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Marine heatwaves and disease alter community metabolism and DOC fluxes on a widespread habitat-forming seagrass species (*Zostera marina*)

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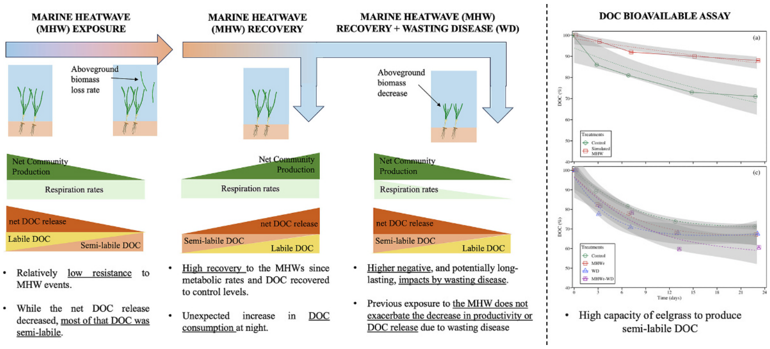
HIGHLIGHTS

- Eelgrass was exposed to a marine heatwave (MHW) followed by wasting disease (WD).
- Simulated MHW reduced DOC fluxes and net production while not changing respiration.
- WD after MHW reduced aboveground biomass, DOC fluxes and net production rate.
- Higher negative impacts of WD than MHW and quick recovery capacity to thermal stress
- Mostly semi-labile DOC released by eelgrass with MHW reducing bioavailability

GRAPHICAL ABSTRACT



- Relatively low resistance to MHW events.
- While the net DOC release decreased, most of that DOC was semi-labile.

- High recovery to the MHWs since metabolic rates and DOC recovered to control levels.
- Unexpected increase in DOC consumption at night.

- Higher negative, and potentially long-lasting, impacts by wasting disease.
- Previous exposure to the MHW does not exacerbate the decrease in productivity or DOC release due to wasting disease

- High capacity of eelgrass to produce semi-labile DOC

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ABSTRACT

Climate change and disease are two major threats to maintaining healthy seagrass habitats. Seagrasses, and the ecosystems they support, play a critical ecological role in global carbon (C) cycles, providing key ecosystem services, such as blue carbon storage. *Zostera marina* (eelgrass), the most widespread seagrass species globally, is increasingly affected by warming and is also regularly infected by the endophytic pathogen *Labyrinthula zosterae*. Both stressors negatively impact plant physiology and population distributions, yet the effects of these stressors on C cycling, and particularly on C metabolism and dissolved organic carbon (DOC) fluxes in eelgrass, remain largely unexplored. Through a mesocosm experiment simulating a marine heatwave (MHW) followed by pathogen challenge with *L. zosterae*, it was observed that the simulated MHW initially decreased daily community DOC fluxes and Net Production Rates (NPR), while not changing Respiration Rates. DOC released into the water column at the end of the MHW also was less bioavailable than DOC from the control treatment. Importantly, community NPR recovered to control levels after the simulated MHW was over, demonstrating the community's resilience to warming. On the other hand, plants challenged with *L. zosterae*, which caused a significant decrease in aboveground biomass, exhibited significant decreases in DOC and NPR up to 20 days after the infection. These results have important implications in blue carbon processes, given that both stressors significantly impact the

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quantity and quality of DOC produced by *Z. marina* communities. These findings also highlight the differing levels of resilience of C cycling in this system by showing that the impacts of the simulated heat wave may be more transient when compared to the effects of disease.

1. Introduction

Seagrasses are marine foundation species that form some of the most ecologically and socio-economically important ecosystems in coastal areas (Short et al., 2011). They are globally distributed and recognized for the critical ecosystem services they provide, including shoreline protection, breeding and nursery habitats for fish and shellfish, biodiversity hotspots, and organic carbon (OC) sequestration (Fourqurean et al., 2012; Campagne et al., 2015; Jiménez-Ramos et al., 2021). Their high OC sequestration ability stems from the high productivity of these plants and from their effectiveness at enhancing the settlement and trapping of suspended allochthonous OC from the water column (Kennedy et al., 2010; de los Santos et al., 2023). The ability of natural ecosystems to act as OC sinks has usually been related to the large deposits of particulate OC (POC) buried in sediments, which are thought to be primarily composed of recalcitrant organic matter (Kennedy et al., 2010). However, recent studies have also highlighted the importance of the recalcitrant fraction of dissolved organic carbon (DOC) as an additional important carbon sink with potential climate regulating capacity (Duarte and Krause-Jensen, 2017; Egea et al., 2023a; Kubo and Tanaka, 2023). DOC is one of the largest exchangeable organic carbon pools in the marine environment, making it a principal part of the global carbon cycle (Hansell, 2013). There are multiple sources of DOC to the ocean, including phytoplankton exudates, leached of organic matter, river inputs (Lønborg et al., 2020) and DOC released by plastic pollution (Egea et al., 2024). Recently, the relevance of seagrass-derived DOC has been highlighted as it represents a significant fraction of their Net Community Production (NCP) (Barrón et al., 2014) and can contribute to carbon sequestration processes in two ways. First, DOC that enters the global ocean conveyor belt can be kept 'trapped' from the atmosphere for hundreds of years (Duarte and Krause-Jensen, 2017). Second, the direct release of recalcitrant DOC (i.e., formed by organic carbon less bioavailable), which can range between 42 and 56 % of the total DOC released from seagrass systems, effectively limits its remineralization to CO_2 by the microbial food web and subsequent return to the atmosphere (Jiménez-Ramos et al., 2022; Egea et al., 2023a).

Seagrasses are under threat worldwide from a growing number of anthropogenic stressors (Orth et al., 2006). While seagrass-dominated communities are usually highly autotrophic (i.e., fix more carbon than they release) and produce large amounts of DOC (up to 33 Tg C yr^{-1}; Barrón et al., 2014), environmental stressors, such as nutrient enrichment (Gypens et al., 2009; Egea et al., 2020), temperature increase (Egea et al., 2023a), or change in the community structure by invasive species (Jiménez-Ramos et al., 2022) and increased herbivory pressure (Jiang et al., 2023; Jiménez-Ramos et al., 2024), can alter these carbon cycling properties. The direction of these balances (i.e., from negative, neutral or positive) depends on the species of seagrass, the environmental context, and the co-occurrence of stressors. For example, in the sub-tropical seagrass *Cymodocea nodosa*, an acute short-term high temperature stress caused increased productivity and a trend to produce more humic-like and less bioavailable DOC (Egea et al., 2023a), whereas the spread of the invasive algae *Halimeda incrassata* resulted in a neutral effect on productivity, but shifted the system from being a net DOC-producer to a net DOC-consumer (Jiménez-Ramos et al., 2022). Such changes in C metabolism and DOC production and its bioavailability can have important ecological implications on the trophic state of these systems and undermine their role as blue carbon sinks (Macreadie et al., 2019). Thus, manipulative experiments considering multiple stressors are increasingly necessary to unravel the outcome responses of seagrasses to drivers of global change.

