

Title: The effects of silicate mineral dissolution on seagrass functioning: Assessing olivine-based approaches to marine carbon dioxide removal

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Abstract

The active removal of carbon dioxide (CO₂) from the atmosphere may be the only viable means of mitigating the worst effects of global warming. Known as carbon dioxide removal (CDR), these approaches involve a variety of techniques to sequester atmospheric CO₂. One technique is marine enhanced rock weathering (mERW), which deploys natural silicate minerals (olivine) along the coastal zones, that dissolve, generate alkalinity and induce atmospheric CO₂ drawdown upon air-sea equilibration. While the geochemistry of olivine dissolution is well known, the effects on foundation species are relatively unexplored. Here, we established a 16-week ecotoxicology experiment that exposed two tropical seagrasses (*Thalassia testudinum* and *Syringodium filiforme*) to one of three treatments: (1) surficial dose of fine-grained olivine; (2) surficial dose of clean aragonite sand; (3) control - no dose. During treatment exposure, seagrass structural and physiological characteristics were measured. Olivine or sand dosing had no effect on shoot density, leaf morphometrics, or photosynthetic yield. Leaf productivity, belowground biomass and carbon / nitrogen content were also unaffected. However, final aboveground biomass was lower in the olivine treatment relative to sand, but only for *T. testudinum*. Tissue trace metal concentrations (nickel and manganese) were elevated with olivine dosing for both species, while other metals showed mixed results. Neither olivine nor sand dosing influenced air-water CO₂ flux. These results suggest that while olivine dosing had minimal effects on tropical seagrasses, elevated trace metals pose a concern, thus it may be necessary to regulate olivine dosing to avoid potential impacts on higher trophic levels.

Keywords: Ocean Alkalinity Enhancement, OAE, CDR, CO₂, Ecotoxicology, olivine, *Thalassia testudinum*, *Syringodium filiforme*

Introduction

Climate change poses an existential threat to the functioning and integrity of the world's natural ecosystems. Global surface temperatures have reached 1.4°C above the 1850-1900 reference period, primarily due to greenhouse gas emissions from energy- and land-use change (World Meteorological Organization, 2026). This has placed increased pressure on terrestrial and marine ecosystems and has largely compromised many of the economic services that they provide (Halpern et al 2015, Hoegh-Guldberg, O. et al 2018). As emissions continue to rise, achieving the UN climate target of limiting global warming to 1.5°C above the reference period will be difficult, even under the most ambitious decarbonization and emissions reduction scenarios (Meinshausen et al. 2022). New approaches that extend beyond emissions reductions will be required, and one proposed strategy is the active removal of carbon dioxide (CO₂) from the atmosphere (National Academies of Sciences, Engineering, and Medicine (NASEM), 2022). Collectively known as carbon dioxide removal (CDR), these approaches involve a variety of techniques to remove and sequester carbon dioxide. To meet current climate goals, it is estimated that nearly 8Gt of CO₂ needs to be removed annually from the atmosphere by mid-century (Edwards et al. 2026). One promising CDR technique is ocean alkalinity enhancement (OAE), which increases surface water alkalinity and shifts seawater carbonate system equilibria from dissolved CO₂ to bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻), thereby enhancing ocean CO₂ uptake at the air-sea interface (Renforth and Henderson 2017). While there are several strategies for OAE (e.g., calcium hydroxide injection - ocean liming), one scalable approach is marine enhanced rock weathering (mERW), which involves the deployment of naturally occurring silicate minerals along the coastal zones, which upon weathering and dissolution, release alkalinity to the overlying water column (Meysman & Montserrat 2017, Geerts et al. 2025). Over

geological timescales (>10,000 years), this process can exert moderate control over the Earth's climate systems (Brantley et al. 2023), and mERW approaches seek to accelerate this process. One commonly proposed mineral for mERW is olivine, a naturally occurring ultramafic silicate ($\text{Mg}_{2-x}\text{Fe}_x\text{SiO}_4$), that slowly dissolves and generates alkalinity in the form of hydroxide ions (OH^-) (Geerts et al. 2025):



It is estimated that this approach has the potential to sequester between 0.3 to 10 Gt of CO_2 yr^{-1} , thereby garnering commercial interest as companies seek to enter the growing carbon market (Feng et al. 2017, Palmiéri & Yool 2024).

Research on the effects of mERW on the biological functioning of marine systems is limited, particularly for foundational species. While olivine is a naturally occurring mineral, deposits often contain transition metals that serve as substitutes for Mg^{2+} or Fe^{2+} in the crystal lattice, thus releasing trace elements such as nickel (Ni), chromium (Cr), cobalt (Co), manganese (Mn), zinc (Zn) and copper (Cu) upon dissolution. Elevated concentrations of these compounds could directly affect marine life and/or bioaccumulate in higher trophic levels, although conclusions from prior experiments largely depend on the study approach and level of exposure (e.g. lab versus field application) (Jankowska et al. 2026). Under laboratory settings, both short- and long-term exposure to olivine have resulted in elevated metal concentrations for the bivalve *Macoma nasuta* and polychaete *Alitta virens* (Jankowska et al 2024), and amphipod *Gammarus locusta* (Flipkens et al 2023). However, under field conditions, long-term olivine exposure showed limited effects on metal accumulation in the oyster, *Crassostrea virginica* (Jankowska et al 2026). Overall, a paucity of work suggests that experiments are needed to address the effects

of olivine-based mERW on the functioning of coastal foundation species, using both lab- and field-based approaches.

Seagrasses are marine plants that are distributed along the world's coastal zones, spanning tropical, temperate and arctic latitudes. They provide key ecological services by structuring the benthic environment and supporting marine fisheries and coastal biodiversity (Orth et al 2006). They also play important roles in improving water quality, preventing sediment erosion and contributing to carbon sequestration, thereby representing a CDR pathway in their own right (Rischer et al. 2026). Given the sensitive nature of seagrass habitats, seagrass beds are unlikely to be intentionally targeted for mERW; however, deployment of mERW feedstocks in coastal areas may result in the inadvertent spread of mERW mineral sands into seagrass habitats. With this in mind, if coastal mERW is going to become a viable strategy for meeting climate targets, research is needed to explore the potential effects on the functioning of these coastal plants.

Using a mesocosm-based ecotoxicology experiment, we assessed the effects of olivine dosing on the functioning of two dominant seagrasses in the Western Atlantic, *Thalassia testudinum* and *Syringodium filiforme*. Replicate tanks were planted with both species and subjected to treatments consisting of either (1) surficial olivine dose; (2) surficial aragonite sand dose; or (3) no dose control. A fourth treatment consisted of olivine dosed to tanks with no seagrass to isolate the effects of olivine on tank water chemistry. During a 110-day exposure period, we measured seagrass structural (leaf morphology, shoot density, above- and belowground biomass), chemical (elemental and trace metal content), and performance (leaf growth, photosynthetic yield, and air-water CO₂ flux) metrics. Water chemistry was also monitored in all tanks to assess the extent of olivine dissolution. Our study represents the first

mesocosm assessment of olivine-based mERW on tropical seagrass functioning, which will help evaluate and inform future field deployments.

Methods

Mesocosm design

Twenty clear acrylic tanks (61 cm long x 33 cm wide x 30 cm high) were established at the Biscayne Bay Campus at Florida International University in March 2022. Each tank was filled with natural seawater collected from Haulover Inlet (~0.1 mi away) and supplied with an aeration bar for circulation. Lighting was provided by a bank of LED light fixtures (Aqua Illumination, Hydra 64, 250W) positioned 50cm above the tank, providing 700-750 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of photosynthetically active radiation (PAR) on a 12:12 light cycle. Temperature control was provided by a single 100W heater in each tank connected to an independent temperature controller (Inkbird, model ITC-308).

Seagrass collection and planting

Fragments of two common seagrasses (*Thalassia testudinum* and *Syringodium filiforme*) were collected at a nearshore location (25.733747,-80.226906) ~30 km from campus on March 15, 2022. Each fragment consisted of 3-4 interconnected shoots and was carefully excavated from the sediment to ensure the root-rhizome complex remained intact. Fragments were transported back to campus in coolers filled with clean seawater and temporarily held in large containers. Prior to transplantation into the acrylic tanks, all fragments were cleansed of attached sediment and inspected for any signs of damage. Afterwards, six separate fragments of *T. testudinum* and five separate fragments of *S. filiforme* were transplanted into each of the 15 tanks (5 tanks remained unplanted). The number of active meristems was also standardized for each tank, with 4 active meristems per tank (2/species). Tanks were initially filled with 5cm of clean

aragonite sand (Caribbean Reef, Bahamas Oolite, grain size 0.25-1.0mm), which was gently rinsed with clean seawater to remove fine particles. After approximately two weeks, an additional 4cm of clean aragonite sand was added to each tank. Water temperature was set to 28°C (continuously monitored with HOBO loggers), and salinity was periodically measured with a YSI Pro20 meter and adjusted to 33ppt with deionized water. Water changes (50-75% volume) were conducted in all tanks once every 2-3 weeks.

Acclimation and baseline metrics

Seagrasses were allowed to acclimate to tank conditions for 180 days, and during this period, baseline metrics were measured weekly. Shoot density was recorded in all tanks and morphometrics (leaf length and width) were measured on four haphazardly selected shoots. Pulse amplitude modulated fluorometry (PAM) was used to assess the photosynthetic health (dark-adapted quantum yield, F_v/F_m) of the same haphazardly selected shoots. During PAM measurements, all plants were initially dark-adapted for 60 minutes by turning off the lighting system. Then, a single saturation pulse ($>8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was administered to the middle portion of the rank 2 leaf of the selected seagrass shoots to record yield. Further baseline measurements included water chemistry sampling and CO_2 flux measurements.

Experimental treatments

After acclimation, experimental treatments were initiated (Fig. S1). Each tank was randomly assigned to one of four treatments ($n=5$): (1) seagrass with 9cm of aragonite sand (control); (2) seagrass with 9cm of aragonite sand + 1cm of additional aragonite sand (+ sand); (3) seagrass with 9cm of aragonite sand + 1cm of olivine sand (+ olivine); (4) no seagrass with 9cm of aragonite sand + 1 cm of olivine sand (+ olivine, no seagrass). Comparisons between treatments 1 and 2 tested for the effects of burial by adding a 1cm layer of sand. Comparisons

between treatments 2 and 3 tested for the effects of olivine relative to sand. Treatment 4 was used to monitor the effects of olivine addition on water chemistry in the absence of seagrasses. Olivine was type AFS 35, sourced from Sibelco, Norway with similar grain size to the aragonite sand (0.25mm - 1mm). See table S3 for geochemical analysis.

