[7]. As ecosystem engineers, seagrasses provide important services to human populations. For example, they are habitats for fisheries, stabilize the sea floor, remove pollutants from their waters, and help to maintain biogeochemical cycles[8]. Like their terrestrial relatives, seagrasses play an import role in sequestering carbon from the atmosphere by incorporating it into their living tissues and burying it as organic matter in their sediments[1]. It is assumed that the majority of this organic carbon is captured in seagrass sediments as plant debris[1,9]. However, seagrasses also leak high concentrations of dissolved organic carbon (DOC) into their environment[10], a carbon source that is turned over by microorganisms in their metabolism.

Here, we show that seagrasses alter the chemistry within their rhizosphere by excreting sugars to their environment. On land, terrestrial plants feed organic compounds to microbial communities in their soils to facilitate the long-term storage of carbon[3]. Comparatively less is known about how seagrasses interact with microbial communities living in their sediments[11,12]. We predict that sugars accumulate and influence the long-term ability of the microbial community to sequester carbon. These sugars shape the microbial taxa and function underneath meadows.

Sediments underneath seagrass meadows are rich in organic sugars

We measured high concentrations (on average 38.9 µM and up to 1145 µM) of organic sugars across four different types of seagrass meadows from three coastal regions (**Figure 1 a,b**) using a new method to detect metabolites in seawater[13]. The primary sugar in the porewater metabolome is the disaccharide sucrose, the most abundant sugar found in seagrass tissues across species[14]. Given that seagrasses occupy between 300,000-600,000 km^2 of coastal regions[1], we conservatively estimate (methods) that there is a global stock between 0.56 to 1.12 Tg of sucrose in the upper 30 cm of sediments colonized by seagrasses. Our observations represent a paradox in microbial ecology, as sugars are thought to be rapidly consumed in heterotrophic metabolism of microorganisms across most natural habitats[15-17]. Until now, the only known habitats that contain high concentrations of simple sugars (e.g., mono- and disaccharides) include plant nectars and fruit juices, biofilms associated with seaweeds, and soils as dissolved organic matter[18,19]. Consequently, the sediments under seagrasses represent a unique sugar habitat spanning hundreds to thousands of continuous stretches of m^2 in aquatic ecosystems.

To describe metabolite composition across both spatial and temporal scales, we collected 570 metabolomic profiles from sediment porewaters underneath and adjacent to a *Posidonia oceanica* meadow in the Mediterranean Sea (**Figure 2a**; methods). Non-targeted and targeted analyses revealed that sediments associated with *P. oceanica*, in comparison to non-vegetated sites, contained significantly ($p < 0.05$) higher concentrations of sucrose, glucose, trehalose, myo-inositol, and mannitol (**Figure 2b,c; Table S1**). Bulk measurements of DOC were also significantly higher ($p < 0.001$) inside the meadow then at the edge or outside sampling spots ($M_{[DOC\text{-}in]}$ = 363.05 µmol L^{-1} ± 69.92 SEM; $M_{[DOC\text{-}edge]}$ = 118.85 µmol L^{-1} ± 7.06 SEM; $M_{[DOC\text{-}out]}$ = 138.94 µmol L^{-1} ± 7.54 SEM) (**Figure S1a; Table S1**).These sugars contributed up to 40 % to

the DOC pool underneath seagrass meadows (**Figure S1b**) and form an energy-rich source of carbon that until now has been poorly characterized at the molecular scale[10].

Sucrose concentrations in seagrass sediments correlated to the availability of light on seasonal and diel temporal scales. Porewater profiles collected across different seasons revealed that sucrose was more abundant in April and October than in July (**Figure S2a**). Our observation that sucrose is significantly ($p < 0.05$; **Table S1**) higher in spring in comparison to other times of the year is consistent with bulk sediment carbohydrate, total organic carbon, and *P. oceanica* productivity measurements in the Mediterranean Sea[20,21]. Furthermore, sucrose abundances from seagrass leaves, but not their rhizomes or roots, are significantly ($p < 0.01$) higher in the day than in the night (**Figure 3a-d, Table S1**). This indicates sucrose is produced by the plants during the day-time hours. By sampling porewater metabolites across the diel cycle, we also observed sucrose concentrations are significantly higher underneath seagrass meadows in the daylight hours than at night ($p < 0.02$)(**Figure 3e, Table S1**). Taken together, our observations indicate that seagrasses produce sugars in their leaves and transport the excess photosynthate to their sediments where is partly consumed on a daily basis.

Our data suggest sugars are released from *P. oceanica* roots into the sediments. Previous studies using bulk-carbon isotopes indicate that CO_2 fixed by seagrasses is deposited into the sediments through their roots[22,23]. Our work using porewater profiles expands on this by showing sucrose abundances are highest at the root-sediment interface regardless of the type of meadow investigated (**Figure 4a, b**; **Figure S2b**). Furthermore, mass spectrometry imaging (MSI) allowed us to visualize the molecular distribution of sugars across tissue sections of *P. oceanica* roots. MSI images, at a pixel size of 10 µM, indicate sucrose is highly abundant at the stele of the root and diffuses through the endodermis to the rhizodermis where it can be released into the

environment (**Figure 4d-f**). On land, terrestrial plants transport sugars to their roots where it ends up in root border cells that separate roots as they grow[24]. Our MSI data suggest similar processes are occurring in seagrasses, providing a mechanism to explain the presence of sugars in our porewater samples.

Collectively, our metabolomic data indicate seagrasses are altering the biogeochemistry of their sediments by pumping a fraction of their primary production into the ground as sucrose. While studies indicate seagrasses pump oxygen through their roots[25], we show that the flux of oxygen into meadow sediments is primarily from the upper diffusive boundary layer of the water column (**Figure 4c**). The deficit in dissolved oxygen in the porewater restricts microorganisms from using aerobic pathways for sucrose respiration. Instead, microorganisms living in anoxic layers ferment sugars to organic acids, which can then be respired by a syntrophic community of sulfate reducers. While oxygen and nitrate are limited in seagrass meadows[26], previous reports from the Mediterranean indicate that sulfate concentrations are high (28 mM) and stable across sites and sediment depths[27]. Experiments incubating sediments with ^{13}C-labeled sucrose indicate microbes living underneath meadows respire between 2.083 mmol of sucrose m^{-2} d^{-1} (**Figure S3**). Although these rates are comparatively higher than those we measured for communities living outside of the meadow (0.895 mmol of sucrose m^{-2} d^{-1}), the community would still need 55.64 hours to completely respire the standing stock of sucrose (5 mmol in the upper 30 cm) without additional inputs from the overlying seagrasses. Coupled with previous observations that there is little to no sulfide occurring underneath these meadows[27], we hypothesize that there are other biochemical interactions blocking the respiration of these sugars.

Seagrass meadows act as a metabolic oasis in an organic desert for microorganisms

Sugars are essential energy sources for all Eukaryotes. Normally, land plants store sucrose in their roots as starch and release a small fraction to their soils[24]. However, the ancestors of seagrasses reduced their ability to produce starch in favor of expanding their genes for sucrose transport. These adaptations allow modern seagrasses to quickly mobilize non-structural carbohydrates for growth, reproduction, osmoregulation, and stress response[28]. Our data support the hypothesis that the expansion of genes for sucrose transport also served as an overflow mechanism to export sucrose during high productivity hours. In terrestrial systems, sugar transport to soils is predicted to serve as a chemo-attractant for beneficial microorganisms, including arbuscular mycorrhizas and beneficial Bacteria and Archaea. These microorganisms trade nutrients for carbon derived from plant photosynthesis[24].

To explore the role of the seagrasses in shaping the microbial community of the sediments, we employed metagenomic analyses. We extracted genomic DNA from sediments collected inside, at the edge, and outside a *P. oceanica* meadow from the anoxic depth layer corresponding to average root depth (between 10 and 15 cm; methods)(**Figure 2a**). We obtained shot-gun libraries and sequenced them to a minimum depth of 141 million reads per sample (Illumina HiSeq; for bioinformatic pipeline see **Figure S4**). In addition to our samples, we downloaded a set of 304 metagenomic libraries from public repositories that cover available terrestrial and oceanic habitats (**Table S2**). Using phyloFlash[29], we reconstructed the 16S small subunit (SSU) of the ribosomal RNA gene from all metagenomes. These metagenomic libraries form clusters related to sampling habitat based on order level taxonomy (ADONIS $R^2 = 0.48$; $p < 0.001$). Our analysis shows that marine sediments host a distinct community of

microorganisms dominated by Proteobacteria. These sediments also contain representatives of Acidobacteria, Actinobacteria, Nitrospirae, and Chloroflexi that are found in terrestrial environments (**Figure 5a, Figure S5**)[30].