Among global change stressors, temperature increase is one of the greatest threats to temperate seagrass meadows (Marbà and Duarte, 2010; Magel et al., 2022). The increase in temperature could benefit seagrasses in certain places and conditions (e.g., species that are not close to their limit of thermal tolerance) by increasing their productivity, growth and biomass (Ontoria et al., 2019; Egea et al., 2023a). In contrast, warming can also lead to a reduction in abundance of individuals or general mortality (Marbà and Duarte, 2010; Strydom et al., 2020). For instance, the dominant seagrass species in the northern hemisphere, *Zostera marina* L. (eelgrass), has experienced die-offs after the occurrence of marine heatwaves (MHWs) (e.g., Moore and Jarvis, 2008; Magel et al., 2022). In addition to stress from high temperatures, disease increases have often been associated with warming in marine and terrestrial species (Harvell et al., 2002). Previous studies linked the exposure to warming in eelgrass to higher prevalence and severity of Wasting Disease (WD) (Sullivan et al., 2018; Aoki et al., 2022). The causative agent of eelgrass WD, *Labyrinthula zosterae* (Muehlstein et al., 1991), acts as a seagrass pathogen when it enters plant cells and consumes the chloroplasts, which can lead to shoot mortality (Short et al., 1987). While *L. zosterae* and other pathogenic *Labyrinthula* spp. are commonly found to co-occur with seagrass populations around the world, their presence does not always coincide with high mortality events, suggesting environmental triggers of virulence (e.g., Brakel et al., 2014; Trevathan-Tackett et al., 2018). Much speculation has been given to the possible environmental drivers of such disease, but conflicting empirical results continue to obscure its mechanisms. For instance, although warming seem to favor WD in some cases (e.g., Sullivan et al., 2018; Aoki et al., 2022), negative or no effect on disease was observed in other studies (Brakel et al., 2019; Jakobsson-Thor et al., 2020). Such changes may be directly related to temperature tolerances of the host (e.g., Schenck et al., 2023) and/or the pathogen (e.g., Olsen and Duarte, 2015), with the former suggesting an interactive effect of stressors, whereby the capacity of seagrass to withstand infection may be reduced after undergoing thermal stress.

Based on the current knowledge, we hypothesize that MHWs will enhance eelgrass productivity and DOC release; additionally, we expect that simulated MHW will increase infection by *L. zosterae* and reduce productivity and DOC release in plants compared to those exposed to ambient temperatures. To test these hypotheses, we used outdoor mesocosms containing transplanted eelgrass shoots to quantify (1) how incidences of a MHW, WD infection, or both stressors in succession alter the system's carbon metabolism and DOC export, and (2) how a MHW impacts subsequent infection and severity of wasting disease in plants.

2. Material and methods

2.1. Plant collection and experimental units assembly

The experiment was conducted in an outdoor mesocosm system at the Experimental Seawater Facility at Hatfield Marine Science Center (HMSC, Newport, Oregon) belonging to Oregon State University (OSU) during summer 2022. Experimental units consisted of twenty-four buckets (30 cm diameter x 60 cm height, 42 L) filled with sediment and *Zostera marina* (eelgrass) plants from Yaquina Bay, OR, U.S.A (lat. 44.624518, long. −124.044372). Water annual temperatures in this small (19 km^2) mesotidal estuary range between 8 and 16 °C and are strongly influenced by coastal currents (Nelson, 2018). Sediment (top ~15 cm) was collected from an established intertidal eelgrass bed, and subsequently sieved through wire mesh with 0.25 cm^2 openings and held in buckets filled with seawater for two weeks in a flow-through system. After two weeks, individual shoots of *Z. marina* were collected during low tide from random locations within the same intertidal eelgrass bed. Healthy-looking shoots without visible signals of *L. zosterae* infection (i.e., brown or necrotic black lesion area in leaves) and intact rhizome-root systems were carefully extracted and transported to the laboratory within 60 min of collection. Following harvesting, plants were processed by trimming leaves to 50 cm in length and the rhizome to a uniform length of five internodes with attached roots back from the apical shoot, which has been demonstrated to allow success transplants of eelgrass for experimentation (e.g., Kaldy et al., 2022). Experimental units were filled with homogenized, the above-mentioned sieved sediment (~10 cm depth), natural seawater, and eight individual processed *Z. marina* shoots (Fig. 1). After the transplant and before the start of the experiment, shoots were acclimated for 10 days in the mesocosms under flow-through conditions with ambient temperature seawater. Three autochthonous adult *Phyllaplysia taylori* individuals were added in each bucket to control for epiphyte growth, and no changes in their abundance were observed between treatments across the duration of the experiment.

2.2. Experimental design

The experimental approach consisted of two consecutive experimental phases (see Fig. 1). In the first experimental phase half of the mesocosm tanks were subjected to a simulated marine heatwave (referred to hereafter as the “marine heatwave phase”). The second experimental phase was then carried out in the same tanks four days after the marine heatwave phase and all experimental units had returned to ambient temperature. This phase consisted of 48 h of direct and continuous pathogen exposure to leaf tissue of half of the replicates to facilitate infection, followed by 20 days of post-infection monitoring and sample collection at defined timepoints (hereafter “Pathogen challenge phase”). Experimental treatment factors and levels followed a full crossed design (Fig. 1). The first experimental phase consisted of a warming treatment with two levels: (1) control (i.e., ambient temperature in summer; set at 15.7 ± 0.04 °C) and (2) simulated MHW (i.e., warming; set at 20.3 ± 0.03 °C). The second experimental phase consisted of a pathogen challenge treatment with two factors and four levels: (1) control (CT; ambient temperature and no pathogen challenge throughout the experiment), (2) MHW recovery (MHWr; warming in the first phase and no pathogen challenge during the second phase), (3) pathogen challenge alone (WD; ambient temperature in the first phase and pathogen challenge in the second phase); and (4) pathogen challenge during the MHW recovery (MHWr-WD; warming in the first phase and pathogen challenge in the second phase).

The twenty-four experimental units (42-liter buckets, Fig. 1c) were randomly allocated within six ~300 L container tanks (three for ambient temperature, and three for the warming treatment, respectively). During the simulated MHW, water temperatures in ambient and warming treatments were controlled by connecting all mesocosm tanks to two large seawater reservoirs (one per treatment; ~2000 L of water from Yaquina Bay) in a closed recirculating system, which fed mesocosm tanks a rate of 3.5 L min^{-1}. Temperature in seawater reservoirs were set using aquaria heaters. The high rate of the seawater renewal in experimental units (every ~12 min) ensured the maintenance of homogenous temperature among experimental units. The six mesocosm tanks holding individual buckets were covered with two layers of window screen mesh (4-mm thick) to provide the light dose in the donor meadow. Ambient light (lumens $ft.^{-2}$) and temperature (°C) conditions inside buckets were continuously monitored (every 10 min) throughout the experimental period by HOBO data loggers (UA-002-64). Light intensity was converted from HOBO values in Lux (lumens m^{-2}) to μmol photons m^{-2} s^{-1} according to a previous calibration with a LiCOR light sensor (LI-1400, LI-COR Biosciences, United States) under laboratory conditions. The average light at middle canopy height inside experimental units was 8.5 ± 1.0 mol photon m^{-2} d^{-1}, which allow the non-light-limited growth of eelgrass in the Pacific Northwest (>7 mol photon m^{-2} d^{-1}; Thom et al., 2008).