Treatment responses (seagrass productivity, biomass and chemical composition)

In addition to the baseline measurements (which continued for the duration of the experiment), treatment responses were assessed in all tanks after 110 days of exposure. Leaf productivity (*T. testudinum* only) was measured on four haphazardly selected shoots in each tank. Leaves were marked with a needle punch near the sediment surface (Zieman 1974) and harvested after 7 days of growth. Leaf production was calculated as the sum of new leaf material produced across all leaves per shoot per day. Furthermore, all biomass in each tank was harvested at the end of the experiment. Active horizontal meristems were counted, and then biomass was partitioned into above / belowground fractions and dried (60° C) to a constant weight. Samples were then ground to a fine powder for elemental (C, N and P), isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and trace metal (Ni, Cr, Co, Fe, Cu, Mn, Pb, As, P) analysis. Elemental and stable isotope content was measured using an elemental analyzer isotope ratio mass spectrometer (EA-IRMS). Isotopic ratios are reported in delta notation with respect to the international standards of atmospheric N_2 and Vienna Pee Dee belemnite (V-PDB) for carbon. Sample replicates had a reproducibility of $\pm 0.08\text{‰}$ for carbon and $\pm 0.2\text{‰}$ for nitrogen. Elemental content was calculated as dry weight and ratios are reported on a mol:mol basis. Phosphorus content was determined by dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis (Fourqurean et al. 1992). Trace metal concentrations were measured on a Thermo Fisher quadrupole inductively-coupled plasma mass spectrometer with Collision Cell Technology

option (Q-ICPMS; Thermo Q). Recovery on internal standards (n=9) was ~0-3% and blanks were below detection. Metal concentrations are presented as µg metal per g of dry biomass.

CO₂ flux measurements

To assess rates of air-water CO₂ flux, an acrylic chamber with Vaisala GMP343 CO₂ sensor (Vantaa, Finland) was fitted onto the mesocosm tanks. Two 5V fans circulated air in the headspace, and a pump cycled air from the headspace through aeration bars placed on the sediment surface. Air-water gas flux was determined on each tank at six times (3 pre- and 3 post-olivine / sand addition) between September 2022 and January 2023, for a total of 129 incubations. Before the first measurement of the day (and hourly thereafter) atmospheric pressure was retrieved from <https://w1.weather.gov> for station KFXE (approximately 32 km from the laboratory) for sensor compensation. In addition, temperature was retrieved from the internal sensor of the GMP343, and a static humidity of 99% was assumed for all incubations. The water level was measured in each tank before every incubation to allow for an exact determination of headspace volume. Compensated as well as raw CO₂ concentrations and temperature measurements were logged at 1 Hz with a ten second averaging filter. Each incubation lasted 10 minutes and started 1 minute after the chamber was installed onto each tank. Rates of gas flux (F) were calculated by determining slopes of CO₂ concentration over time, then using the following formula (Podgrajsek et al. 2014):

$$F = \frac{dCO_2}{dt} \frac{V}{RTA}$$

where the first term is the slope of the CO₂ concentration over time t, V is the chamber volume, R is the universal gas constant, T is average temperature inside the chamber over the incubation period, and A is the chamber area. Because gas exchange rate is influenced by the concentration

gradient between $[\text{CO}_2]_{\text{water}}$ and $[\text{CO}_2]_{\text{air}}$ (Mannich et al. 2019), slopes were standardized for intercomparison by using only data from 200 seconds at concentrations below 700 ppm. Only slopes with a linear model fit of $R^2 > 0.95$ were retained to calculate flux estimates ($n=116$). To account for the change in seagrass photosynthetic material in the tanks over time, CO_2 flux was normalized by total seagrass leaf area at the time of incubation.

Water chemistry analysis

Two weeks prior to olivine/sand addition, water samples were collected in all tanks for nutrients, carbonate chemistry and trace metal analysis. Samples were collected with a peristaltic pump and passed through a $5\mu\text{m}$ pre-filter, followed by a $0.45\mu\text{m}$ PES (Supor) filter. Samples for carbonate chemistry were collected in 40mL glass vials with no headspace and immediately poisoned with 20 μL of saturated mercuric chloride (HgCl_2). Samples for nutrient analysis were collected in precleaned 125mL plastic bottles and immediately frozen. Trace metal samples were collected in acid cleansed plastic bottles and spiked with 625 μL of trace metal grade concentrated HCl. Trace metals were analyzed using a NexION 2000P ICP-MS instrument (Perkin Elmer) with a SC-DX SeaFAST S2 (Elemental Scientific) autosampler following the protocol of Lagerstrom et al. (2013). Nutrient samples were analyzed via automated flow injection and the precision of nitrite was $0.63\text{--}3.88\ \mu\text{mol L}^{-1}$, nitrate+nitrite was $1.06\text{--}3.01\ \mu\text{mol L}^{-1}$, ammonia was $0.98\text{--}2.50\ \mu\text{mol L}^{-1}$, and silica was $0.24\text{--}2.82\ \mu\text{mol L}^{-1}$. pH (total scale) was measured immediately after sample collection and was determined spectrophotometrically using the meta-cresol purple (mCP) indicator dye method (Clayton and Byrne 1993). Total alkalinity was determined spectrophotometrically using the bromocresol green method (Breland and Byrne 1993) with a precision of $\pm 8\text{--}9\ \mu\text{mol/kg}$.

Statistical analysis

The two types of seagrass response data: repeated baseline metrics (shoot density, morphometrics, Fv/Fm) and one-time treatment responses (leaf productivity, meristem density, above/belowground biomass, leaf elemental and isotopic composition) were analyzed with separate statistical approaches. Repeated metrics were analyzed with generalized linear mixed effects models (GLMMs) using the glmmTMB package in R with either a gaussian (shoot density / leaf morphometrics) or beta (quantum yield) error distribution. The model structure followed a BACI (before-after control-intervention) framework and included the fixed effects of treatment (+olivine, +sand, control), period (pre-, post-olivine/sand addition), and their interaction. Thus, a significant treatment $\times$ period interaction suggests that any treatment differences were altered by either olivine or sand addition. We also included ‘tank’ and ‘week’ as random factors in the model. Because most repeated metrics were continuously changing throughout the experiment (as plants acclimated and expanded), some data (shoot density and leaf morphometrics) were de-trended prior to analysis to assist with model validation (using the DHARMA package). Thus, statistical analysis was conducted on weekly deviations as opposed to absolute values for those metrics. Seagrass species were analyzed separately. End-experiment metrics were analyzed with a non-parametric Kruskal- Wallis test and post hoc differences were assessed with a Dunn’s multiple comparison test.

To assess the effects of treatment, biomass type, and their interaction on tissue trace metal content, nonparametric factorial analysis via the Aligned Rank Transform (ART) method in R was used (ARTool package; Kay & Wobbrock, 2020). This approach allows for the testing of main and interaction effects in nonparametric datasets. When significant effects were found, estimated marginal means (EMMs) and pairwise post hoc comparisons were computed using the emmeans package with Holm adjustment. For Cu and Zn in *S. filiforme*, a two-way ANOVA was

applied with treatment and biomass as fixed factors. Significant effects were further examined using Tukey's HSD post hoc test. To assess the effects of treatment, period, and their interaction on CO₂ flux, a similar nonparametric analysis via ART was applied, and pairwise post hoc comparisons were computed using contrast tests with Holm adjustment (Kay & Wobbrock, 2020). Seawater carbonate chemistry and nutrient / trace metal concentrations are reported as a time series with means $\pm$ SE.

Results

Seagrass shoot density, leaf morphology and photophysiology

For the baseline metrics of shoot density, leaf morphology and photophysiology, seagrass response to +olivine / +sand was minimal for both *T. testudinum* and *S. filiforme* (Table 1). There was no treatment $\times$ period interaction for trends in shoot density, leaf length, leaf width, or photosynthetic quantum yield (Figs. 1 and 2), indicating that olivine / sand did not cause any differences in plant morphometrics among treatments. Furthermore, the main factor of treatment was not significantly different for any baseline metric. There was a period effect for *T. testudinum* leaf length ($p < 0.0001$), indicating that for all treatments leaf length initially decreased and then increased after the start of the treatments (Fig. 1). Period was also significant ($p = 0.0001$) for *S. filiforme* quantum yield (F_v/F_m), indicating that yields were slightly higher after treatment start (Fig. 2).

Seagrass leaf productivity, biomass and elemental content

Treatment responses of leaf productivity, above-belowground biomass, and isotopic / elemental content were also minimal for both species. There were no treatment differences in absolute or relative leaf productivity (*T. testudinum* only, Fig. 3), active meristems (both species, Fig. S2) or belowground biomass (both species, Table 2). Neither were there any differences in

leaf C, leaf N, leaf P, leaf $\delta^{13}\text{C}$, or leaf $\delta^{15}\text{N}$ (Table 2). However, for *T. testudinum*, aboveground biomass was higher in +sand relative to +olivine (Dunn's posthoc, $p=0.03$, Fig. 4), but neither of these treatments were different from the controls ($p>0.05$). For *S. filiforme*, there were no differences in aboveground biomass across treatments.

Seagrass biomass trace metal content

Seagrass trace metal content varied by species, tissue type (above- vs. below-ground), and treatment (Fig. 5). Nickel showed the strongest response, with effects of treatment, biomass type and their interaction ($p<0.05$, Table S1, S2). In *S. filiforme*, Ni concentrations were higher in +olivine (aboveground: $20.2 \pm 8.6 \mu\text{g g dw}^{-1}$; belowground $10.2 \pm 3.6 \mu\text{g g dw}^{-1}$) as compared to the other treatments ($0.6\text{--}2.1 \mu\text{g g dw}^{-1}$ range for above and below-ground biomass, combined). Similarly, *T. testudinum* accumulated markedly more Ni in +olivine, reaching $78.9 \pm 15.0 \mu\text{g g dw}^{-1}$ in above-ground tissues and $18.9 \pm 11.6 \mu\text{g g dw}^{-1}$ in the below-ground tissues as compared to other treatments ($1.3\text{--}3.7 \mu\text{g g dw}^{-1}$ range for above and below-ground biomass, combined). Cr also increased with +olivine, particularly in below-ground tissues for both species, but variability was high (Fig. 5, Table S1 and S2). Cobalt concentrations were generally low in *S. filiforme* but substantially higher in *T. testudinum*, especially in above-ground tissues under control and +sand ($>30 \mu\text{g g dw}^{-1}$), with no treatment effect ($p>0.05$, Table S1). Other metals were unaltered by +olivine ($p>0.05$, Table S1), except for Fe and Mn in *T. testudinum* which were elevated with +olivine compared to other treatments ($p<0.05$, Table S1, Figs. S3-S4).