The community composition of our metagenomes is structured by the presence of seagrasses (**Figure 5b**). We compared the diversity and composition of sediment samples collected inside, at the edge, and outside a *P. ocenicea* meadow. In parallel, our amplicon sequencing approach of the full length 16S rRNA gene (PacBio, 22.5 mB / sample) revealed that microbial communities associated with our sediments are highly diverse, mirroring known complexities in terrestrial habitats that contain thousands of taxa per gram of soil[4]. However, we observed fewer amplicon sequence variants (ASVs) inside the meadow then at the edge or outside (M_{inside} = 1793 ± 79 SEM; M_{edge} = 2224 ± 23 SEM; M_{out} = 1983 ± 51 SEM)(**Figure 5c**). Seagrass sediments, which primarily contain sucrose, are dominated by fewer microorganisms supporting the hypothesis that metabolite diversity drives microbial composition.

Regardless of how we assessed the taxonomic composition of our metagenomes, we show that vegetated sediments host habitat-specific assemblages of Bacteria and Archaea (**Figure S6**). Coupled to previous work from a *Zosteria marina* meadow in the Pacific[31], our data indicate that seagrasses select for specific members of microbial taxa living underneath meadows. Using a random forest classifier, we further show that specific members of the sediment community are predictive of the presence of seagrasses on both local (out of bag error 11.11 %; RF *p-value* < 0.002) and global scales (model accuracy = 0.77; methods). We found that members from Asgardaeota, Crenarcheaota, Euryarchaeota, Bacteriodetes, Chloroflexi, Deltaproteobacteria, Spirochaetes, and Poribacteria were predictive of the presence of seagrasses and more abundant in vegetated versus non-vegetated sediments (**Figure S7**). Considering

members of these taxonomic groups occur in vegetated sediments across in multiple studies[32,33], these data suggest that these taxa contribute to the core microbiome living in marine rhizospheres (**Figure S5**).

To further explore the function of microbial communities living in sediments adjacent to and underneath seagrass meadows, we reconstructed 124 genome bins from a co-assembly of all nine metagenomic libraries (> 70 % complete, < 20 % corrected contamination, **Table S3**; **Figure 4d**) (methods). While these metagenomic assembled bins (MAGs) do not represent the full taxonomic composition of the sediments, they reflect the most prevalent members making up the microbial community (**Figure 5d**). To determine the linkage of each bin to sampling location, we investigated and compared contig coverages across libraries. We found 15, 20 and 21 bins exclusively associate with sediments inside, at the edge, and outside the *P. oceanica* meadow (**Figure S8**).

Our analyses revealed that MAGs associated with seagrass meadows were functionally distinct from those found in non-vegetated sediments (**Figure 6a**). We used eggNOG-mapper (v 2.0.0)[34] to annotate predicted proteins from each site-specific bin with clusters of orthologous groups (COGs). MAGs found underneath meadows, in comparison to those at the edge or outside sampling sites, encoded a higher proportion of proteins involved in energy production and conservation, signal transduction, and translation. In addition, these meadow bins contained fewer genes involved in carbohydrate metabolism (**Figure 6b)**. These results were surprising considering that we observed high concentrations of sugars underneath meadows (**Figure 2c**) and the structural foundation of *P. oceanica* is composed of carbohydrates locked away as plant debris.

Microbial taxa occurring below seagrass meadows lack the required gene set to degrade both simple and complex carbohydrates. By comparing predicted proteins to the carbohydrate activate enzyme (CAZyme) database, we show that MAGs occurring inside the meadow have fewer CAZymes than non-meadow MAGs (**Figure 7**). Specifically, meadow-MAGs reduced the proportion of glycoside hydrolases (GH) in their genomes. GHs break apart glycoside bonds leading to the remineralization of carbohydrates. Looking at substrate use patterns of GH families, our analyses revealed that MAGs occurring at the edge and outside sampling sites, in comparison to those inside the meadow, contained more genes for the degradation of common plant carbohydrates, including sucrose, cellulose, and pectin (**Figure 7**). Unlike reports from terrestrial systems[35], our results indicate that fewer microorganisms living in the marine rhizosphere have genes to degrade organic carbon and the number of genes per organism are low. Alternatively, seagrass meadows support a specialized community of microbial taxa that can only use select substrates in their metabolism (e.g., sucrose). This is supported by our combined observations that taxonomic diversity of microorganisms living in meadow sediments is low (**Figure 5c**) and the associated microbial communities use sucrose in their metabolism (**Figure S3**).

Conclusions

We know very little about how seagrasses interact with below ground microorganisms and how this is linked to long term carbon storage[11,12]. Our sampling effort represents an extensive survey of sediment chemistry underneath seagrass meadows and allowed us to determine seagrasses are the source of sugars in marine rhizospheres. Here, we show that seagrass sediments harbor a large source of sugar in the form of sucrose that is not fully used in microbial metabolism. Given that the microbial communities underneath meadows are limited in

their ability to degrade these sugars, these energy rich compounds are accumulating below seagrasses. Previous studies hypothesized that sugar excretion from seagrasses would feed a beneficial consortia of microbial partners[36], like those occurring in terrestrial rhizospheres. Our analyses did not find evidence to support this hypothesis. Instead, the sediment environment, likely mediated by the presence of the meadows, restricts the microbial metabolism of sugars.

Unfortunately, seagrasses are disappearing at a rate of 110 km^2 yr^{-1}, largely due to anthropogenic activities including land use changes that lead to eutrophication and habitat loss[37]. Given the importance of seagrasses in storing carbon, it is critical to consider how changes in environmental conditions alter associated sediment communities. Future changes in the relationship between marine plants and the microbial assemblages within the rhizosphere, likely driven by shifts in macronutrients, will alter the ability of seagrasses to sequester carbon.

Methods

Porewater sampling. Porewater was collected from vegetated and non-vegetated sediments for metabolomic analyses from seagrass meadows in the Mediterranean (42° 48’29.4588” N; 10° 8’ 34.4436” E), Carrie Bow Cay, Belize (N 16° 04’ 59”, W 88° 04’ 55”), Twin Cayes, Belize (N 16° 50’ 3”, W 88° 6’ 23”), and Kiel, Germany (54° 27’ 26.56256” N; 10° 11’ 33.1908” E). Using a steel lance (1 m long, 2 µM inner diameter) outfitted with a wire mesh (0.0063 mm) to prevent sampling sediment and seagrass biomass, porewater was carefully extracted from sediments into sterile syringes. A porewater profile consisted of top to bottom sampling of the sediments every 5 or 10 cm down to 30 or 40 cm depending on sampling location. Following porewater extraction, samples were frozen at -20 °C until processing for metabolomic analyses.

Porewater profiles were collected at higher spatial and temporal scales from two *Posidonia oceanica* meadows on Elba Island, Italy. Profiles (n=3) from replicate (n=3) transects were collected inside, 1 m at the edge and 20 m outside a meadow in Sant'Andrea Bay (42° 48'29.4588" N; 10° 8' 34.4436" E ; 6-8 m water depth). These collections were repeated in April, July, and October of 2016 to describe metabolite composition across *P. oceanica's* growing season. Porewater samples were collected at a fixed sediment depth (-10 cm) across a 24 hour diurnal cycle in Galanzana Bay (42° 44'9.438" N; 10° 14' 16.3032" E; approximately 2 m water depth). Using replicate fixed lances (n=10) sediment water was extracted (2 mL) at time points centered around high and low solar irradiance periods (10:00, 12:00, 14:00, 20:00, 22:00, 0:00, 2:00 and 5:00) on 23-24 May 2017. Following collection, samples were immersed in liquid nitrogen and subsequently stored at -20 °C until further processing.