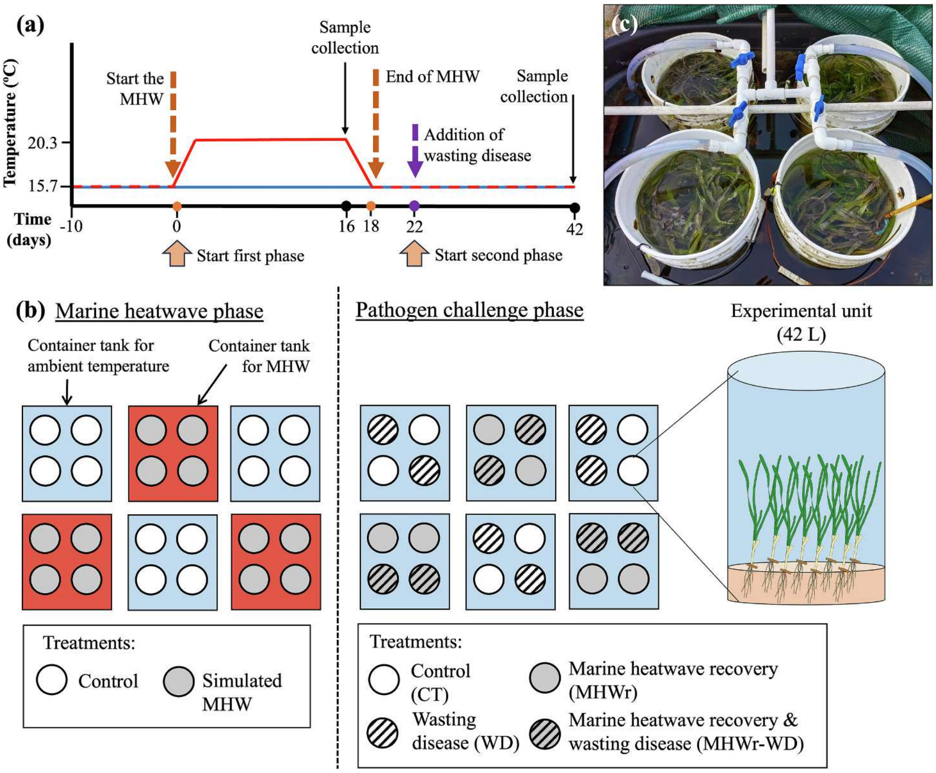

Fig. 1. Simplified diagram of (a) the temporal sequence and (b) the experimental design of the experiment conducted. (c) A representative container tank with four experimental units. MHW: Marine heatwave, WD: Wasting Disease.

The warming treatment simulated a summer heatwave according to the definition of Hobday et al. (2016) (i.e., anomalously warm water event features by values higher than the 90th percentile of the local long-term climatological observations during at least five days) and an analysis of historical temperature data from Yaquina estuary (NOAA Station SBEO3, Appendix A, Fig. S1). Water under the MHW conditions averaged 20.3 ± 0.03 °C for 14 days (i.e., a rise of ca. +4.6 °C with respect the average 15.7 ± 0.04 °C of control buckets; Appendix A, Fig. S2). The simulated heatwave was preceded and followed by two days of warming or cooling ramp (approx. ±2 °C/day). In order the asses the effects of MHW on C metabolism, DOC fluxes, plant biomass, and disease, samples were collected at the end of the simulated MHW ($t = 16$; see methodological details in the following section).

Four days after the end of the MHW ($t = 22$), the pathogen challenge phase was initiated under flow-through conditions with ambient temperature seawater. Controlled inoculations of *L. zosterae* were conducted in half of the experimental units that had been exposed to ambient and warmed seawater temperatures in the first experimental phase, respectively, following the method described in Brakel et al. (2019). In brief, pathogen challenge was performed by clamping medical gauze pieces (1.5 × 1.5 cm) that were either sterile (i.e., "mock" infections) or that had been impregnated with *L. zosterae* for 48 h by pressing them onto a serum–seawater agar (SSA) plate that was full of this pathogen. For each pathogen/mock challenge, sterile or infected gauze pieces were carefully attached to the second rank blade (i.e., second youngest leaf) of shoots with a clear, flexible plastic tubing (12.7 mm thick, 1.5 cm long) that was split vertically as a clamp to cover and press the gauze pieces onto the leaf. After 48 h, plastic-tubing clamps and gauze pieces were removed, and plants were maintained under control (ambient) temperature conditions and flow-through water conditions until the end of the experimental period ($t = 42$) when the final sample collection took place.

2.3. Sample collection and measurement of response variables

To measure DOC fluxes, three water samples (using a 50 mL acid-washed syringe) at the end of each experimental phases ($t = 16$ and 42 days) were collected from the middle of the water column of each experimental bucket at three times: i) just before sunset, ii) right after the following sunrise, and iii) ~8 h after sunrise (midday). In this way, net DOC flux in dark and light periods can be discerned (Egea et al., 2023a). To transform the experimental units into a closed system during the sampling period, seawater renewal was halted. DOC exchange between experimental units and the atmosphere was evaluated through performing a control assay at the end of the experiment period (i.e., once experimental units were emptied of plants and sediments and filled only with seawater from the estuary), and found to be negligible. DOC samples were filtered through pre–combusted (450 °C for 4 h) Whatman GF/F filters (0.7 μm) and were mixed with 80 μL of H_3PO_4 (diluted 30 %) and held at 4 °C in acid–washed glass vials encapsulated with silicone-PTFE caps until further analyses.

Carbon metabolism (i.e., changes in dissolved oxygen (DO) concentrations) was assessed using gas-tight chambers (~4 L) that were carefully placed in each experimental unit on the days after DOC sampling had finished ($t = 16$ and 42). Chambers were similar to those used in previous studies (e.g., Jiménez-Ramos et al., 2023): rigid polyvinyl chloride cylinders (12 cm in diameter) sunk ~8 cm depth into the sediment with a gas-tight polyethylene transparent plastic bag fitted to each cylinder enclosing the eelgrass. During the incubation, the DO concentration was measured three times (as described above for DOC), in order to discriminate between Gross Primary Production rate (GPP) and Respiration Rate (RR). DO concentrations (mg L^{-1}) were recorded using a self-adhesive oxygen sensor spot (SA7-541-212, PyroScience) fixed on the inner wall of the each chamber and an optical oxygen meter (FireSting-O_2 1-channel, PyroScience) connected to a hand-held bare optical fiber (Spfib-bare, PyroScience). In addition, a hand-held temperature sensor (TSUB21, PyroScience) connected to the optical oxygen meter was introduced to the experimental tanks beside each chamber to record the in situ temperature (°C). After the carbon metabolism samplings, all chambers were carefully removed.

Wasting disease lesions were examined on leaf tissue of two randomly collected shoots from each experimental unit (i.e., 12 shoots per treatment) at the end of the experiment (i.e., 20 days after infection; $t = 42$). Typical wasting disease symptoms were noted as black necrotic irregular lesions on eelgrass leaves that were carefully distinguished from senescing tissue on older leaves (Aoki et al., 2022). Infected leaves were placed between two sheets of transparent plastic film, scanned at 600 dots per inch (DPI) using an Epson Perfection V550 scanner. A set of high-resolution images were processed using Image-J software (Rasband, 1997-2016) to quantify wasting disease severity (i.e., lesioned leaf area divided by total leaf area per shoot). Sheath length and leaf area (leaf length and width) of leaves were measured with a ruler.

Finally, upon disassembly of the experimental setup, eelgrass biomass within experimental units was harvested, rinsed and dried at 60 °C to quantify the photosynthetic (i.e., aboveground) and subterranean (i.e., belowground) biomass (g dry weight; g DW) per treatment. The aboveground biomass loss rate per treatment was determined by dividing the accumulated biomass of dead leaves (collected manually through the experimental period) by the experimental time.