CO₂ flux measurements

There was no treatment $\times$ period interaction for water-air CO₂ flux (Figs. 6 and S9), indicating that differences in CO₂ flux pre- / post olivine-sand addition were similar across treatments. However, the main factor of treatment was significant ($p=0.02$), and post-hoc

analysis revealed higher CO₂ flux in the control treatment compared to +sand, but +olivine was not different from either other treatment (Fig. 6). Furthermore, there was a period effect ($p < 0.001$), indicating that CO₂ fluxes were generally lower after sand/olivine addition, even for the control tanks.

Water column chemistry

Nutrients- Pretreatment dissolved nutrient concentrations were comparably low (Fig. S5). Nitrite concentrations ranged from $0.0011 \pm 0.0003 \text{ mg L}^{-1}$ (control) to $0.0039 \pm 0.0025 \text{ mg L}^{-1}$ (+sand) and increased following treatment initiation in the control ($0.0049 \pm 0.0032 \text{ mg L}^{-1}$) and +olivine ($0.0051 \pm 0.0026 \text{ mg L}^{-1}$) tanks, while remaining stable in +sand ($0.0039 \pm 0.0039 \text{ mg L}^{-1}$). Combined nitrite-nitrate concentrations were low and variable, with no consistent directional change following treatment initiation. Ammonium concentrations remained stable in the control and +olivine treatments, while +sand was elevated after addition ($0.0460 \pm 0.2085 \text{ mg L}^{-1}$), however with high variability. The most pronounced response was observed for dissolved Si. Before addition, Si concentrations were uniformly low and indistinguishable across treatments, ranging from 0.031 ± 0.016 to $0.036 \pm 0.040 \text{ mg L}^{-1}$. Following olivine addition, Si increased approximately 15-fold to $0.519 \pm 0.342 \text{ mg L}^{-1}$, a pattern generally consistent with olivine dissolution, whereas the control ($0.038 \pm 0.017 \text{ mg L}^{-1}$) and +sand ($0.061 \pm 0.102 \text{ mg L}^{-1}$) treatments remained at pre-addition levels.

Trace metals - Pretreatment seawater trace metal concentrations were similar across all tanks, indicating comparable baseline conditions (Figs. S6-7). Following treatment initiation, the most prominent treatment-specific divergences were observed for Ni, Mn, and Co with +olivine. Ni concentrations increased following olivine addition, rising from $0.623 \pm 0.108 \text{ } \mu\text{g kg}^{-1}$ to $28.25 \pm 7.66 \text{ } \mu\text{g kg}^{-1}$, a 45-fold increase, while remaining stable in the control (0.519 ± 0.095 to

0.496 ± 0.102 µg kg⁻¹) and +sand (0.541 ± 0.060 to 0.639 ± 0.245 µg kg⁻¹) treatments. Mn followed a similar pattern, increasing from 0.542 ± 0.162 µg kg⁻¹ to 10.14 ± 6.48 µg kg⁻¹ with +olivine, with no change in the control or +sand treatments. Cobalt also doubled following olivine addition (0.397 ± 0.127 to 0.778 ± 0.341 µg kg⁻¹), consistent with co-dissolution of these elements from the olivine mineral matrix. Iron showed high variability with no clear treatment-specific trend. Zinc, Cu, and Pb concentrations remained relatively stable and did not differ substantially between treatments or time periods. Cd, Mo, and U declined across all three treatments following addition, suggesting a temporal or seasonal signal independent of treatment. Cd decreased from approximately 0.021 ± 0.004 µg kg⁻¹ to 0.014 ± 0.005 µg kg⁻¹ across all treatments, Mo declined from 6.47 ± 1.59 µg kg⁻¹ to 5.39 ± 1.37 µg kg⁻¹, and U decreased from 4.08 ± 0.34 µg kg⁻¹ to 2.63 ± 0.65 µg kg⁻¹, with control and +sand treatments following the same trajectory as +olivine, indicating these declines were not driven by mineral addition.

Carbonate chemistry - After starting the treatments, total alkalinity ranged from a minimum of 2199 ± 42 µmol kg⁻¹ in +sand to 2333 ± 56 µmol kg⁻¹ in +olivine (Fig. S8). pH_T ranged from 7.99 ± 0.015 in the +olivine to 8.13 ± 0.019 in the +sand. pCO₂ ranged from 309.5 µatm in the +sand to 474.5 µatm in the no seagrass, + olivine treatment. Finally, DIC ranged from 1832 ± 42 µmol kg⁻¹ in the +sand treatment to 2035 ± 50 µmol kg⁻¹ in the no seagrass, + olivine treatment.

Discussion

Olivine addition had minimal effects on the structure and function of two dominant seagrasses in the tropical Western Atlantic. Changes in baseline and treatment metrics were relatively unaltered after the 110d exposure. However, we note some evidence that leaf biomass decreased in the olivine treatment (relative to +sand), only for *T. testudinum*. Furthermore,

olivine addition increased tissue metal concentrations, primarily Ni and Mn. Overall, our work shows that while seagrass structure and function may be minimally affected by olivine, increases in tissue metal concentrations were evident and may pose a concern for field applications.

Olivine addition could impact seagrasses via the sudden application of several centimeters of fine-grained sand, which could bury photosynthetic biomass or alter levels of sediment anoxia. Our results demonstrate that for modest applications (1cm layer), structural / functional effects are minor. Leaf productivity, F_v/f_m , leaf $\delta^{13}C$ and CO_2 drawdown were unaltered by treatment, suggesting that photosynthetic impairment or excessive leaf burial was not evident. Furthermore, shoot density, leaf length and leaf width were also unaltered by treatment. While there were shifts in leaf length for *T. testudinum*, this was reflected across all treatments and likely represented residual acclimation to tank conditions (potentially reflected as an initial response to mesocosm rooting depth, which may have been shallower than the rooting depth at the collection site). After development of belowground biomass (~week 30, Fig. 1), *T. testudinum* leaf length increased. Seagrasses commonly cope with burial events from storms, barrier island migration or burrowing animals and can display plastic responses (changes in aboveground canopy architecture and sheath length) to mitigate any negative effects (Cabaco et al 2008, Marba et al 1994, Marba and Duarte 1994). We examined responses from two large-bodied species, which are generally more tolerant of minor burial events (Cabaco et al 2008). Smaller-bodied species (e.g., *Halophila*) may be more affected, as prior work has demonstrated that 2 cm of burial can induce 50% shoot mortality for smaller species and nearly 100% mortality for *H. ovalis* (Cabaco et al 2008). Burial thresholds are positively correlated with shoot mass and rhizome diameter (Cabaco et al 2008); thus, these metrics may be useful in future assessments of how olivine application may affect specific meadows.

The strongest response to olivine was an increase in tissue trace metal concentrations. Seagrasses can accumulate trace metals via leaf and root uptake (Prange & Dennison 2000); thus, metal content and the associated toxicity of olivine may directly affect seagrass functioning and the food webs they support. In our experiment, tissue Ni content was most consistently affected by olivine; thus, we primarily focus our discussion on this element. Nickel is an essential micronutrient; however, prior work has demonstrated that excessive amounts can lead to plant chlorosis, necrosis and other negative effects such as impaired seedling growth and reduced photosynthetic rates (Hassan et al., 2019). In our tanks, seawater Ni concentrations increased to 30-40 $\mu\text{g kg}^{-1}$ with olivine, which is beyond chronic exposure limits (8.2 $\mu\text{g kg}^{-1}$) established for aquatic life by the US Environmental Protection Agency. Accordingly, seagrass tissue Ni concentrations increased to 78.9 and 10.2 $\mu\text{g g}^{-1}$ DW for *T. testudinum* and *S. filiforme*, respectively. Field reports of seagrass tissue Ni concentrations range from <10 $\mu\text{g g}^{-1}$ DW for the seagrass *Cymodocea nodosa* (Malea & Kevrekidis 2013) to 77.3 $\mu\text{g g}^{-1}$ DW for *C. nodosa* at polluted sites near ore smelting plants (Nicolaidou & Nott, 1989). Studies on the physiological effects of Ni toxicity in seagrasses are limited; however, work has demonstrated acute damage to subcellular components (actin filaments and endoplasmic reticula) in *Halophila stipulacea*, even at relatively low exposures (8-14 $\mu\text{g g}^{-1}$ DW)(Malea et al 2021). Oxidative stress and epidermal cell death have also been documented in *H. stipulacea* (Malea et al 2021). While our work made no assessments of ultracellular functioning or structure, we did assess higher-order processes (e.g., photosynthetic functioning, leaf productivity) which integrate across multiple facets of cellular function. In contrast with prior work, we found limited effects of metal toxicity. Photosynthetic yield, shoot density, leaf morphometrics, leaf productivity (*T. testudinum* only), CO_2 drawdown, belowground biomass and elemental content were all unaffected. It is important

to note that we report total seagrass tissue Ni concentrations and do not differentiate between extracellular Ni (externally bound to cell walls) and intracellular Ni concentrations (uptake and accumulation into the protoplasm). Thus, the limited effects of trace metal toxicity detected in our experiment may result from the majority of metal ions being externally bound, as previously documented in other studies (Malea et al 2021). Seagrasses also possess mechanisms of metal detoxification, thus any Ni incorporated intracellularly may have further been detoxified via complexation with organic molecules or compartmentalization within vacuoles (Hall 2002, Li et al 2023). However, it is important to note that the current experiment was conducted in a closed-tank system with limited water exchanges. Under natural conditions of mERW-based olivine application, a substantial proportion of dissolved olivine products would likely be diluted by tidal and wind-driven flushing. Therefore, these findings should be interpreted with caution, as previous studies have highlighted that laboratory tank experiments may not accurately reflect responses observed under natural field conditions (Jankowska et al., 2026). Future experiments should explore the impacts of olivine on seagrasses under open-system conditions where the overlying seawater is continually renewed.

Olivine also increased Mn, Fe and Cr tissue concentrations. However, these effects were idiosyncratic (evident for either a single seagrass species or biomass compartment). While Mn and Fe are considered essential micronutrients for seagrass functioning (involved in photosystem complexes and chlorophyll synthesis), Cr is not. Tissue Mn concentrations increased 10-fold with olivine as compared to field reported values for seagrasses along the Mexican Caribbean coast (Solis et al 2008). Meanwhile, tissue Fe and Cr concentrations were similar in range to prior field reports (Govers et al 2014), thus the magnitude of olivine effects were minimal for these elements. Interestingly, no olivine-associated Co tissue accumulation was observed, despite

increases in water column concentrations and prior studies reporting bioaccumulation in marine invertebrates exposed to olivine (Flipkens et al., 2023). This suggests that seagrass may preferentially accumulate essential trace metals, such as Mn and Fe, over non-essential metals such as Co. Overall, despite increases in tissue concentrations for certain elements (primarily Ni and Mn), shifts in seagrass physiology or structure were largely undetected. We do note that there was a significant effect of treatment on leaf biomass (for *T. testudinum* only) at the end of the experiment, which was predominantly driven by increases in the sand treatment. While the cause of this response is unclear, we do have some evidence that leaf biomass was already higher in the sand treatment even before treatment initiation in early-October (Fig. S10). Thus, this response may have been due to factors unrelated to olivine/sand addition.