Seagrass plant sampling. Individual *P. oceanica* plants from Galanzana Bay were across a diurnal cycle (at 10:00, 12:00, 14:00, 20:00, 0:00, and 5:00) in parallel with sediment porewater samples. Immediately following collection (< 5 min), whole plants were immersed in liquid nitrogen to halt changes in metabolite composition. Plants were stored at -80°C until extraction.

Metabolomics. Metabolomic profiles were obtained from sediment porewaters and prepared for GC-MS using a recently described method[13].

Metabolites were extracted from seagrass tissues using a modified method for plant-based metabolite profiling[38]. Briefly, tissues form frozen plants were separated into leaves, rhizomes, and roots and ground under liquid nitrogen into a find dust using a mortar and pestle. Approximately 400 mg of ground plant tissue was aliquoted into Eppendorf vials. Pre-cooled methanol (1.4 mL) was added to each aliquot and vortexed for 10 s. Tissues were extracted at

70°C for 10 min in a thermomixer at 950 rpm and subsequently centrifuged to pellet the tissue at 11,000 *g* at 4 °C. The supernatant was transfer to 14 mL glass vials. To separate the polar and non-polar compounds, 750 µL of chloroform and 1.5 mL of high grade water was added to each sample, vortexed for 10 s and centrifuged for 15 min at 2,200 *g*. 25 µL of the upper, polar-phase was dried to completeness in a vacuum concentrator without heating (approximately 1 h) after which the aliquots were stored at -80°C until metabolite dativization.

To remove condensation formed during extract storage, directly before preparation for GC-MS analysis we further dried extracts in a vacuum concentrator for 30 min. Metabolite derivatization was performed by adding 80 µL of MeOX dissolved in pyridine (20 mg mL^{-1}) to the dried pellet and incubating for 90 min at 37 °C using a thermal rotating incubator under constant rotation at 1350 rpm. Following recent advancements in signal improvement[39], the pyridine was removed from the sample at room temperature under a gentle flow of N_2 gas (approximately 1 hour). Following the addition of 100 µL of BSTFA, each extract was vortexed, and incubated for another 30 min at 37 °C using a thermal rotating incubator under constant rotation at 1350 rpm. 100 µL was transferred to a GC-MS vial for further GC-MS data acquisition.

GC-MS data acquisition. To obtain metabolite profiles from both porewater and seagrass extracts, all derivatized samples were analyzed on an Agilent 7890B GC coupled to an Agilent 5977A single quadrupole mass selective detector. The injector temperature was set at 290 °C. Using an Agilent 7693 autosampler, 1 µL was injected in splitless mode through a GC inlet liner (ultra inert, splitless, single taper, glass wool, Agilent) onto a DB-5MS column (30 m × 0.25 mm, film thickness 0.25 µm; including 10 m DuraGuard column, Agilent). The inlet liner was

changed after every 50 samples when processing porewater extracts and after 100 samples when processing seagrass tissues to avoid damage to the GC column. Metabolite separation on the column was achieved with an initial oven temperature of 60 °C followed by a ramp of 20 °C min^{-1} until 325 °C was reached which was then held for 2 mins. Helium carrier gas was used at a constant flow rate of 1 mL min^{-1}. Mass spectra were acquired in electron ionization mode at 70 eV across the mass range of 50–600 *m/z* and a scan rate of 2 scans s^{-1}. The retention time for the method locked using standard mixture of fatty acid methyl esters (Sigma Aldrich).

Metabolome data analysis. In order to identify which compounds are driving differences between vegetated and non-vegetated sediments, we used a non-targeted metabolomic approach to assess differences in metabolite composition from porewater samples collected inside and outside a *P. oceanica* seagrass meadow. Raw GC-MS data from samples collected in October 2016 were imported into R (v3.5.2) as converted mzXML files (MsConvert)[40] and processed using XCMS (v2.99.6)[41]. Individual peaks were picked using the matchedFilter algorithm with a full width at half maximum set to 8.4, signal to noise threshold at 1, *m*/*z* width of 0.25 (step parameter), and *m*/*z* difference between overlapping peaks at 1. Resulting peaks were grouped, retention times corrected and regrouped using the density (bandwidth parameter set to 2) and obiwarp methods. Following peak filling, the CAMERA (v1.32.0)[42] package was used to place *m*/*z* peaks into pseudo-spectra by grouping similar peaks with the groupFWHM function. A single *m/z* value was selected to represent each CAMERA group using the following criteria: 1) *m/z* value > 150, 2) occurs across samples with the highest frequency and, if two or more ions are tied in terms of number of samples detected, 3) has the highest mean intensity. Ions with very low mean intensities (< 0.001) were considered noise and removed from the analysis. Ion

abundances were normalized to the ribitol internal standard and compared using a volcano plot to show ions with significant differences (alpha < 0.1) between sampling location and high fold change values (log(FC) > 2). These peaks were subsequently identified using the Mass Hunter Suite and through comparison to the NIST database.

Using our non-targeted analysis as a guide, identified sugars were further quantified in both porewater and seagrass metabolomic profiles using Mass Hunter Quantification Suite (Agilent). Salinity matched calibration curves were used to determine absolute sugar concentrations across porewater samples. For all statistical analyses, calculated sugar concentrations above the instrument detection limits were conservatively assigned the maximum calibration concentration (200 µM). Values below the detection limits were considered noise and given a value of 0 µM. The relative ion abundances of plant sugars were normalized to tissue wet weight and compared using an analysis of variance (ANOVA) across sampling time. Sugar concentrations from sediments were compared across spatial and temporal scales using ANOVA models where sugar concentration was a function of (1) sampling location*sampling depth, (2) sampling month and (3) time of day blocked by sampling spot. Sugar concentrations were transformed to meet the assumptions of the model.

Estimation of global sucrose abundances. To determine the amount of sucrose underneath seagrass meadows, the minimum and maximum concentrations across all sampling depths underneath each of the four types of meadows investigated were used to provide a conservative range. These values were extrapolated to the known area of seagrass meadows worldwide[1]. By calculating the total L of porewater given a porosity of 0.42 within the upper 30 cm underneath seagrass meadows, it was possible to estimate the total g of sucrose occurring below seagrasses on a global scale using the following equation:

$$g\ of\ sucrose\ = [sucrose] \times\ total\ L\ of\ porewater$$

Seagrass root mass spectrometry imaging. Seagrass root pieces were embedded into pre-cooled (4°C) 2 % carboxymethylcellulose block and snap frozen in liquid nitrogen. Roots were cross-sectioned into 12 µM thick slices with a cyrostat (Leica CM3050 S, Leica Biosystems Nussloch GmbH; chamber and object holder temperature at -25 °C). Individual sections were obtained by pressing the sample between two frozen polylysine glass slides to ensure a flat surface for mass spectrometry imaging (MSI) analysis. Before MSI sample preparation, overview images of the root sections were acquired with a stereomicroscope (SMZ25, Nikon, Düssedorf, Germany) and, for the high-resolution figure in the manuscript, with an Olympus BX53 compound microscope (Olympus, Tokyo, Japan) at a resolution of 0.36 µm/ pixel, operated through the software cellSens (Olympus, Tokyo, Japan). For matrix application, super 2,5-Dihydroxybenzoic acid solution (30 mg mL^{-1} in 50 % methanol:water with 0.1 % trifluoroacetic acid) was applied to each section using a robotic sprayer (SunCollect, SunChrome, Wissenschaftliche Geräte GmbH, Germany). Spraying consisted of 10 layers at a flow rate of 10 µL min^{-1} (layer 1) and 15 µL min^{-1} (layers 2-10) resulting in a homogeneous layer of crystalline matrix with grain sizes around 1 µm.

MS-images were obtained using an atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI) ion source ("AP-SMALDI10", TransMIT GmbH, Germany), coupled to a Fourier transform orbital trapping mass spectrometer (Q Exactive™ HF, Thermo Fisher Scientific GmbH, Bremen, Germany). MSI images were collected by scanning the matrix covered tissue sections at a step size of 10 µm without overlapping of the laser spots. Mass spectra were acquired in positive-ion mode with a detection range of *m/z* 50 to 750 and a mass resolving power of 240,000 at *m/z* 200.