2.4. Laboratory analyses and flux calculations

DOC concentrations were obtained by catalytic oxidation at high temperature (720 °C) and chemiluminescence using a Shimadzu TOC-VCPH analyzer. Certified reference material for DOC (Low and Deep), provided by D. A. Hansell and W. Chen (University of Miami), was used to evaluate the accuracy of the estimates. Hourly respiration rates (RR_H) and hourly DOC export rates during night period (DOC_{HN}) were estimated as the difference in DO or DOC concentrations, respectively, between sunset and the subsequent sunrise sampling events between two consecutive days divided by the time span between them. Hourly Net Production Rates (NPR_H) and hourly DOC export rates during daylight period (DOC_{HD}) were estimated from the difference in DO or DOC concentrations, respectively, between sunrise and midday (~8 h after sunrise) samplings of a given day divided by the time span between these sampling events. Hourly Gross Primary Production rates (GPP_H) were calculated as the sum of the hourly rates of RR_H and NPR_H. All results were normalized through the number of shoots within each incubator and then converted to area estimates by multiplying the obtained results by the mean field shoot density (80 shoots m^{-2}). Oxygen metabolic rates were converted to C units assuming photosynthetic (PQ = moles O_2: moles CO_2) and respiratory quotients (RQ) of 1, a widely used value for benthic macrophyte communities (e.g., Egea et al., 2023a). Finally, daily rates of Gross Primary Production (GPP_D) were calculated by multiplying GPP_H by photoperiod, whereas daily rates of respiration (RR_D) by extrapolating the RR_H to 24 h. Likewise, daily rates of net DOC flux were calculated by summing the DOC_{HD} multiplied by light hours with the DOC_{HN} multiplied by the darkness hours. Daily Net Production Rates (NPR_D) were calculated as the difference between GPP_D and RR_D. The equations used for these estimates are indicated in the supplementary material.

A bioassay to estimate the bioavailability of DOC produced in each treatment was performed at the end of each experimental phase. The term bioavailable fraction of DOC (i.e., accessible to microbial degradation) was defined as the DOC used by heterotrophic microorganisms within days, according to previous studies on degradation rates of DOC (e.g., Chen et al., 2020; Jiménez-Ramos et al., 2022). Following the methods of previous studies (e.g. Jiménez-Ramos et al., 2022; Xie et al., 2024), a microbial bioassay was performed using hermetically sealed glass bottles as incubations (500 mL; one per experimental unit). 500 mL of seawater from each experimental unit was collected using 50 mL acid-washed syringes. 450 mL of the collected seawater was filtered through

0.2 μm membranes to allow DOC from experimental unit to pass through but preventing microbial input. The remaining 50 mL were filtered through a 0.8 μm filter to eliminate bacterial predators, such as small flagellates, but keeping the community of free-living cells primarily composed of prokaryotic organisms. Then, both fractions were added to incubations at a ratio of 9:1 (0.2:0.8 μm filtered). DOC bioavailability assays were maintained in the dark at room temperature until microbial communities reached stationary phase. Ammonium (NH_4Cl) and phosphate (NaH_2PO_4) were added to incubations at the beginning to final concentrations of 10 and 2 μM, respectively, to avoid growth limitation by nitrogen or phosphorus availability (Jiménez-Ramos et al., 2022). Beginning at time zero, samples were collected from each incubation bottle and every 24–48 h to measure DOC concentrations and bacterial abundances throughout the bioavailability assay (Appendix A, Fig. S3). Bacteria were counted with a FacsAriaII (Cell Sorter) flow cytometer as described previously in Gasol and Del Giorgio (2000). After 24 days of incubations, the reduction of DOC with respect to their initial values (%) was considered as the highly labile fraction of DOC (i.e., rapidly degraded by microbes), whereas the remaining DOC in the bioassay once the curve of microbial consumption reached stationary phase was considered as semi-labile fraction of DOC (i.e., resistant to rapid microbial degradation), which can be used as a proxy for recalcitrant (i.e., formed by organic carbon with lower biodegradability) fraction of seagrass-derived DOC, according to previous studies on degradation rates of DOC (e.g., Chen et al., 2020; Jiménez-Ramos et al., 2022; Kubo and Tanaka, 2023).

2.5. Data analyses

General Linear Models (GLMs) were used separately for the two experimental phases to examine the effects of treatments (MHW on phase one, and combination of MHW and pathogen challenge on phase two) tested as fixed, categorical variables. For each response variable, a particular family error structure and link function was selected to achieve the assumptions of linearity, homogeneity of variances and the absence of overdispersion, which were checked by visual inspection of residuals and Q–Q plots after modeling (Harrison et al., 2018). GPP_D, DOC_{HN}, DOC_{HD}, net DOC flux, wasting disease severity and effects on eelgrass biomass were modeled using Gaussian distribution with identity link, whereas RR_D and NPR_D were modeled using Gamma distribution with inverse link. Assumptions of normality and homoscedasticity were evaluated by examining the residuals of all linear models. For each experimental phase (i.e., the MHW and Pathogen Challenge phases), DOC bioavailability results were modeled by GLMs and generalized additive models (GAMs) using the R package 'mgcv' with time as random factor. GAMs are an extension of GLMs particularly useful for time-series and/or for identify any potential non-linear response of change in response variable (Wood, 2017). For all model sets, the best-fitting models were determined using the Akaike's Information Criterion (AIC), allowing the selection of the most parsimonious model. GLMs generated with Gamma distribution were selected for the first phase (i.e., the MHW phase). For the second phase, GAMs generated with a Gamma distribution, an inverse link function, a cubic regression spline and a smoothing parameter ($k = 4$) were selected over GLMs as best-fitting model in AIC. Pairwise comparisons were tested using estimated marginal means with a Bonferroni correction ('emmeans' R package; Lenth et al., 2019). Statistical analyses were computed with R statistical software 4.4.1 (R Core Team, 2024).

3. Results

3.1. Community metabolism

The marine heatwave (MHW) treatment caused a significant decrease in daily rates of both Gross Primary Production (GPP_D) and Net Production Rate (NPR_D) relative to controls (0.9- and 0.75-times, respectively) (Fig. 2a; Appendix A, Table S1), but this pattern disappeared during the recovery period (i.e., during the second experimental phase when temperature was back to ambient levels). In contrast, a significant reduction in GPP_D and NPR_D (0.5- and 0.6-times, respectively) was observed for plants that experienced wasting disease (WD) stress during the recovery of MHW (i.e., MHWr-WD treatment) compared to those recovering from the MHW alone (i.e., MHWr treatments) (Fig. 2b; Appendix A, Table S1). Similarly, plants undergoing pathogen challenge alone (i.e., WD treatment) exhibited significant reductions in GPP_D and NPR_D relative to control plants (ca. 0.7-times decrease). Daily rates of Respiration Rates (RR_D) did not change with warming during the initial phase or after the MHW was over. In samples challenged with *L. zosterae,* a slight, but not statistically significant, reduction in RR_D was observed (0.7- and 0.5-times lower than WD and MHWr-WD treatments, respectively).