It was surprising that we did not detect strong increases in air-water CO₂ flux with olivine addition (Fig. 6), however our intent was to primarily use these measurements as a means of assessing photosynthetic activity and not geochemical changes in water chemistry. It is possible that the large biological-driven changes in CO₂ fluxes obscured any signal of CO₂ uptake that was solely attributable to olivine addition. Other studies using batch reaction experiments (seawater-olivine incubations, no biological component) have demonstrated both alkalinity increases and CO₂ drawdown with olivine addition.

Marine enhanced rock weathering (mERW) has been proposed as one potential mechanism for increasing the CO₂ uptake capacity of the coastal oceans, thus serving as a strategy to mitigate the worst effects of global warming and climate change. However, scaling-up such an approach first requires the proper assessment of any biological-ecological risks associated with deployment. Given widespread seagrass presence across the coastal zone, we examined the ecotoxicological effects of olivine addition on the functioning of two common

seagrasses in the Western Atlantic. While overall effects were minimal, increases in tissue metal concentrations (Ni, Mn, Fe, Cr) were evident. As prior work has highlighted concern regarding the effects of metals in aquatic and marine environments (Schneider et al 2018), the transfer of olivine-sourced metals throughout coastal food webs warrants further evaluation, particularly for large-scale field deployments.

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Table 1 - Effects of olivine addition on seagrass shoot density, leaf morphometrics and photosynthetic yield. Results from generalized linear mixed-effects models.

Response	Factor	Chisq	Df	Pr(>Chisq)
<i>T. testudinum</i>				
Shoot density (# tank ⁻¹)	Treatment	0.2216	2	0.8951
	Period	0.1595	1	0.6896
	Treatment:Period	0.4011	2	0.8183
Leaf length (mm)	Treatment	0.7387	2	0.6912
	Period	24.5939	1	<0.0001
	Treatment:Period	1.0258	2	0.5988
Leaf width (mm)	Treatment	0.0632	2	0.9689
	Period	0.7737	1	0.3791
	Treatment:Period	0.0168	2	0.9916
Photosynthetic yield (Fv/Fm)	Treatment	0.341	2	0.8433
	Period	0.6942	1	0.4047
	Treatment:Period	0.8865	2	0.6420
<i>S. filiforme</i>				
Shoot density (# tank ⁻¹)	Treatment	2.4883	2	0.2882
	Period	0.9425	1	0.3316
	Treatment:Period	0.6962	2	0.706
Leaf length (mm)	Treatment	1.2323	2	0.5400
	Period	0.0038	1	0.9512
	Treatment:Period	2.1901	2	0.3345
Photosynthetic yield (Fv/Fm)	Treatment	2.8684	2	0.2383
	Period	14.8295	1	0.0001
	Treatment:Period	2.6714	2	0.2629

Table 2- Effects of olivine addition on seagrass leaf productivity, biomass and elemental content. Results from a non-parametric Kruskal-Wallis test.

Response	Chisq	Df	Pr(>Chisq)
<i>T. testudinum</i>			
Leaf productivity, Absolute (mg shoot ⁻¹ d ⁻¹)	2.34	2	0.3104
Leaf productivity, Relative (mg g ⁻¹ d ⁻¹)	0.72	2	0.6977
Active meristems (# tank ⁻¹)	5.51	2	0.0635
Standing crop (g tank ⁻¹)	6.48	2	0.0392
Belowground biomass (g tank ⁻¹)	1.86	2	0.3946
Leaf %C (dry mass basis)	4.94	2	0.0845
Leaf %N (dry mass basis)	4.58	2	0.1013
Leaf %P (dry mass basis)	4.11	2	0.1276
Leaf δ ¹³ C (dry mass basis)	1.68	2	0.4317
Leaf δ ¹⁵ N (dry mass basis)	4.34	2	0.1142
<i>S. filiforme</i>			
Active meristems (# tank ⁻¹)	0.84	2	0.6579
Standing crop (g tank ⁻¹)	1.68	2	0.4317
Belowground biomass (g tank ⁻¹)	2.94	2	0.2299
Leaf %C (dry mass basis)	5.12	2	0.0771
Leaf %N (dry mass basis)	5.22	2	0.0733
Leaf %P (dry mass basis)	3.207	2	0.2011
Leaf δ ¹³ C (dry mass basis)	0.39	2	0.8246
Leaf δ ¹⁵ N (dry mass basis)	4.06	2	0.1311

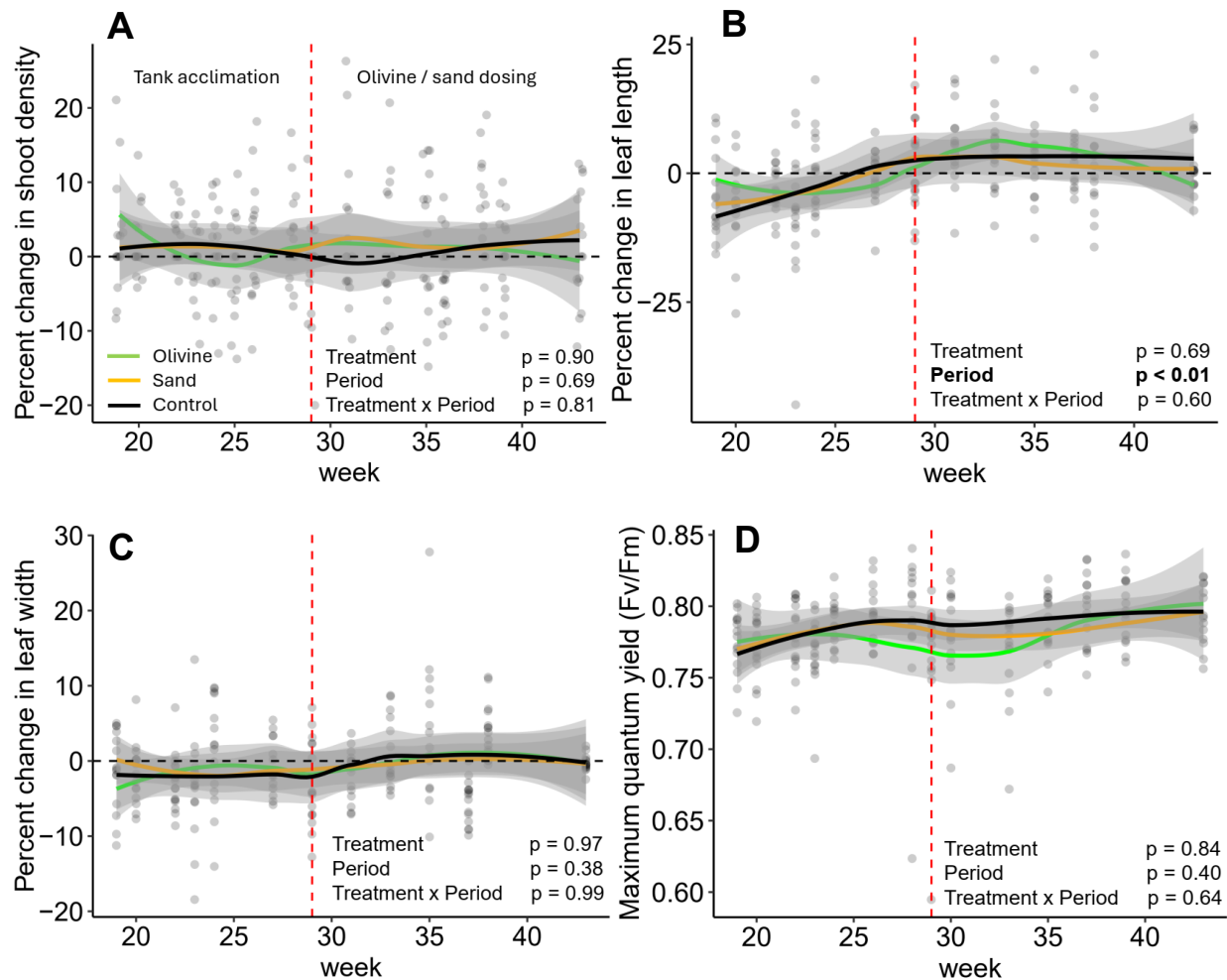


Figure 1. *Thalassia testudinum* responses to olivine addition. (A) Weekly percent change in shoot density. (B) Weekly percent change in leaf length. (C) Weekly percent change in leaf width. (D) Maximum quantum yield (Fv/Fm). Dashed red line indicates the week of olivine / sand- addition. Jittered points represent individual tanks and colored lines represent locally weighed smoothers of treatment means. Statistical results of generalized linear mixed effects models are shown.