Sediment DNA extraction. Metagenomic DNA was extracted from sediment cores (n=3) collected inside, at the edge, and 20 m outside a *P. oceanica* meadow in Sant'Andrea Bay, Italy in October 2016. Directly after collection, cores were sectioned into 5 cm slices and frozen at -20°C. Samples were stored at -80°C until further processing. From the 10 - 15 cm sediment slice, community DNA was extracted from 0.2 g of sorted sandy sediments following a combined approach to remove extracellular DNA prior to extraction[43,44].

Briefly, exDNA was removed by suspending frozen sediment into 200 µL of carbonate dissolution buffer. The solution was incubated at room temperature for 1 h at 600 rpm. After which, 1.6 mL of 10X tris hydrochloride buffer (300 mM; pH 10) was added to each sample and incubated for an additional hour. All samples were centrifuged at 10,000 x g for 20 minutes to separate dissolved exDNA from sediments containing intact microbial cells. Remaining cellular DNA was extracted from pelleted sediments in 500 µL of extraction buffer[44]. Following 3 freeze thaw cycles, 10 µL of proteinase K (20 mg μL^{-1}) was added to each sample and incubated for 30 min at 37 °C. Each sample was then incubated (2 h, 50 °C) with 50 µL of 20 % SDS buffer and then centrifuged at 4000 x *g* for 10 min. After re-extracting the pellet in both buffers (166 µL of extraction buffer and 16 µL of 20 % SDS at 65 °C for 20 min), sample supernatants were combined with an equal volumes of chloroform: isoamylalcohol (24:1 v/v) and centrifuged (10,000 x *g*, 10 min). This step was repeated a second time and the resulting aqueous phase containing DNA was combined with 600 µL of isopropanol and stored overnight at 4 °C. To pellet DNA, samples were centrifuged (14,000 x *g*, 30 min) and supernatant was discarded. The DNA pellet was washed in pre-cooled 80 % ethanol and centrifuged (14,000 x *g*, 10 min). The resulting supernatant was removed and DNA pellet was dried in a vacuum concentrator

(Eppendorf concentrator plus, 10 min, V-AL). DNA was eluted in DEPC water and stored at -20 °C until metagenomic library preparation.

DNA sequencing. Metagenomic library preparation and sequencing was performed by the Max Planck Genome Centre. Prior to library generation, genomic DNA quality was assessed using an Arlene 2100 Bioanalyzer (Agilent) and subsequently purified with DNeasy PowerClean Pro Cleanup Kit (Qiagen). The cleaned genomic DNA was fragmented, on average, into 300 base pair (bp) fragments. An Illumina compatible library was generated for each sample following manufacturer's instructions. For each library, 100 million x 150 bp paired end reads were sequenced on an HiSeq 3000.

Full length 16S rRNA. The sediment DNA was purified with DNeasy PowerClean Pro Cleanup Kit, Qiagen to remove components which were inhibiting amplification without a cleanup step. Then 16S amplicons were amplified with universal primer pair GM3F/GM4R and Phusion DNA polymerase, NEB and the chemical regime of the NEB manual includes DMSO with annealing temperature for the first two cycles at 42°C, 20 s followed by 45°C, 20 s for the remainder 34 cycles. The number of PCR cycles suited to get sufficient DNA for downstream applications was empirically determined by quantitative PCR (ViiA 7 Real-Time PCR System, ThermoFisher) in the presence of 1x Evagreen (20x Evagreen, Biotium, Fremont, U.S.A). Amplicons were purified with DNA Clean & Concentrator Kits, Zymo Research or AMpure Beads (Beckman Coulter) and quantified by Qubit HS kit (ThermoFisher). Next, PacBio libraries were prepared as recommended in protocol "2 kb Template Preparation and Sequencing" of Pacific Biosciences and using adaptors with 16mer barcode sequence to demultiplex sequencing data. Libraries were pooled equimolar and sequenced on Sequel I of Pacific Biosciences on a single cell with 20

hours run time, polymerase binding kit 3.0, sequencing chemistry version 3.0 and SMRT cell 1Mv3. Resulting reads were error corrected using circular consensus (CCS) reads (10 passes) using the SMRTlink (v 6.0.0) software.

Resulting 16S CCS reads were processed using Dada2[45] in R to obtain amplicon sequence variants (ASVs). Briefly, amplicon reads were imported into R and filtered to control for expected sequence length (between 1000 and 1600 bp) and high quality reads (minQ=3). Following error estimation, taxonomy was assigned to each unique ASV by comparison against the GTDB database. The resulting count table was imported into phyloSeq[46] for subsequent analyses including estimate of sequence richness and comparison of taxonomic profiles across samples.

Metagenomic analysis. Sediment metagenomic libraries were assembled both individually and as a co-assembly to facilitate down-stream analyses. Briefly, both individual libraries and concatenated read datasets were cleaned of Illumina adaptors and low quality reads using bbduk (v38.34, http://jgi.doe.gov/data-and-tools/bb-tools/) and normalized to an average depth of 100X per *k-mer* with bbnorm (v38.34; http://jgi.doe.gov/data-and-tools/bb-tools/). Reads with *k*-mers less then 2 were considered sequencing errors and were discarded. SPAdes (v3.12.0)[47] was used to error correct all reads and assemble individual libraries, while the co-assembly was obtained using MEGAHIT (v1.1.3)[48] with *k*-mers from 21 to 51 in steps of 10. Sequencing coverage of each contigs were calculated by mapping the error corrected reads back to the co-assembly using bbmap (v38.34, http://jgi.doe.gov/data-and-tools/bb-tools/).

The metagenomic co-assembly was binned using three automated binning programs (MetaBAT v0.32.5; concoct v1.0.0 and MaxBin v2.2.6)[49-51] and subsequently dereplicated and

aggregated with DAS Tool (v1.1.1)[52]. Bin quality was assessed using CheckM qa method (v1.0.7)(**TableS3**)[53]. Bins with completeness > 70 % and corrected contamination for strain heterogeneity < 20 % were considered to be high quality and used in subsequent analyses. High quality bins were given a taxonomic classification using the GTDBTk classify work flow (v0.2.1)[54]. Open reading frames (ORFs) from each bin were predicted with Prodigal (v2.6.4)[55] and annotated by both comparison to the CAZymes database using dbCAN (v2.0)[56] and assigned COG functional categories with eggNOG (v1.0)[34].

Metagenomically assembled bins (MAGs) were assigned to specific habitats by comparing fold coverage statistics for each bin across libraries. Contig coverage values for bin were imported into R. Large contigs (length > 5000 bp) were compared using a one-way ANOVA across sampling libraries. Models separated by sampling site (adjusted *p-value* < 0.1) were compared using a Tukey's post-hoc test to determine sampling origin. MAGs occurring either in multiple habitats or in single libraries were discarded from the analysis.

Taxonomic composition. To determine the taxonomic composition of sediment metagenomic libraries, both phyloFlash (v2.0, ref) and Kaiju (v1.4.5, ref) were used to classify the cleaned reads dataset. phyloFlash assembles the SSU rRNAs to explore the taxonomic composition, while Kaiju compares predicted protein sequences to the NCBI non-redundant protein database. Phyloseq (1.26.1, ref) was used to further process all three taxonomic composition datasets, including the phyloFlash, kaiju and full length 16S results. Data processing included removal of all non-bacteria or archaea taxa and normalization of count data to relative abundances. For all three datasets, principle coordinates analysis (PCoA) was used to assess differences in taxonomic compositions among sampling sites.

In order to compare the taxonomic composition of the sediment metagenomic datasets to publicly available shotgun libraries from a diverse range of habitats (**TableS2**), the ena_phyloFlash.pl script was used to download and process each sampling dataset. Additional read datasets, specifically sampled in seagrass habitats and other complex soil ecosystems, were downloaded separately and processed with phyloFlash from MG-RAST. All processed datasets were compared using the phyloFlash_complare.pl script to obtain a sample by taxonomic composition matrix on the order level. Samples containing less then 500 and more then 1M reads per library were discarded from the analysis. Non-bacteria or archaea reads were removed from the resulting sample matrix and sample count data were converted to relative abundances. A PCoA was used to compare the composition between samples using order level taxonomic assignments. A permutational multivariate analysis of variance test (ADONIS) was used to determine if the sampled habitat drove community assemblage patterns. Communities for both analyses were compared at the order taxonomic level.