3.2. DOC fluxes

Overall, DOC fluxes during daylight hours were higher than during night hours in all treatments (GLM, $p < 0.005$) and daily DOC fluxes were positive. Plants subjected to the MHW exhibited a significant reduction in DOC production during daylight hours compared to control plants (ca. 0.6-fold) (Fig. 3a and b; Appendix A, Table S1), but these reductions disappeared once the MHW was over. In contrast, during this second experimental phase, communities recovering after the simulated MHW exhibited negative DOC fluxes (i.e., consumption) during night hours, whereas nighttime DOC fluxes were positive in all other

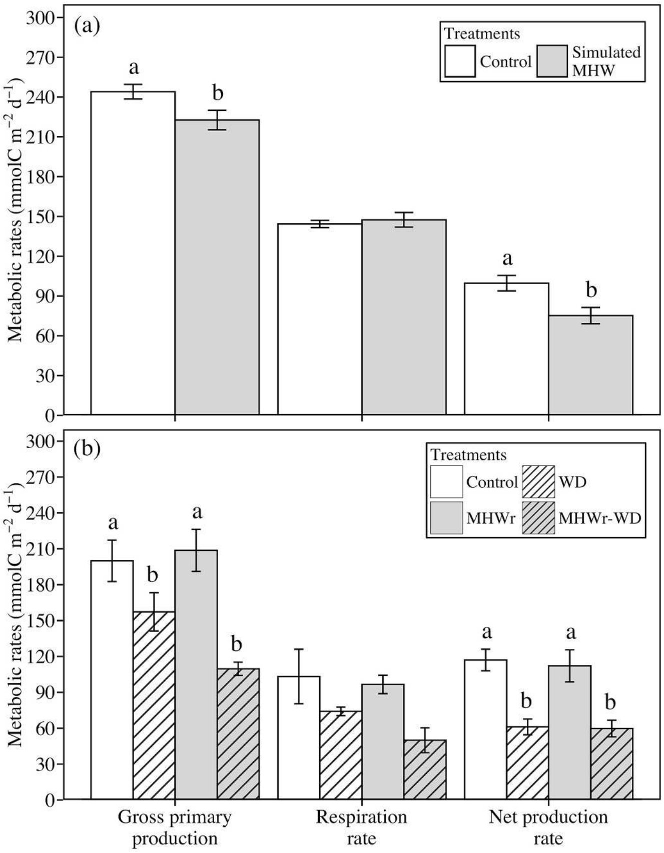

Fig. 2. Community Metabolic rates (mean ± SE) of Gross Primary Production (GPP), Respiration Rate (RR) and Net Production Rate (NPR) of *Zostera marina* communities for (a) the test of MHW (i.e., first experimental phase), and (b) for the pathogen challenge after the MHW had ended (i.e., second experimental phase). Different letters indicate significant differences among treatments for each response variable.

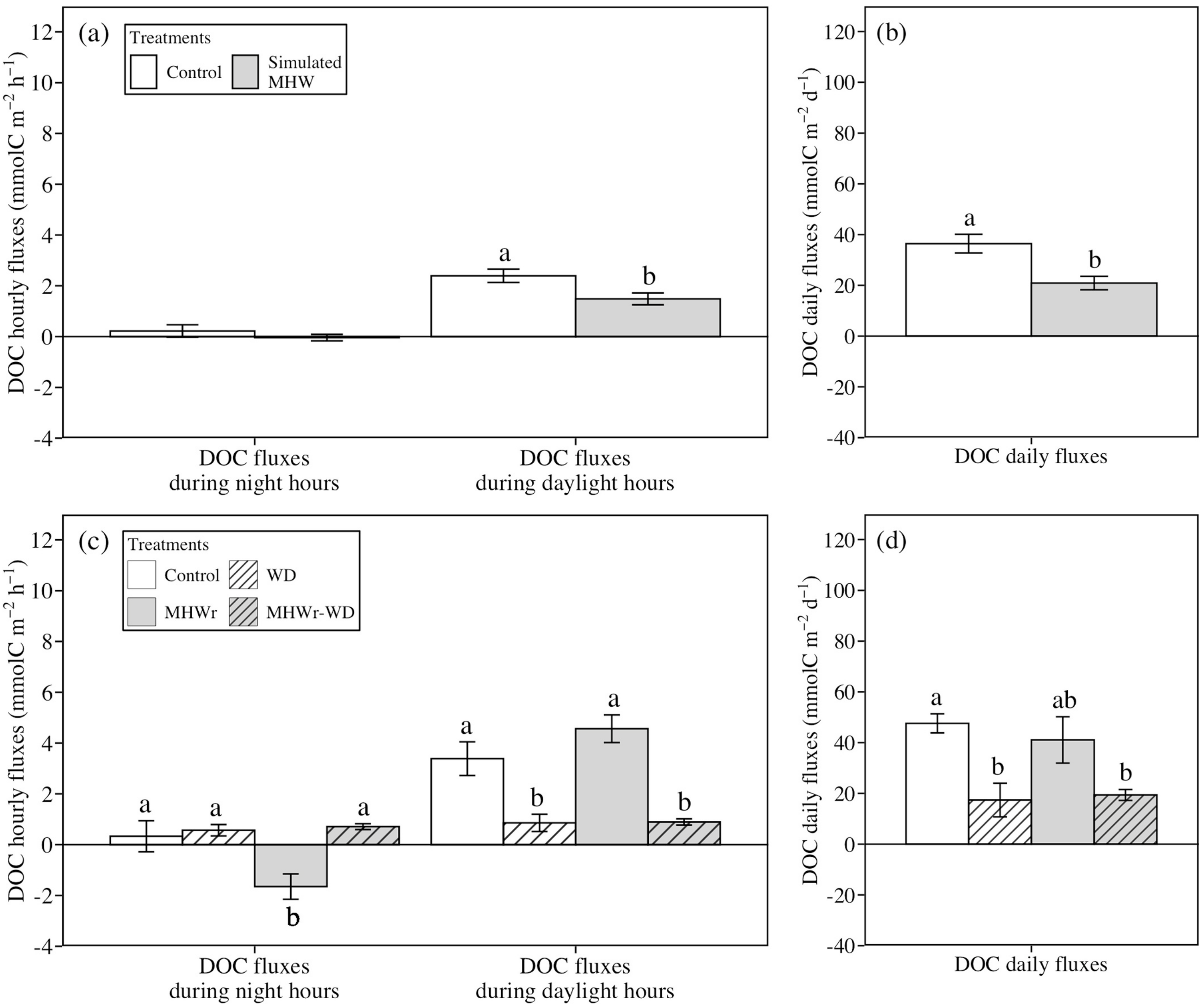

Fig. 3. DOC hourly fluxes (mean ± SE) at night and daylight hours (left panels), and overall daily fluxes (right panels) in *Zostera marina* communities for (a, b) the test of MHW (i.e., first experimental phase), and (c, d) for the pathogen challenge after the MHW had ended (i.e., second experimental phase). Different letters indicate significant differences among treatments for each response variable.

treatments during this experimental phase (Fig. 3c; Appendix A, Table S1). On the other hand, a marked reduction in DOC fluxes during daylight hours was observed for plants that were exposed to wasting disease compared to uninfected plants in the control treatment (regardless of whether they had undergone prior warming), leading to a sharp decrease in the daylight and daily DOC fluxes in both treatments exposed to *L. zosterae* (Fig. 3d; Appendix A, Table S1).

3.3. Bioavailability of DOC

The DOC pool from control treatments was rapidly respired during the first 14–16 days after bacterial inoculation, after which the rate of DOC loss from incubations slowed down, leaving ca. 71 % of initial DOC pool remaining after 24 days (Fig. 4a). In contrast, DOC consumption for the MHW treatment was significantly lower (88 % of initial DOC remaining). Moreover, a slowing down of DOC consumption during the second experimental phase after ca. 16 days (ranging between ca. 60 % to ca. 71 % of DOC remaining; Fig. 4c) was observed, but no statistically significant differences were detected among the different warming or pathogen challenge treatments (Appendix A, Tables S2 and S3); i.e., the plants for which warming had ceased, returned to similar levels of DOC as controls, and the pathogen challenge did not alter the bioavailability of the DOC produced. The initial phase of DOC consumption in all incubations coincided with an initial phase of bacterial growth in all treatments (Fig. 4b and d), with relatively higher microbial abundances coinciding with treatments with the lowest levels of DOC remaining in the bioassay incubations (e.g., control samples from the first experimental phase and MHWr-WD samples from the second experimental phase).