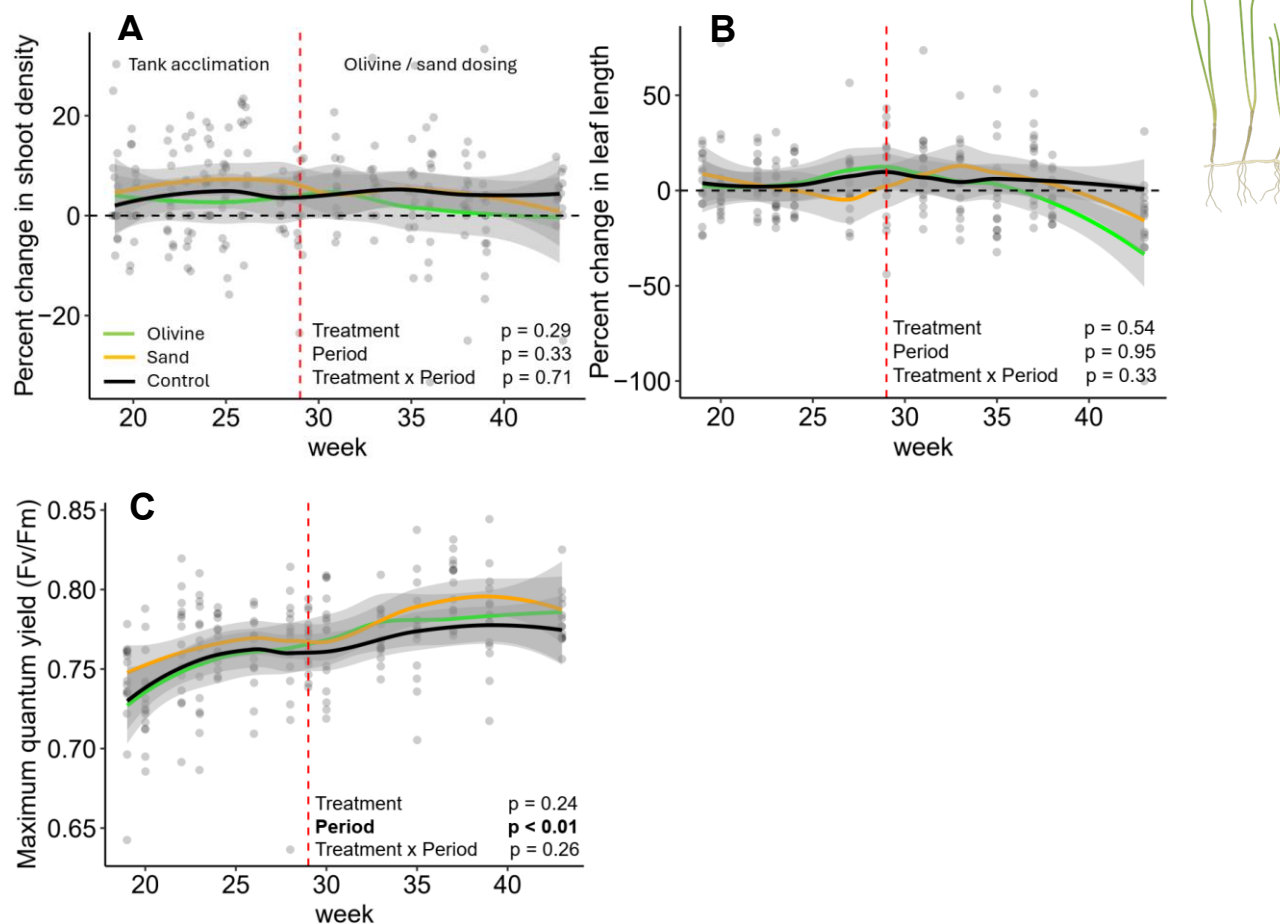


Figure 2. *Syringodium filiforme* responses to olivine addition. (A) Weekly percent change in shoot density. (B) Weekly percent change in leaf length. (C) Maximum quantum yield (Fv/Fm). Dashed red line indicates the week of olivine / sand addition. Jittered points represent individual tanks and colored lines represent locally weighed smoothers of treatment means. Statistical results of generalized linear mixed effects models are shown.

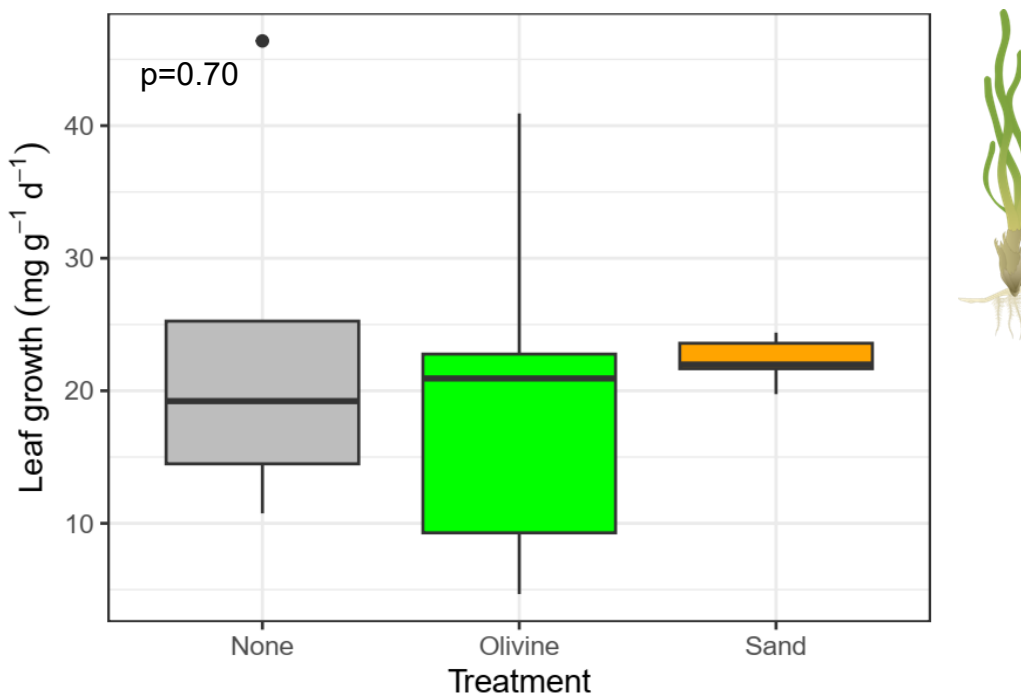
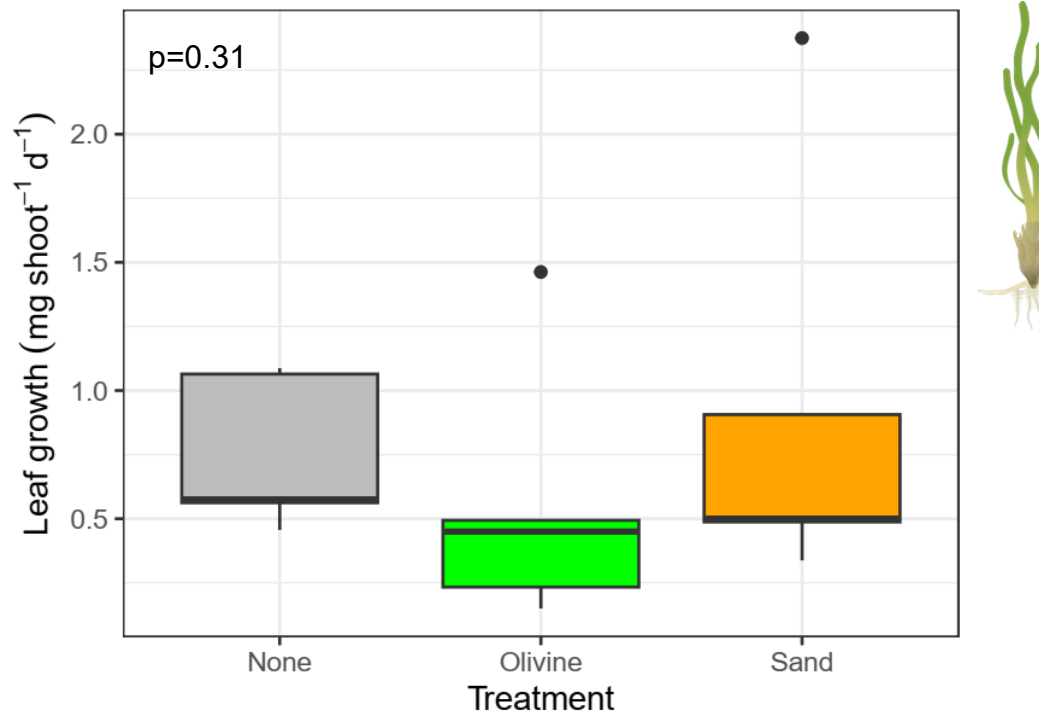


Figure 3. Treatment effects on *Thalassia testudinum* absolute (mg shoot⁻¹ d⁻¹) and relative (mg g⁻¹ d⁻¹) leaf productivity.

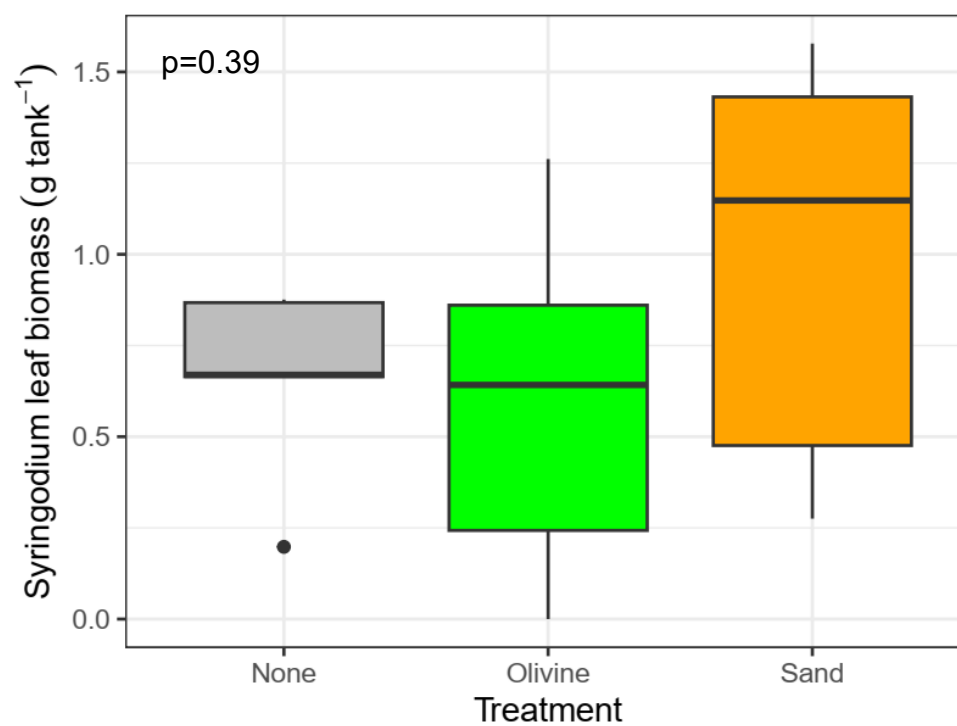
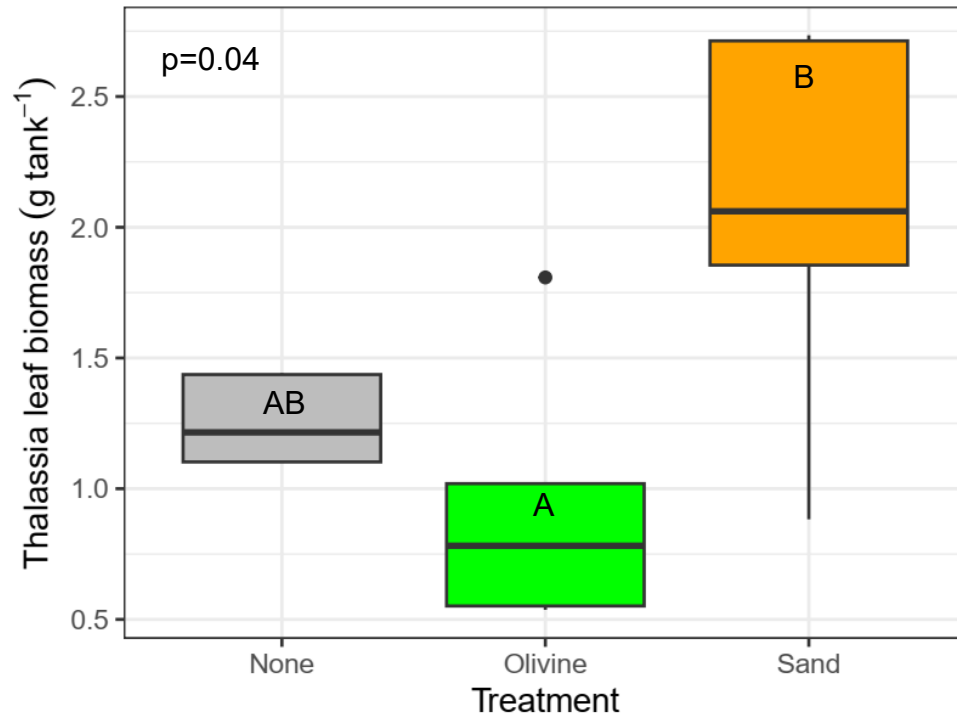