To determine which taxa are predictive of the presence of seagrasses, a random forest classifier was built using sediment samples collected inside, at the edge, and outside a *P. oceanica* meadow (out of bag error 11.11 %). The classifier was used to predict if sediment metagenomes from public repositories (as processed above with phyloFlash) were associated with the presence of seagrass meadows. The model was tested for significance using a permutation test of 1005 permutations and 501 tree splits, and cross validated using the "leave-one-out" method. Important features were identified at a mean decrease in Gini level of 0.02.

Microbial activity. Sediments were collected using push-cores inside (n=3) and at the edge (n=3) of a *P. oceanica* meadow in Sant'Andrea Bay, Italy. 50 mL of sediment from the core's oxic (upper 5 cm) and anoxic layers (bottom 25 cm) was added to replicate 500 mL Schott

bottles containing either double filtered (0.2 µM) anoxic (bubbled with N2 gas) or oxic seawater. After an overnight acclimation step to ensure oxygen is drawn down in the anoxic treatments, fully labeled $^{13}C^{12}$-sucrose was added to each bottle at a final concentration of 50 µM. In parallel, a dead controls were prepared by combining sediments from all cores with 4 % paraformaldehyde. All experimental bottles and dead controls were sampled by collecting 6 mL of seawater into 6 mL exetainers containing 50 µL of mercury chloride at each time point (0, 1, 3, 6, and 12 h post sucrose inoculation). Sampling seawater was replaced by freshly filtered anoxic or oxic seawater. Following the conclusion of the experiment, total sediment wet weights were recorded.

To determine the rate of $^{13}CO_2$ production, 2 mL of each sample was acidified with 20 % phosorphic acid[57] in preparing for cavity ring-down spectroscopy (G2201-i coupled to a Liaison A0301, Picarro Inc., connected to an AutoMate Prep Device, Bushnell). Sucrose uptake rate by each sediment bottle was determined by measuring the ratio of $^{13}C\text{-}CO_2$ to $^{12}C\text{-}CO_2$ and calculating changes in the isotopic ratio over time as normalized to g of sediment.

DOC sampling & analysis. Dissolved organic carbon (DOC) samples were collected in parallel with porewater metabolomic samples from inside, at the edge and 20 m outside a *P. oceanica* seagrass meadow in October of 2016. Taking care to avoid oversampling porewater space, approximately 20 mL of each porewater sample was collected into polypropylene syringes. Samples were filtered through pre-combusted (500 °C, 4 h) Whatman GF/F filters (0.7 µM) into 20 mL acid-washed and pre-combusted scintillation vials. Samples were acidified to pH 2 using 25 % hydrochloric acid and stored at 4 °C until analysis. DOC concentrations were measured by high temperature catalytic oxidation on a Shimadzu TOC-VCPH analyzer. Instrument trueness

and precision was tested against Deep Atlantic seawater reference material (42 µM DOC; acquired from the Hansell lab at University of Miami, FL, USA) and low carbon water (1 µM DOC) and was better than 5 %.

Code availability

All code used for data processing and analysis is available at https://github.com/esogin/sweet_spots_in_the_sea

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Figures

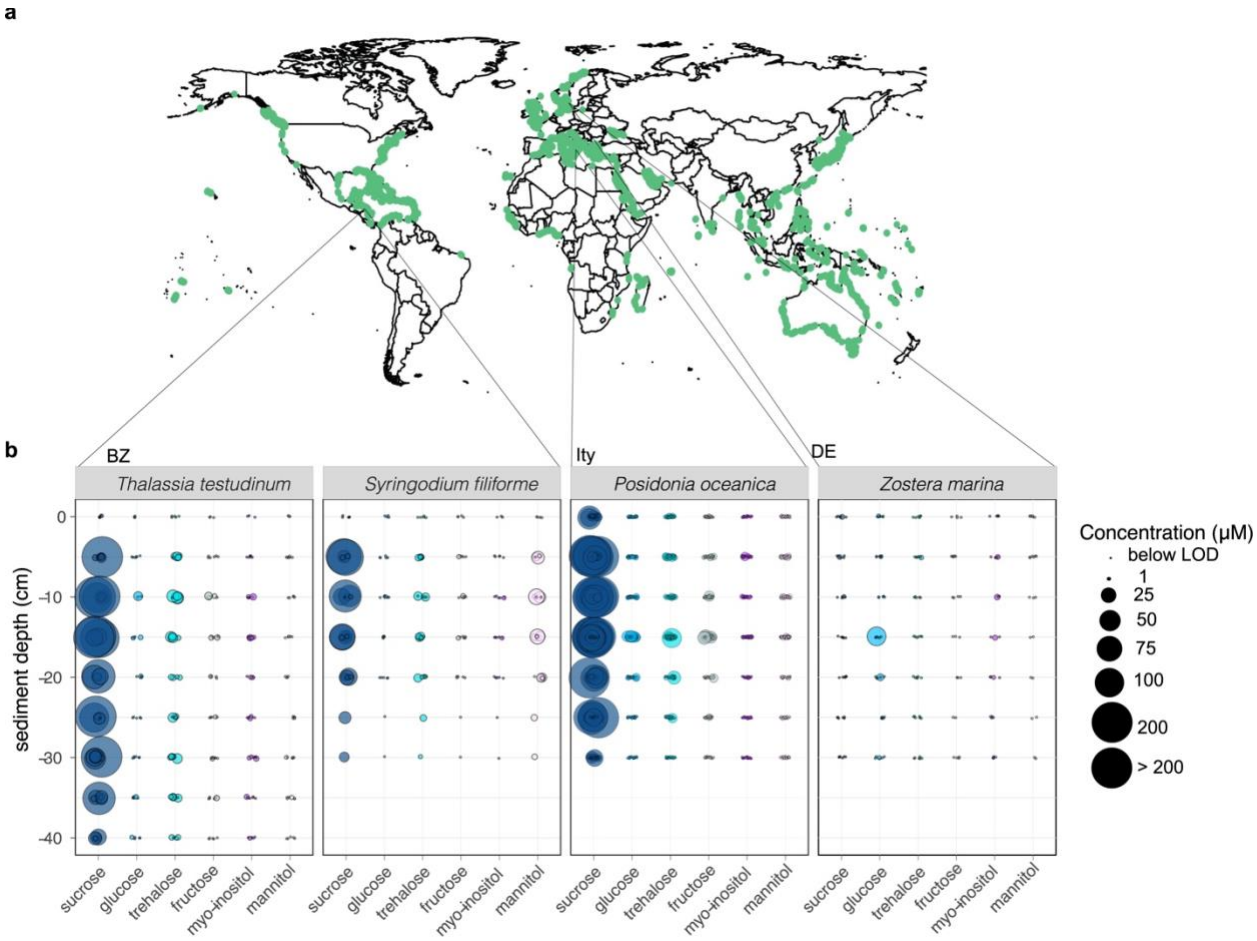


Figure 1| Metabolomic analysis of sediment porewaters underneath seagrasses reveal high concentrations of sugars worldwide. **a,** Seagrasses occur across coastal regions in both oligotrophic (e.g., Caribbean and Mediterranean) and eutrophic (e.g., Baltic Sea) waters. **b,** A bubble plot showing pore-water measurements (n=244) underneath meadows in both Belize (BZ) and Italy (Ity) are rich in sugars, while sugars are less abundant underneath meadows in the more eutrophic Baltic Sea, Germany (DE). The size of each point represents the concentration of each sugar across the depth gradient. For each seagrass meadow, between 6 (*S. filiforme*, *T. testudinum*, and *Z. marina*) and 14 (*P. oceanica*) individual profiles were sampled. To facilitate understanding, samples where sugars were not detected are not plotted, values below our quantification limits are sized to the smallest point while values above 200 µM are sized to the largest point.