3.4. Plant biomass and disease severity

Whereas there were no effects of warming or pathogen challenge on seagrass belowground biomass, aboveground biomass exhibited a marked decrease after infection with *L. zosterae*, with significant decreases observed in the MHWr-WD treatment compared to all others (0.6-fold lower than control; Fig. 5a and b; Appendix A, Table S1). Stress treatments tended to increase the aboveground biomass loss rate, but differences compared to control treatments were not statistically significant (Fig. 5c; Appendix A, Table S1). On the final day of the

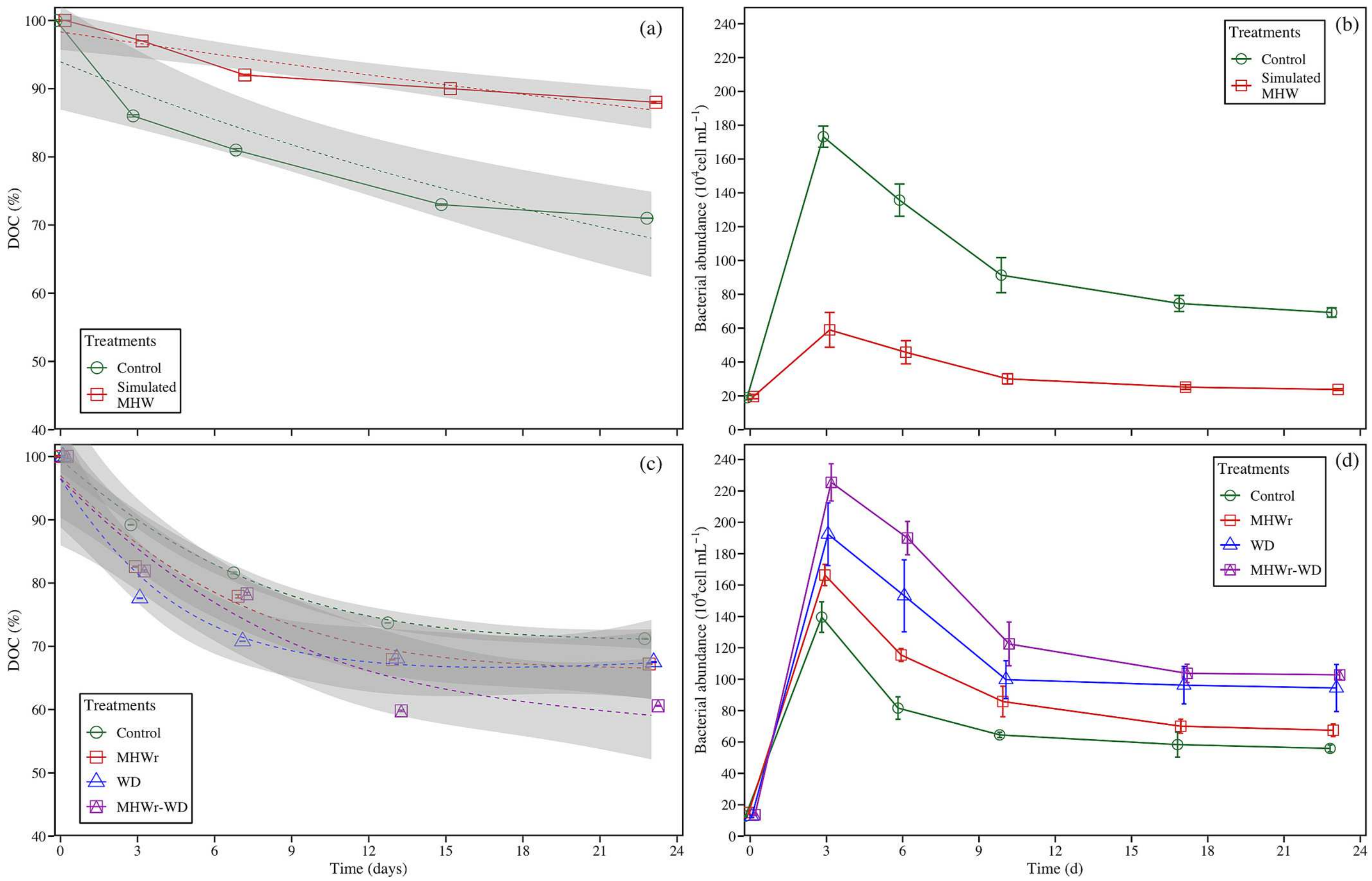

Fig. 4. Time course of DOC pool consumption (panels *a* and *c*; percentages of their respective initial concentrations ± SE) and bacterial abundance (panels *b* and *d*; mean ± SE) during the incubation period in the DOC bioavailability assays. Dashed lines on left panels show the best-fitted models applied to data, with shaded areas indicating the 95 % model confidence intervals.

experiment ($t = 42$) the severity of wasting disease lesions was very low for leaves from the control treatment (5 ± 1.7 % of leaf area), but increased significantly in the WD and MHWr-WD treatments (23.9 ± 3.5 % and 55.1 ± 7.7 %, respectively). Notably, the latter treatment demonstrated a marked synergy between the MHW and the subsequent pathogen challenge with lesions ca. 11-fold higher than controls (Fig. 5d; Appendix A, Table S1).

4. Discussion

4.1. *Effects of the MHW event on carbon metabolism and DOC fluxes*

The simulated marine heatwave (MHW) caused a decrease in the Net Production Rate (NPR), which appears to be a consequence of lower Gross Primary Production (GPP) rates, rather than increasing respiration rates (RR) (Fig. 2a; Appendix A, Table S1). At the same time that these plants underwent a decrease in GPP and NPR, they also exhibited a significant reduction in DOC production during daylight hours, and, consequently, in the overall daily DOC fluxes (Fig. 3a and b; Appendix A, Table S1). These findings contradicted our expectations since recent previous studies in other temperate seagrasses, such as *Cymodocea nodosa,* reported enhanced photosynthetic performance (Deguette et al., 2022) and increases in GPP and CR (Egea et al., 2019, 2023a) during MHW events. However, as a tropical-originated species, *C. nodosa* has an optimum temperature range relatively higher than that of *Zostera marina* (from 24.5 to 31.0 °C and 15.3–23.3 °C in *C. nodosa* and *Z. marina*, respectively) (Lee et al., 2007; Máñez-Crespo et al., 2020). Furthermore, eelgrass usually exhibits responses to thermal stress when exposed to MHW scenarios. For instance, at water temperatures higher than 20 °C, this species usually experiences detrimental effects on its photosynthetic performance (Winters et al., 2011; Franssen et al., 2014). These results are in accordance with negative daily carbon balances for this seagrass species (e.g., under temperatures higher than its optimal range; Marsh et al., 1986) and underline that reduction in productivity may occur under heatwave events.