Figure 4. Treatment effects on *Thalassia testudinum* and *Syringodium filiforme* leaf biomass (g tank⁻¹).

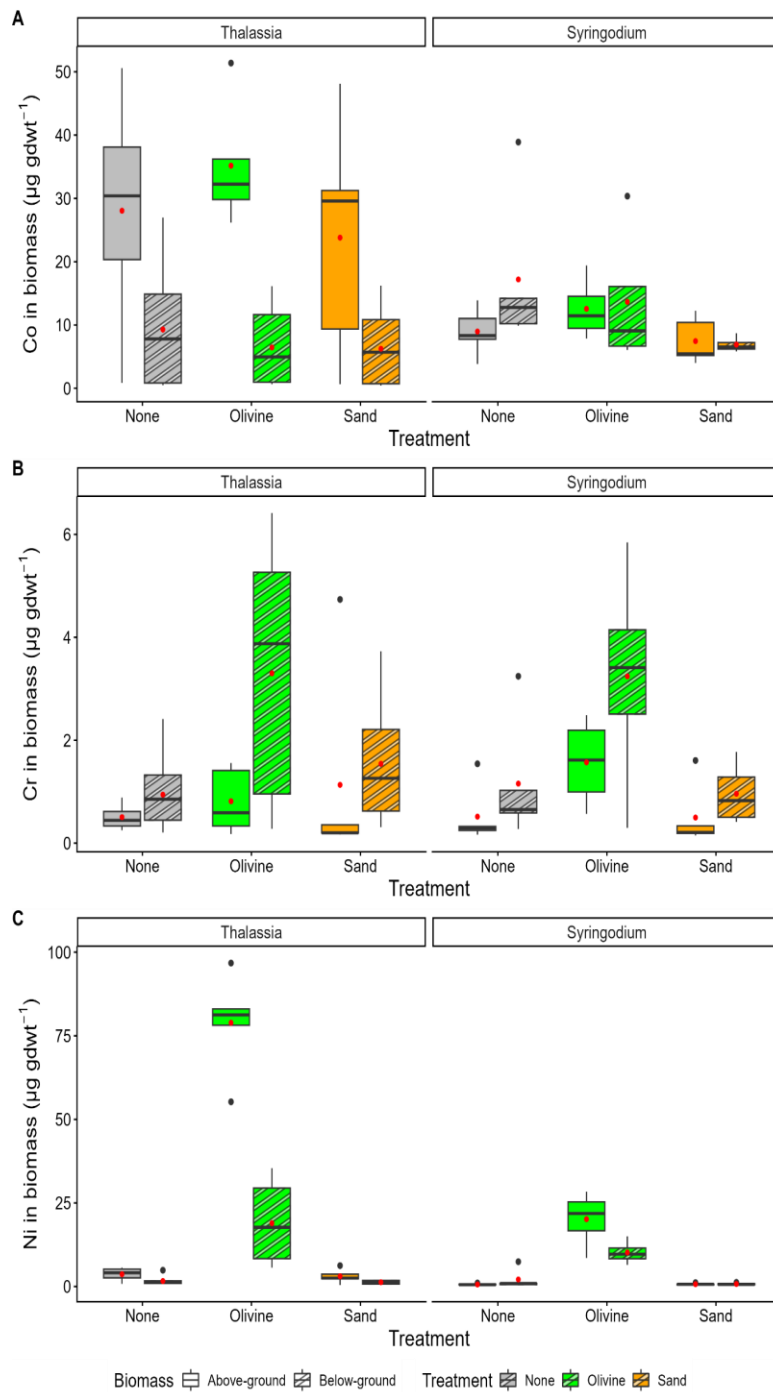


Figure 5 Seagrass biomass metal concentration ($\mu\text{g g dw}^{-1}$) measured at the end of the experiment: **(A)** Co, **(B)** Cr, and **(C)** Ni. Mean (red dot), median (horizontal line), 25th and 75th percentile (box), minimum and maximum value (vertical line), and outliers (black dot) are presented.

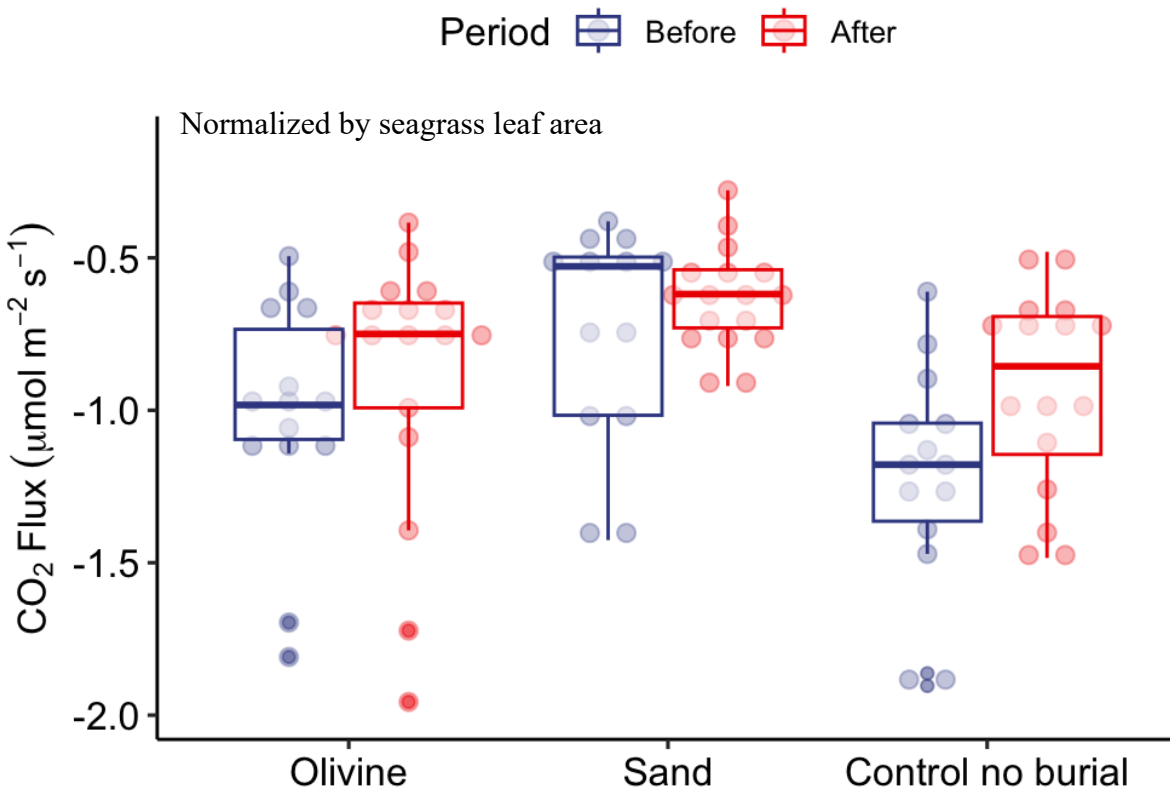


Figure 6. Water-air flux of CO₂ measured in the tank head space, normalized by seagrass leaf area ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Points are individual flux measurements, point and box colors indicate measurement period (before or after olivine/sand addition). Mean (bold line), 25th and 75th percentile (box), minimum and maximum value (vertical line), and outliers (filled dots) are presented.

Supporting information for

Table S1 Results of two-way ArtANOVA for metal concentration in two seagrass species among Treatments, Biomass type, and their interaction. P values provided, with significant tests marked with bold font and an asterisk * showing significance at $p < 0.05$, ** significance at $p < 0.01$, and *** significance at $p < 0.001$. Only significant results for pair-wise tests are provided.