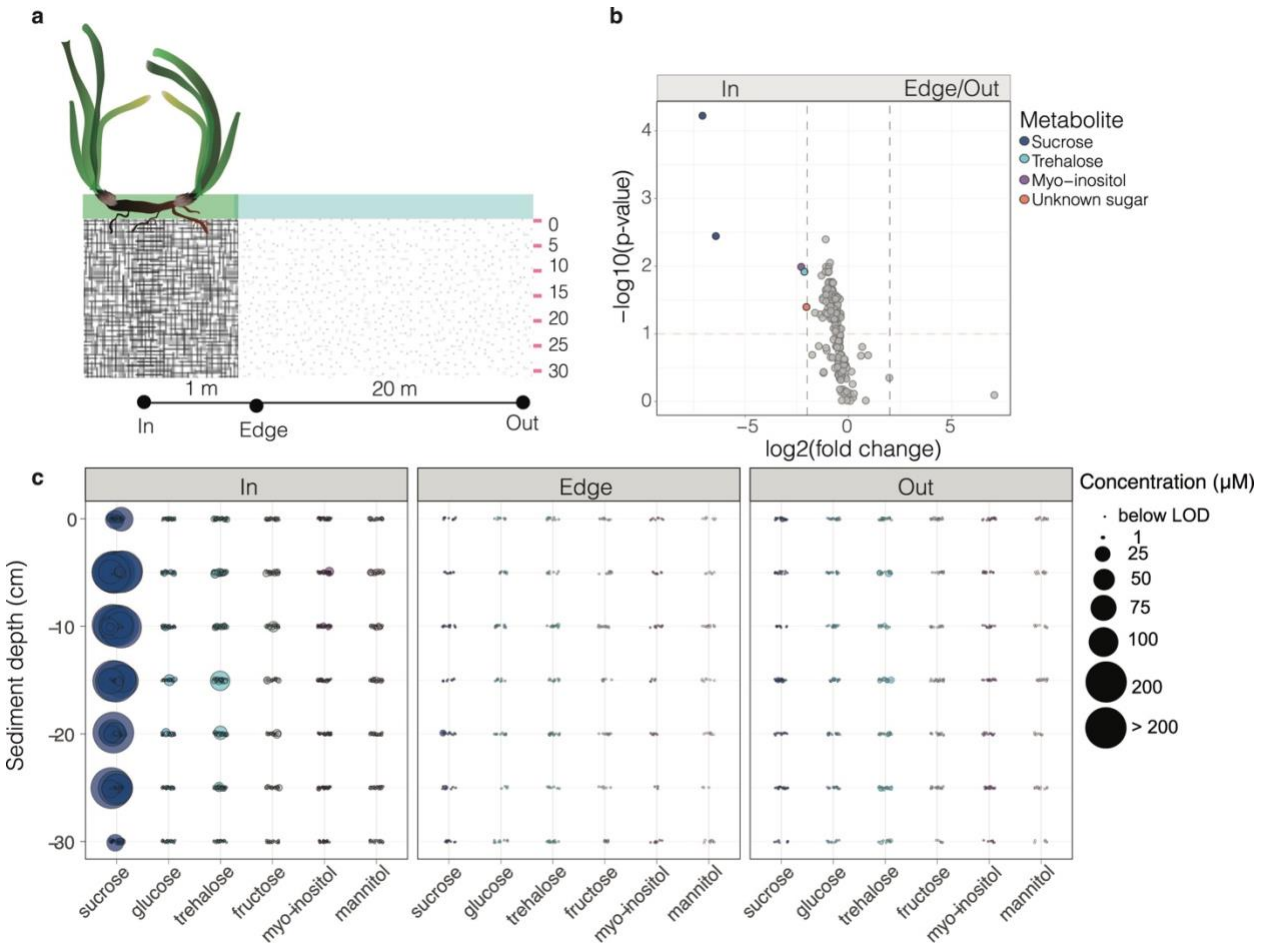


Figure 2| Sugars are more abundant underneath seagrass meadows than in non-vegetated sediments. a, Sediment porewater profiles were collected underneath, 1 m and 20 m outside of a *P. oceanica* meadow in Sant'Andrea Bay, Elba, Italy. Individual profiles consisted of samples collected every 5 cm from 0 to -30 cm underneath the meadow. **b,** A volcano plot of log2(fold changes) and log10(p-values) for each metabolite detected using a non-targeted approach. These data show sugars are more abundant in the porewater metabolome inside than at the edge or outside the seagrass meadow. **c,** A bubble plot shows sugar concentrations across sediment depth gradients. For each sugar, the concentration was significantly higher inside the meadow than outside or at the edge (two-way ANOVA comparing sugar concentrations as a function of sediment depth and location). To facilitate understanding, samples where sugars were not detected are not plotted, values below our quantification limits are sized to the smallest point while values above 200 μM are sized to the largest point.

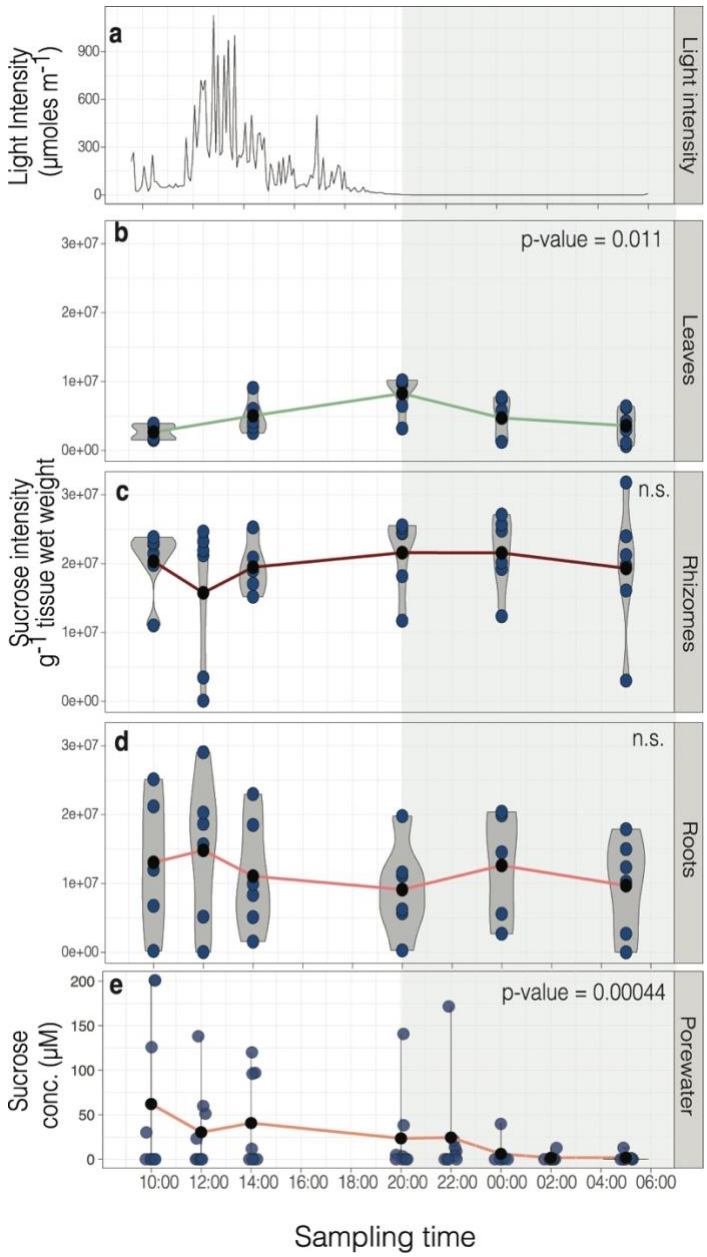


Figure 3| Sucrose abundances vary across a day/night cycle in plant leaves and sediment porewater. a, *In-situ* light levels across a 24 hour period in Galenzana Bay, Italy (> 2 m water depth). (**a-d**) Violin plots show the relative abundance of sucrose g_{-1} tissue wet weight from plant **b,** leaves**, c**, rhizomes, and **d,** roots at each sampling time point**.** Linear models show that sucrose abundances are significantly more abundant in plant leaves at dusk (t= 20:00) than at other times of day. **e,** Violin plots showing sucrose concentrations as measured in sediment porewaters at each sampling time point. A linear mixed effects model with a random effect of sampling spot show sucrose concentrations are significantly higher during the daylight hours then after midnight (t = 0:00) and before dawn (t = 5:00). The means of each sampling time point (black points) are connected by solid lines. For **e,** sucrose concentrations greater than the limits of quantification are represented as 200 µM.