The negligible effect of the simulated MHW on RR found here contrasts with a number of studies showing an increase in seagrass photosynthesis and RR up to a certain threshold with warming (e.g., Pedersen et al., 2016; Nguyen et al., 2021). However, inhibition or decrease of photosynthesis due to temperature increase can occur in *Z. marina* by photorespiration (e.g., Buapet and Björk, 2016; Celebi-Ergin et al., 2022). When temperature rises, O_2 solubility increases faster than CO_2 solubility (Rasmusson et al., 2020) and the RuBisCO enzyme reduces its affinity for CO_2, resulting in a loss of plant productivity and increased energy consumption (Jordan and Ogren, 1984). Photorespiration occurs during both light and dark periods in seagrasses (Celebi-Ergin et al., 2022), but, given the high production of O_2 in the day by photosynthesis and the inherent enzyme affinity for O_2, RuBisCO appears to favor photorespiration during the daylight, and CO_2-fixation at night (Rasmusson et al., 2020). Thus, we hypothesize that the temporal and temperature controlled changes in the CO_2:O_2 soluble concentration ratio, along with an overall increase in electron flow through the photosynthetic machinery, may have led to an increase in photorespiration during the day (Buapet et al., 2013; Buapet and Björk, 2016). In contrast, when the light reactions of photosynthesis are inactive and dissolved O_2 concentration is no longer elevated at nighttime, the increase in photorespiration ceased. While high temperatures can increase dark respiration (Nguyen et al., 2021), significant changes in RR at night

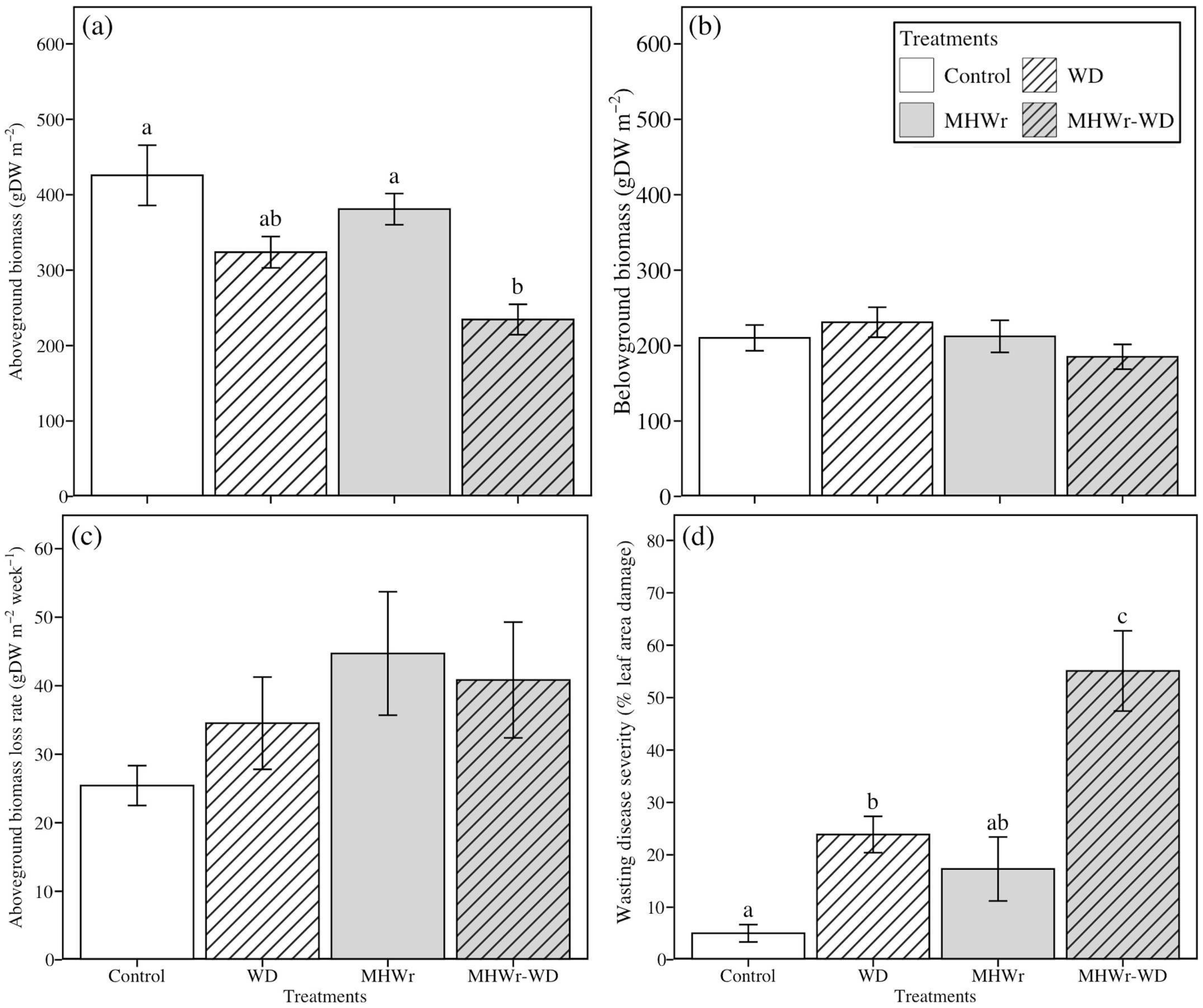

Fig. 5. *Zostera marina* (a) aboveground biomass, (b) belowground biomass, (c) aboveground biomass loss rate and (d) wasting disease severity for shoots and leaves collected from each treatment at the end of the experimental period (t = 42 days). Different letters indicate significant differences among treatments for each response variable.

were not observed. Such lack of effect on dark respiration may be a consequence of lower DOC availability (both in terms of net DOC concentration and its bioavailability; Figs. 3a and 4a) at night in the MHW treatment (which would support microbial community respiration; Allesson et al., 2016) and lower O_2 availability as a result of eelgrass photorespiration. An alternative explanation for the results is that the medium-term experimental design might have disguised the RR peak, since RR often are more sensitive and respond more rapidly to increasing temperatures than photosynthetic rates (Staehr and Borum, 2011).