	Thalassia			Syringodium		
Factors	df	F value	p	df	F value	p
Ni						
Treatment	2	35.973	1.3998e-09 ***	2	26.78	1.6678e-06 ***
Biomass type	2	49.298	2.0040e-08 ***	1	8.06	0.00984104 **
Treatment: Biomass type	4	56.549	2.9809e-12 ***	2	10.55	0.00067381 ***
Pair-wise	All biomass types – O ≠ S, CNB All treatments – AGB ≠ BGB			AG biomass – O ≠ CNB BG biomass – O ≠ S, CNB All treatments – AGB ≠ BGB		
Co						
Treatment	2	0.53109	0.59216	2	2.83	0.081358
Biomass type	2	27.36911	6.0171e-06 ***	1	0.61	0.446775
Treatment: Biomass type	4	0.79907	0.45697	2	1.06	0.366303
Pair-wise	AGB ≠ BGB					
Cr						
Treatment	2	2.6572	0.0828190	2	4.23	0.028468 *

Biomass type	2	7.5630	0.0089851 **	1	6.65	0.017546 *
Treatment: Biomass type	4	2.0531	0.1419859	2	1.18	0.327091
Pair-wise	AGB $\neq$ BGB			-		

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Factors	df	F value	p	df	F value	p
Cu						
Treatment	2	0.44832	0.641952	2	0.55	0.5871
Biomass type	2	28.021	4.9415e-06 ***	1	3.80	0.0646
Treatment: Biomass type	4	2.61830	0.085707	2	0.50	0.6126
Pair-wise	AGB ≠ BGB					
Fe						
Treatment	2	9.3128	0.00049404 ***	2	1.60	0.2255227
Biomass type	2	31.4169	1.8307e-06 ***	1	10.15	0.0044459 **
Treatment: Biomass type	4	9.2791	0.00050543 ***	2	1.63	0.2202759
Pair-wise	All treatments – AGB ≠ BGB AGB – O ≠ CNB, S			-		
Mn						
Treatment	2	11.115	0.00015132 ***	2	4.23	0.028468 *
Biomass type	2	35.741	5.5500e-07 ***	1	6.65	0.017546 *

Treatment: Biomass type	4	69.675	1.3364e-13 ***	2	1.18	0.327091
Pair-wise	All treatments – AGB ≠ BGB AGB – O ≠ CNB, S BGB – O ≠ CNB, S, CNB ≠ S			-		
Pb						
Treatment	2	0.15323	0.85845	2	0.79	0.46466
Biomass type	2	2.27324	0.13968	1	0.25	0.62377
Treatment: Biomass type	4	0.58115	0.56403	2	0.29	0.75111
As						
Treatment	2	1.084974	0.3478912	2	1.43	0.26171
Biomass type	2	9.830706	0.0032571 **	1	31.52	1.4272e-05 ***
Treatment: Biomass type	4	0.062659	0.9393584	2	2.15	0.14170
Pair-wise	AGB ≠ BGB			-		
P						
Treatment	2	0.86955	0.4271058	2	0.5460	0.58728
Biomass type	2	8.31640	0.0063647 **	1	4.7633	0.04057 *
Treatment: Biomass type	4	1.97626	0.1522236	2	0.3646	0.69880
	AGB ≠ BGB			-		

Table S2. Mean $\pm$ SD trace metal concentrations in different seagrass biomass fractions ($\mu\text{g g dw}^{-1}$) measured at the end of the experiment in two species and three treatments.

Treatment	Species	Biomass	Ni ($\mu\text{g g dw}^{-1}$)		Cr ($\mu\text{g g dw}^{-1}$)		Co ($\mu\text{g g dw}^{-1}$)		Cu ($\mu\text{g g dw}^{-1}$)		Fe ($\mu\text{g g dw}^{-1}$)		Mn ($\mu\text{g g dw}^{-1}$)		Pb ($\mu\text{g g dw}^{-1}$)		As ($\mu\text{g g dw}^{-1}$)		P ($\mu\text{g g dw}^{-1}$)	
			mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Control no burial	Syringodium	Above-ground	0.62	0.29	0.52	0.58	8.97	3.76	11.31	3.64	158.86	172.04	4.60	1.49	2.37	2.00	0.11	0.25	733.32	277.88
Control no burial	Syringodium	Below-ground	2.11	2.98	1.16	1.20	17.19	12.26	17.50	8.58	358.74	183.73	15.12	29.70	2.35	1.72	0.58	0.20	608.11	191.56
Olivine	Syringodium	Above-ground	20.16	8.56	1.57	0.88	12.55	5.01	11.06	3.18	106.83	27.50	37.65	9.43	3.95	3.14	0.00	0.00	734.88	111.49
Olivine	Syringodium	Below-ground	10.21	3.59	3.24	2.28	13.65	11.38	13.29	4.97	341.24	186.86	9.66	8.83	1.88	0.59	0.42	0.14	621.40	256.05
Sand	Syringodium	Above-ground	0.71	0.28	0.50	0.62	7.45	3.63	11.55	1.09	124.45	75.90	8.69	2.29	2.09	2.39	0.15	0.14	704.75	124.90
Sand	Syringodium	Below-ground	0.78	0.32	0.96	0.62	6.90	1.26	13.81	4.28	239.45	49.02	2.44	0.69	1.81	1.44	0.34	0.08	447.62	139.15
Control no burial	Thalassia	Above-ground	3.69	2.18	0.51	0.28	28.05	20.68	11.03	1.93	112.34	16.10	16.97	10.85	1.89	1.12	0.43	0.12	1024.91	225.05
Control no burial	Thalassia	Below-ground	1.65	1.23	0.94	0.68	9.29	9.29	21.98	8.83	166.21	38.48	6.18	12.03	2.80	1.35	0.65	0.31	830.78	218.69
Olivine	Thalassia	Above-ground	78.89	15.01	0.81	0.63	35.17	9.77	7.22	1.61	137.14	30.76	228.78	98.64	1.97	0.49	0.49	0.14	1108.54	134.63
Olivine	Thalassia	Below-ground	18.93	11.61	3.30	2.37	6.45	6.04	23.37	5.95	413.75	210.26	6.64	3.85	2.75	1.29	0.75	0.20	669.13	166.07
Sand	Thalassia	Above-ground	3.06	2.14	1.13	2.02	23.80	18.88	12.62	8.08	133.60	30.03	19.78	16.19	2.34	0.99	0.61	0.20	793.64	292.14
Sand	Thalassia	Below-ground	1.29	0.53	1.54	1.12	6.24	6.50	18.42	6.64	203.08	72.62	23.80	71.67	2.50	1.17	0.78	0.34	799.14	440.49

Table S3. Geochemical analysis of the source olivine (Sibelco AFS35) used in the experiment.

Data Type (Source)		Sibelco AFS35
Mineralogy (wt%)	Olivine (Mg92)	85.0
Q-XRD (Qmineral)	Orthopyroxene	6.7
	Chlorite	5.2
	Mica	0.0
	Talc	0.9
	Serpentine (Antigorite)	2.2
Major Elements (wt %)	MgO	50.20
XRF (Sibelco)	SiO ₂	41.40
	Al ₂ O ₃	0.42
	MnO	0.09
	Fe ₂ O ₃ (T)	7.40
	Loss on Ignition (LOI)	0.60
Trace Elements (ppm)	Cr	1779
ICPMS and XRF (Sibelco and ASU)	Ni	2515
	Co	100
Total Carbon (TC, wt%)		0.02
Organic Carbon (OC, wt%)		BDL
Inorganic Carbon (IC, wt%, by difference)		0.02
Grain Size (µm)	d10	324
Laser Particle Sizer (Qmineral)	d50	528
	d90	833
Specific Surface Area (BET, m ² /g) (Qmineral)		0.245
Specific Gravity (g/cm ³ , Sibelco)		3.25
Bulk Density (g/cm ³ , Sibelco)		1.7

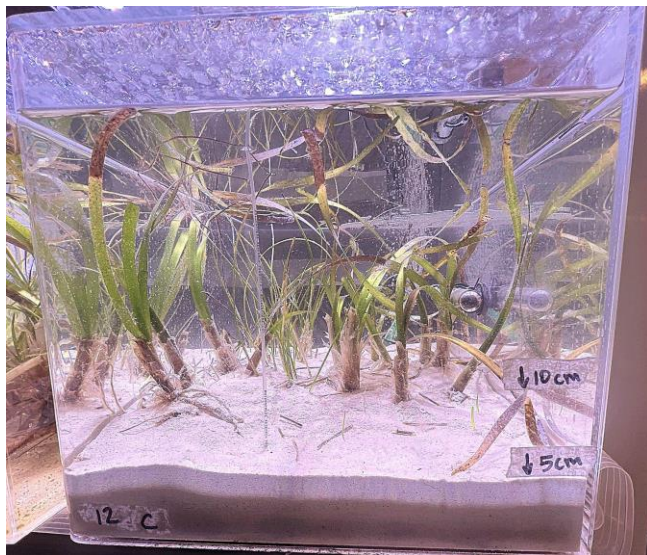
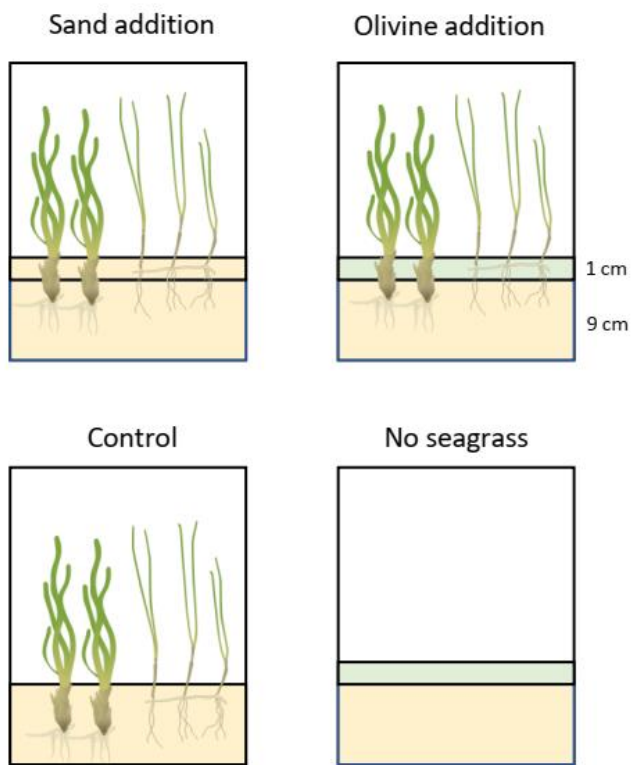


Figure S1: Visual depiction of treatments and photograph of an experimental tank during planting