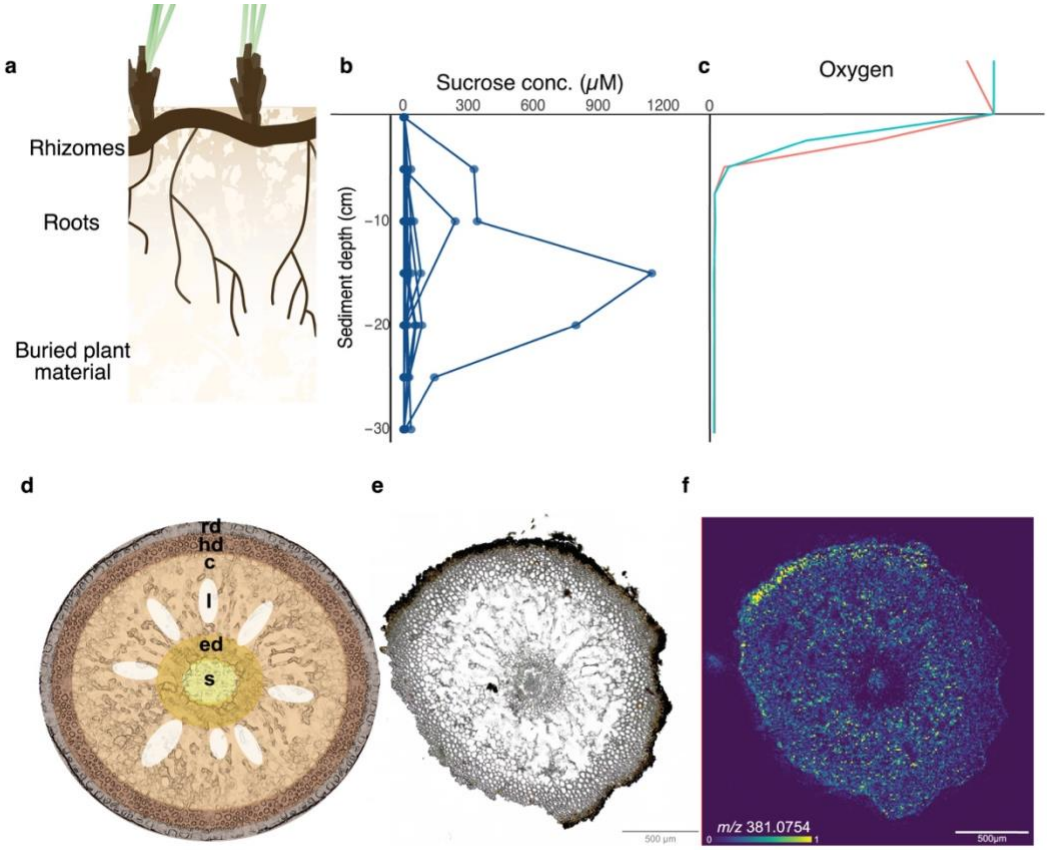
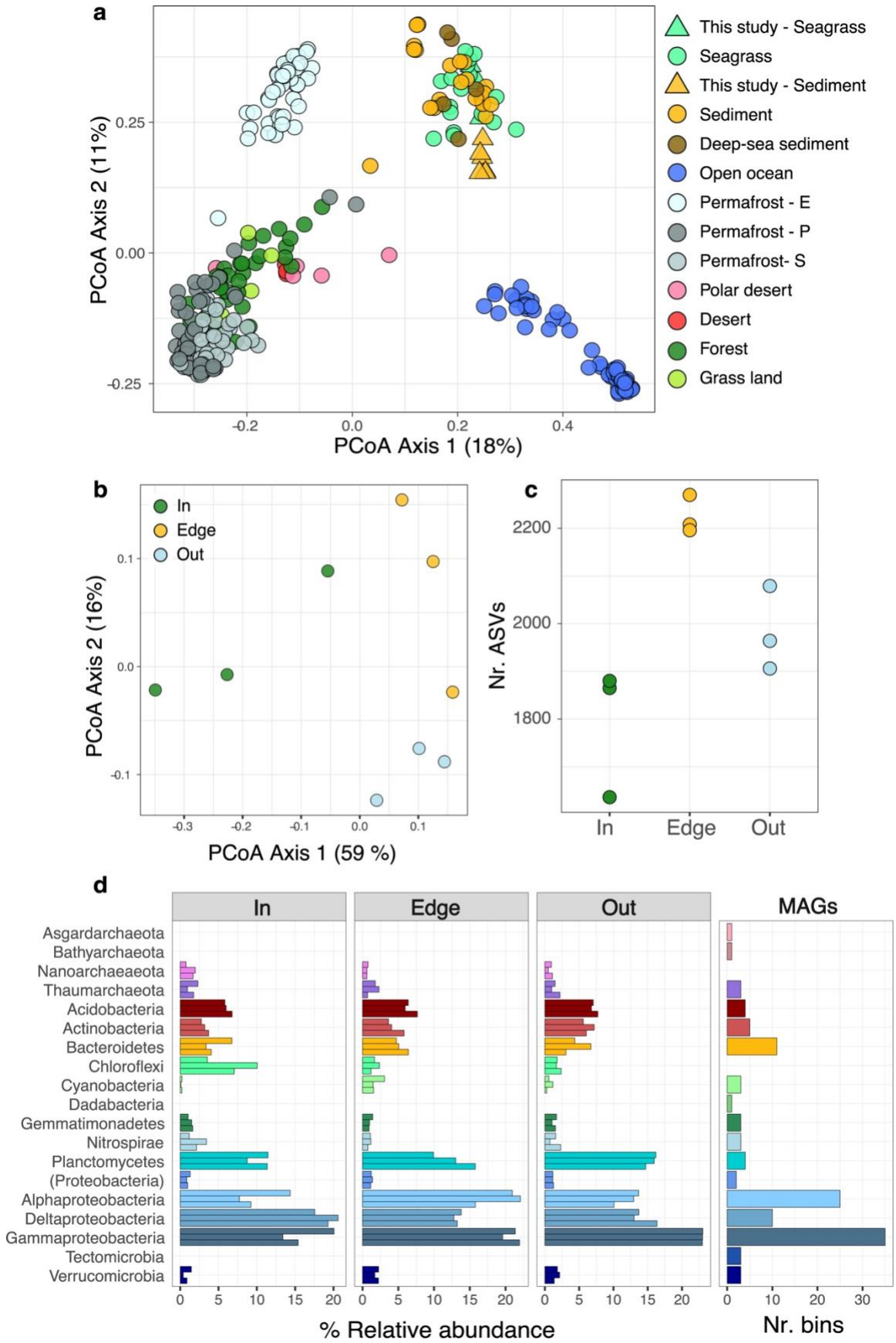


Figure 4| Seagrasses deposit sucrose into their sediments through their roots. a, A schematic showing that *P. oceanica* roots extend between 5 and 20 cm into the mat. **b,** Root depth is correlated to peaks in sucrose concentrations. **c,** Oxygen profiles do not reveal peaks associated with presence of roots. **d**,**e,** The structure of *P. oceanica* roots reflect the **f,** molecular distribution of sucrose (*m/z* 381.0754). **f,** Sucrose ion abundances from low (blue) to high (yellow) are relative to total signal intensity of each 10 µm pixel. rd = rhizodermis, hd = hypodermis, c = cortex, l = lacunae, ed = endodermis, s = stele.


a
This study - Seagrass
Seagrass
This study - Sediment
Sediment
Deep-sea sediment
Open ocean
Permafrost - E
Permafrost - P
Permafrost- S
Polar desert
Desert
Forest
Grass land
PCoA Axis 2 (11%)
PCoA Axis 1 (18%)
b
In
Edge
Out
PCoA Axis 2 (16%)
PCoA Axis 1 (59 %)
c
Nr. ASVs
In
Edge
Out
d
In
Edge
Out
MAGs
Asgardarchaeota
Bathyarchaeota
Nanoarchaeaeota
Thaumarchaeota
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Dadabacteria
Gemmatimonadetes
Nitrospirae
Planctomycetes
(Proteobacteria)
Alphaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Tectomicrobia
Verrucomicrobia
% Relative abundance
Nr. bins

743

Figure 5 | Seagrass meadows shape microbial community composition. a, A principle coordinate analysis (PCoA) comparing the community composition on the order level of environmental metagenomes across a broad range of habitats shows marine sediments harbor a distinct microbial community. **b,** Similarly, a PCoA analysis comparing the taxonomic composition of sediment metagenomes collected inside (green), at the edge (yellow), and outside (blue) a meadow reveal that sediments underneath seagrasses have a unique assemblage of microbes. These communities are less diverse as show in **c,** where the number of amplicon sequence variants (ASVs) from the full length 16S approach is lower inside the meadow then at the edge or outside. **d,** Sediment communities are dominated by delta-, alpha- and gammaproteobacteria, the dominant community members are reflected in the taxonomic identity of the 124 metagenomic assembled bins (MAGs). In **d,** only the 15 most abundant bacterial phyla are shown in plots comparing in, edge, and out communities.