4.2. Effects of WD during the recovery of the MHW event

Interestingly, once temperature conditions recovered to ambient levels (i.e., when the simulated MHW event was over), eelgrass production rates of the plants that had previously undergone the simulated MHW were restored to control levels, suggesting that *Z. marina* has a relatively quick capacity to recover indicators of carbon metabolism to non-stress levels after thermal stress, which is in accordance with previous studies denoting high recovery of eelgrass photosynthetic rate after MHW events (e.g., Winters et al., 2011; Jueterbock et al., 2020). Similarly, and further highlighting the link between C metabolism and DOC production, DOC daylight hour fluxes and net daily fluxes were statistically indistinguishable from controls after the MHW was over. Concomitant to this recovery in daily DOC fluxes, an unexpected increase in DOC consumption at night during the MHW recovery phase was found (Fig. 3c). Although seagrasses typically act as a net source of DOC (i.e., positive net DOC flux) to the marine environment, some studies have reported that seagrass communities can also take up DOC (van Engeland et al., 2013; Liu et al., 2020; Egea et al., 2023b). For example, DOC exudate from seagrasses can be rapidly utilized by bacteria community in leaves (phyllosphere) and sediments (rhizosphere) (Kaldy et al., 2006; Liu et al., 2020). The DOC consumption by bacteria can be boosted by higher temperatures, as it was reported by Kaldy et al. (2006) during summer months in seagrass rhizosphere. In addition, degradation rates of dissolved organic matter, including DOC, seem also be stimulated by increased temperature (Lønborg et al., 2020), resulting in a negative net DOC flux. However, following this hypothesis, the MHWr-WD treatment should have also exhibited negative DOC fluxes at night given the fact that those plants were exposed to the same MHW conditions. We attribute this contrasting result between these treatments to two separate processes. First, the decrease in aboveground biomass experienced by MHWr-WD plants (Fig. 5a) would have yield a relatively lower phyllosphere abundance (i.e, microorganisms associated with leaves), since the surfaces of seagrasses provide habitat for microbial communities (Champenois et al., 2024; Tasdemir et al., 2024). Microbial phyllosphere in seagrasses exert a relevant role in DOC fluxes (Liu et al., 2020). Therefore, the relatively lower surface of eelgrass available for phyllosphere to develop, may reduce the community DOC uptake in MHWr-WD compared to MHWr. Furthermore, we suspect that WD modified the microbiome diversity –as it was observed in previous studies (Beatty et al., 2022)–, which could also explain the changes in the use of DOC by phyllosphere. Second, the pathogen infection probably resulted in an increase of detrital and dead leaves –denoted by higher trend of aboveground biomass loss rate (Fig. 5c). Previous studies denoted the important carbon transfer from eelgrass detrital leaves (Vähätalo and Søndergaard, 2002) and seagrass wrack (i.e., detached leaves and stems; Lavery et al., 2013) to the community DOC pool. Moreover, the mechanism of particulate OM degradation often triggers intermediate stages of dissolved OM production by leaching of POC into DOC fraction (Burdige et al., 2016; Egea et al., 2023c; Yamuza-Magdaleno et al., 2024). The observed DOC consumption was limited to night hours, perhaps because the DOC released by primary producers in the community during light-hours overlapped this trend to higher DOC consumption in MHRr treatment. These are open questions in this study that should be further assessed in future research.

Similar to the simulated MHW treatment, infection by *L. zosterae* led to a significant decrease in GPP and NPR. In this case, pathogen consumption of plant chloroplasts and the creation of large areas of necrotic tissue can greatly reduce plant photosynthetic area and capacity (Short et al., 1986), likely accounting for the observed decrease in primary production. Infection with *L. zosterae* also strongly decreased daily release of DOC primarily due to significant decreases in the release of DOC during daylight hours (Fig. 3c and d). Interestingly, the results suggest that previous exposure to the MHW does not exacerbate the decrease in productivity or DOC release due to wasting disease (Fig. 2b and 3d). Although the MHW and the pathogen challenge treatments both decreased DOC fluxes and primary productivity, the results suggest that the pathogen challenge seems to lead to much greater and longer-lasting reductions (e.g., NPR decreased by ca. 25 % during the MHW, whereas WD treatments decreased it ca. 48 %), highlighting the significant negative, and potentially long-lasting impacts that wasting disease may have on shoot physiology and *Z. marina* populations.

Higher prevalence and severity of WD as a result of *L. zosterae* infection has often been linked to warming (e.g., Sullivan et al., 2018; Aoki et al., 2022). Interestingly, while no major changes in aboveground biomass were observed under warming alone (ca. 11 % lower than control), eelgrass suffered significant decreases in leaf biomass after infection, and this effect was worsened when the infected plants had previously undergone the simulated MHW (ca. 45 % lower than control) (Fig. 5a). Consequently, although the C metabolism of plants that have undergone WD alone or WD after warming were similar in the short-term experiment evaluated here (Fig. 2b), the significantly higher disease susceptibility under warming (Sullivan et al., 2018; Aoki et al., 2022; this study) coupled with the decrease in photosynthetic biomass recorded, might lead to combined effects of these stressors that exacerbate the decrease on community production in the long-term, which is in accordance with the initial hypothesis. However, further experimentation investigating longer-term effects will be needed to resolve this hypothesis.

4.3. Results on bioavailability of DOC fluxes

The carbon lability experiments show that *Z. marina* exhibits a high capacity to produce a significant proportion of semi-labile fraction of DOC under ambient conditions, as indicated by the high values of DOC remaining in control samples (as high as ca. 71 %; Fig. 4). In addition, the results demonstrate that eelgrass's contribution to the ocean DOC pool can be altered when exposed to different stressors. For instance, under thermal stress, not only was the net DOC release lower compared to controls, but a larger proportion of it showed lower bioavailability. We attribute the decreased bioavailability of DOC under thermal stress to changes in the chemical structure of DOC directly produced by seagrass and/or to changes in the microbial community structure and function that impact the turnover and nature of the residual carbon in the experiments. Recently, it has been shown that temperature increases favor the release of seagrass-derived humic-like components (Egea et al., 2023a), possibly through the higher production of seagrass secondary metabolites (Rotini et al., 2013), which would favor the first mechanism. Alternatively, temperature increases are known to rapidly alter microorganisms associated to seagrass (Vogel et al., 2021) and generally increase bacterial abundance, production, and respiration (Joint and Smale, 2017), which would presumably increase the consumption of labile DOM by marine prokaryotes. However, these processes can also enhance the release of recalcitrant DOM as a by-product of microbial metabolism of labile DOC from macrophytes (Chen et al., 2020; Kubo and Tanaka, 2023; Xie et al., 2024). Since we cannot differentiate the direct sources of the remaining organic carbon in the experiments and since we did not perform a more in-depth analysis of the diversity and function of the microbial communities in the samples, we cannot rule out either mechanism. Further experimentation specifically characterizing the long-term degradation and compositional changes of seagrass-derived DOC, and changes in the microbiome functional and taxonomic diversity due to warming will be needed to definitively resolve these hypotheses.

4.4. Conclusions and ecological implications

Eelgrass is among the most sensitive seagrass species to warming and wasting disease (Muehlstein, 1992; Magel et al., 2022). Both stressors triggered reductions on primary productivity and DOC fluxes, with WD exhibiting much greater effects, in addition to reductions in aboveground biomass and WD severity. The results, therefore, provide evidence of higher negative impacts of WD than of the MHW for eelgrass, although future research should continue to investigate the interactive effects of these stressors in order to enhance the understanding about the key controlling factors for eelgrass C dynamics under changing environment. The reduction on primary productivity has important ecological implications, since this may impair the ecosystem's potential as a carbon sink (Duarte and Cebrián, 1996; Mazarrasa et al., 2021), and may affect food webs and biodiversity –e.g., lower seagrass growth potential, and reductions in plant biomass impacting trophic transfer of C and habitat structure (Jiménez-Ramos et al., 2019, 2021).

This study is the first to confirm that only a minor fraction of the DOC exuded by *Z. marina* is highly labile and readily bioavailable to microbial decomposition, and that this proportion undergoes significant changes based on environmental stress. The modifications on bioavailability of DOC found here by MHW and WD are also ecologically relevant. While highly labile DOC from vegetated coastal communities constitutes an important source of carbon in the microbial food web and remineralization to CO_2 (Moran et al., 2022; Egea et al., 2023c), the production of recalcitrant DOC (i.e., formed by organic carbon less bioavailable) in the marine environment can contribute to relatively long term storage of organic C in deep ocean waters (Duarte and Krause-Jensen, 2017; Jiménez-Ramos et al., 2022; Cai and Jiao, 2023; Egea et al., 2023a). These findings provide new insights regarding the effects of MHWs and WD on seagrass' production and DOC release, highlighting that WD may not only negatively impact eelgrass but alter the ecological functioning of the communities it creates.

CRediT authorship contribution statement

Luis G. Egea: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rocío Jiménez-Ramos:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mary K. English:** Writing – review & editing, Methodology, Investigation. **Fiona Tomas:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Ryan S. Mueller:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177820.

Data availability

Data will be made available on request.

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