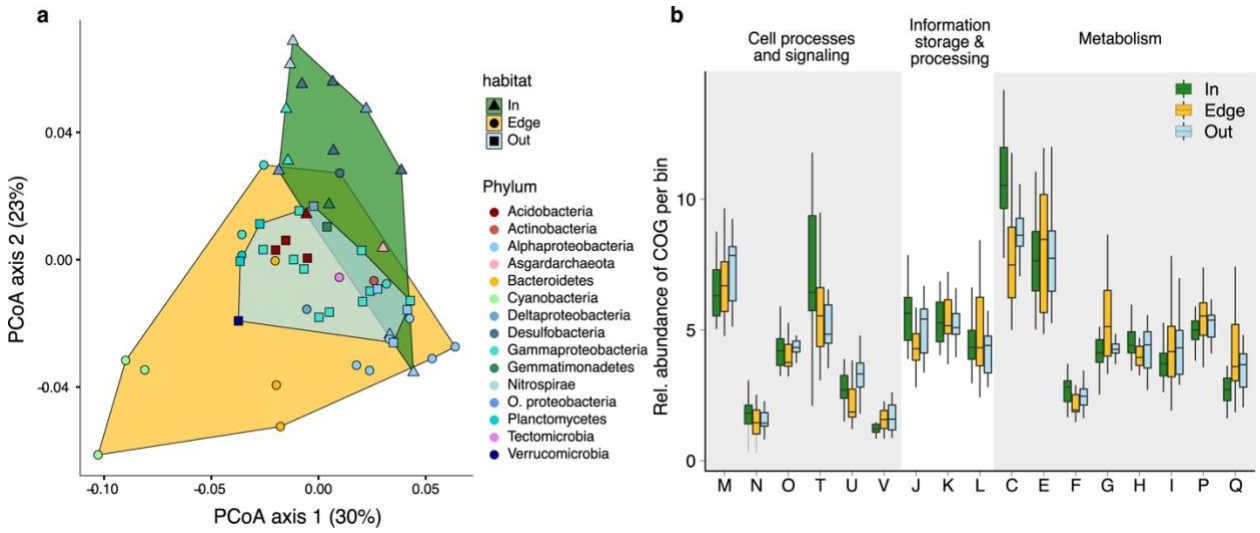


Figure 6 | Seagrass meadows select for the function of MAGs. **a,** a PCoA analysis comparing the relative abundance of cluster of orthologous genes (COG) shows that bins inside the meadow contain a different composition of COGs then bins at the edge or outside the meadow. **b,** Boxplots showing the relative abundance of COG categories per bin in (**a**) indicate the microbes occurring underneath meadows have higher relative abundances of genes involved in nucleotide metabolism and transport (F), signal transduction (T), translation (J), and energy production and conservation (C) but have fewer genes involved in carbohydrate transport and metabolism (G).

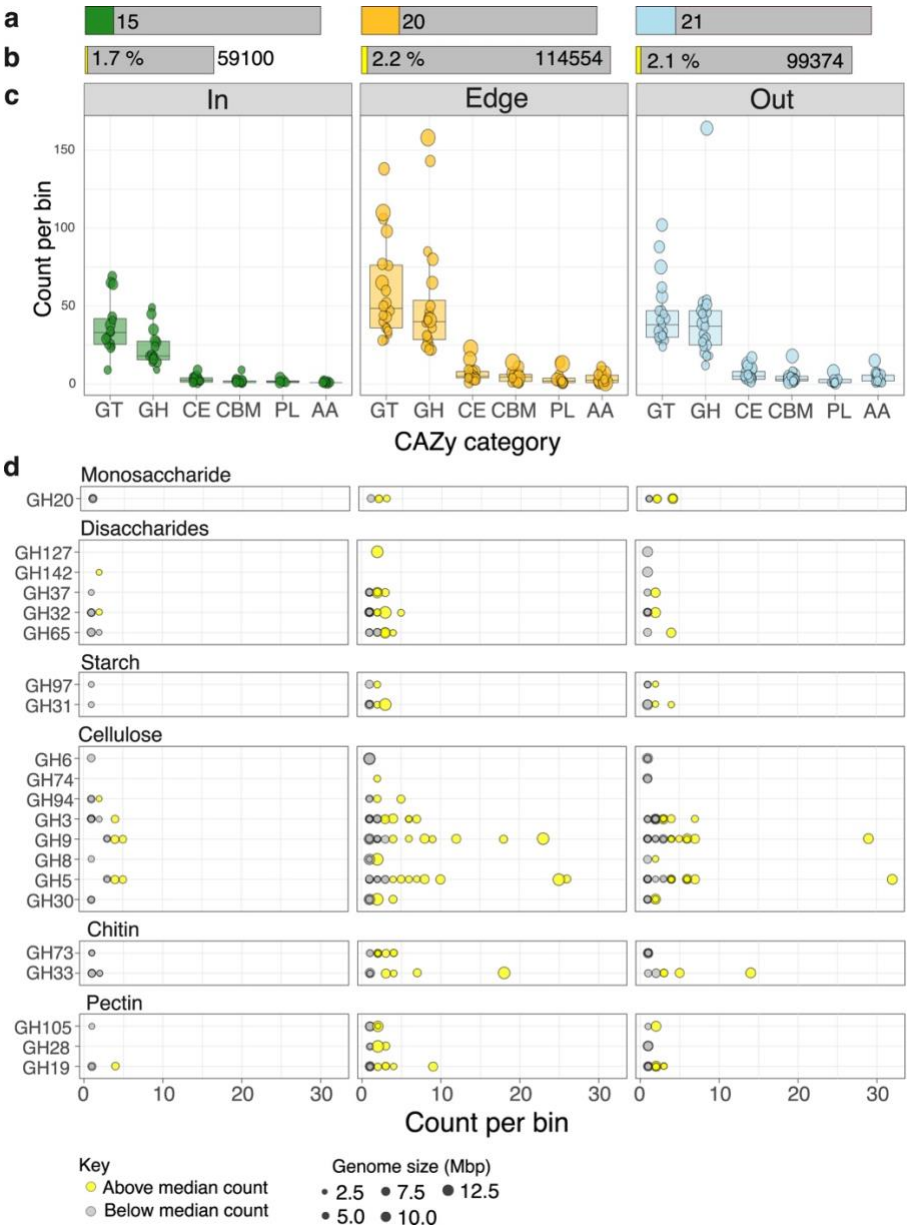


Figure 7| Bins occurring inside the meadow contain fewer carbohydrate activated enzymes (CAZys). The number of MAGs specific to each environment (**a**) and percent of their protein coding sequences (**b**) compared in **c** and **d** is proportional across habitats. **c,** Boxplots show that bins occurring inside the meadow have fewer CAZy genes, and in particular GHs, than those occurring at edge or outside the meadow. **d,** Gene counts per bin (points) of each GH family sorted by their substrates show that bins occurring inside the meadow contain fewer GH-genes than those at edge or outside. Individual points are colored in yellow if they contain gene counts above the family median. For both **c, d**, points representing bins are sized in scale with genome size. GT= glycosylTransferases, GH= glycoside hydrolases, CE= carbohydrate esterases, CBM= carbohydrate-binding molecules, PL=polysaccharide lyases, AA= auxiliar activities.