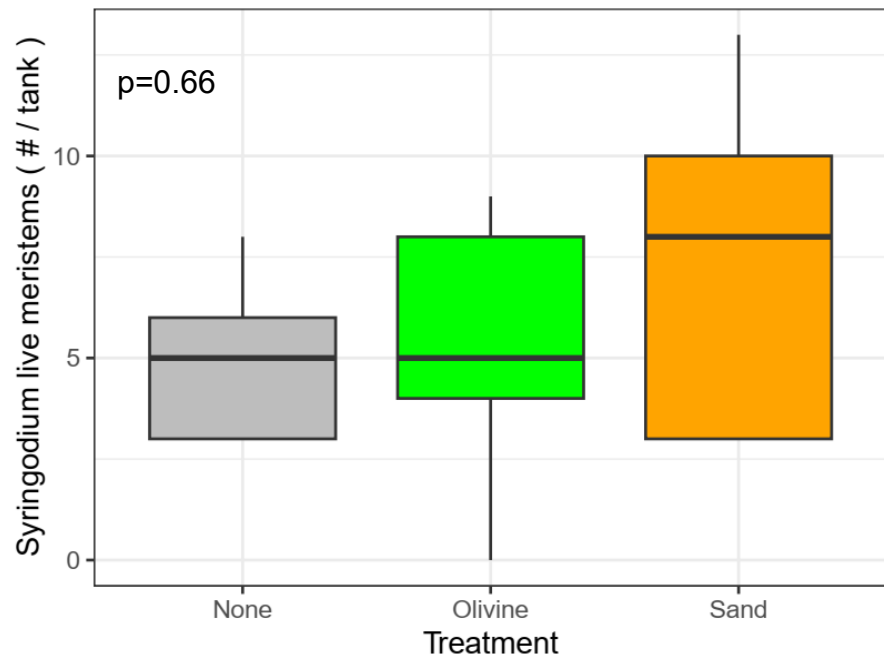
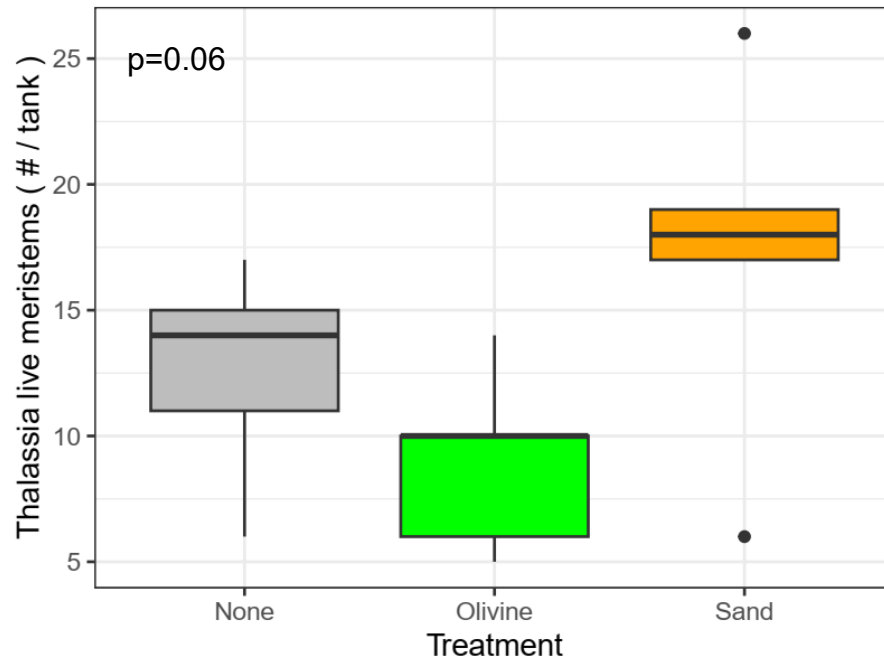


Figure S2: Treatment effects on number of active meristems

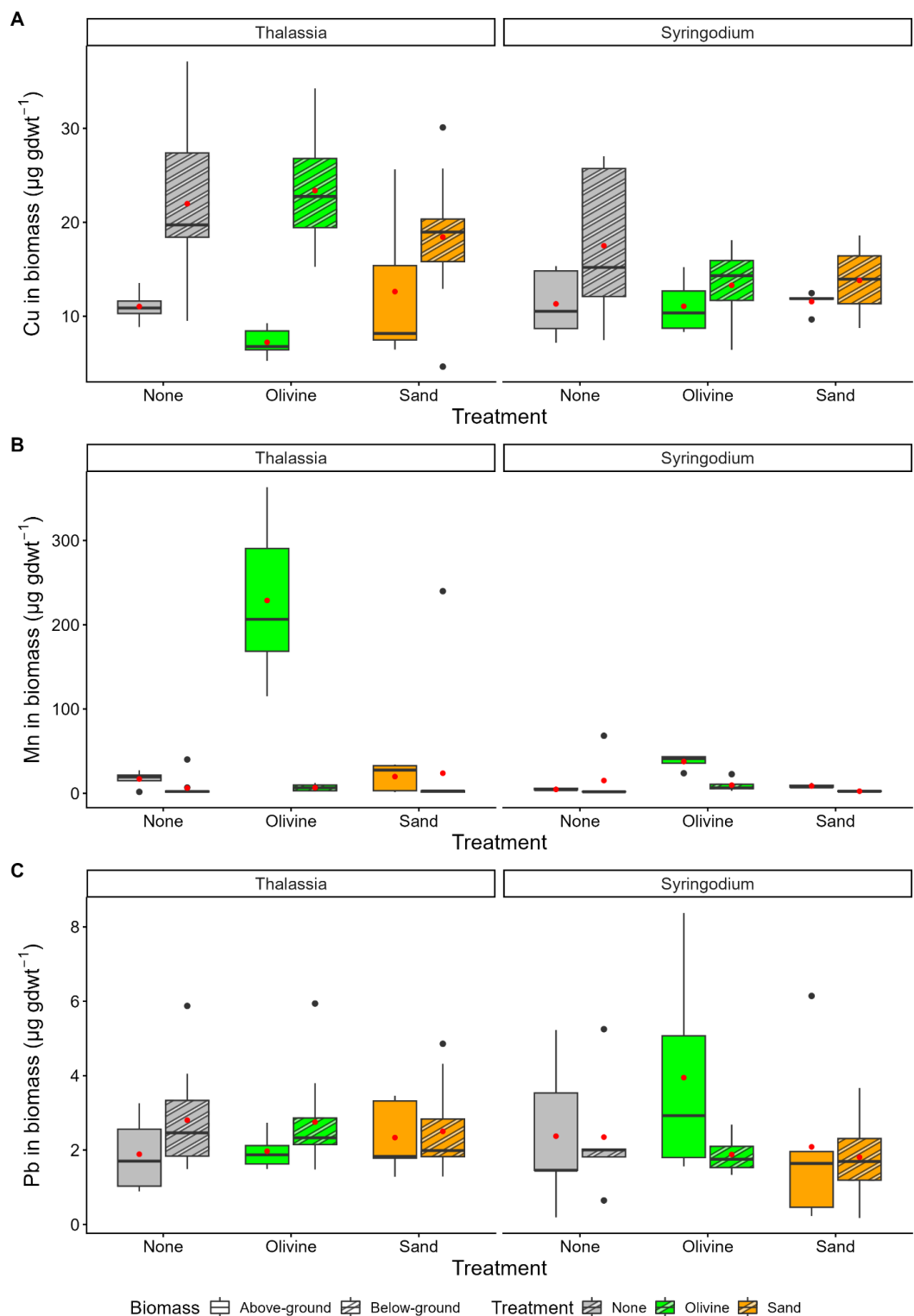


Figure S3: Seagrass biomass metal concentration ($\mu\text{g g dw}^{-1}$) measured at the end of the experiment: Cu (A), Mn (B), and Pb (C). Mean (red dot), median (horizontal line), 25th and 75th percentile (box), minimum and maximum value (vertical line), and outliers (black dot) are presented.

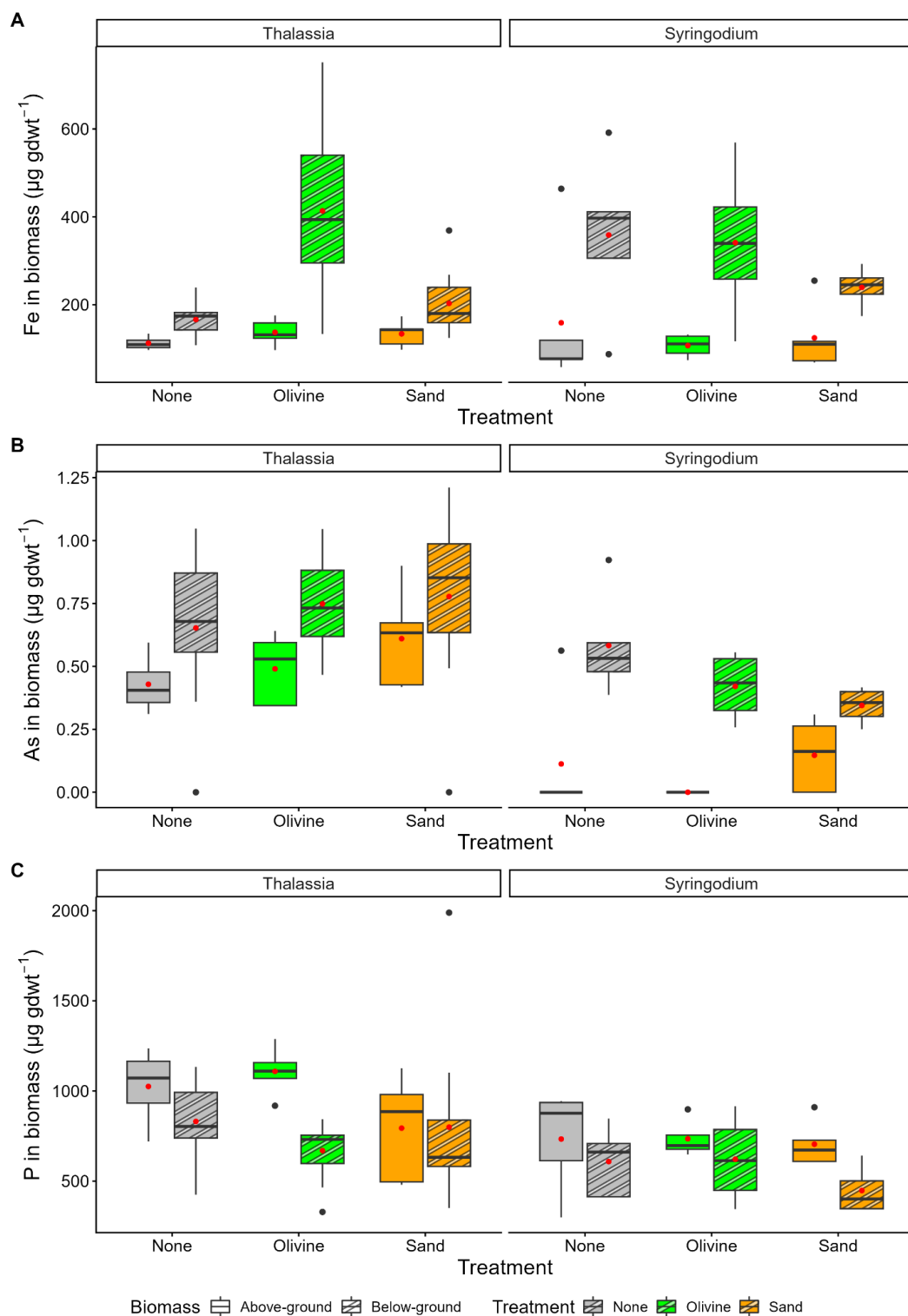


Figure S4: Seagrass biomass metal concentration ($\mu\text{g g dw}^{-1}$) measured at the end of the experiment: Fe (A) and As (B), and P (C). Mean (red dot), median (horizontal line), 25th and 75th percentile (box), minimum and maximum value (vertical line), and outliers (black dot) are presented.

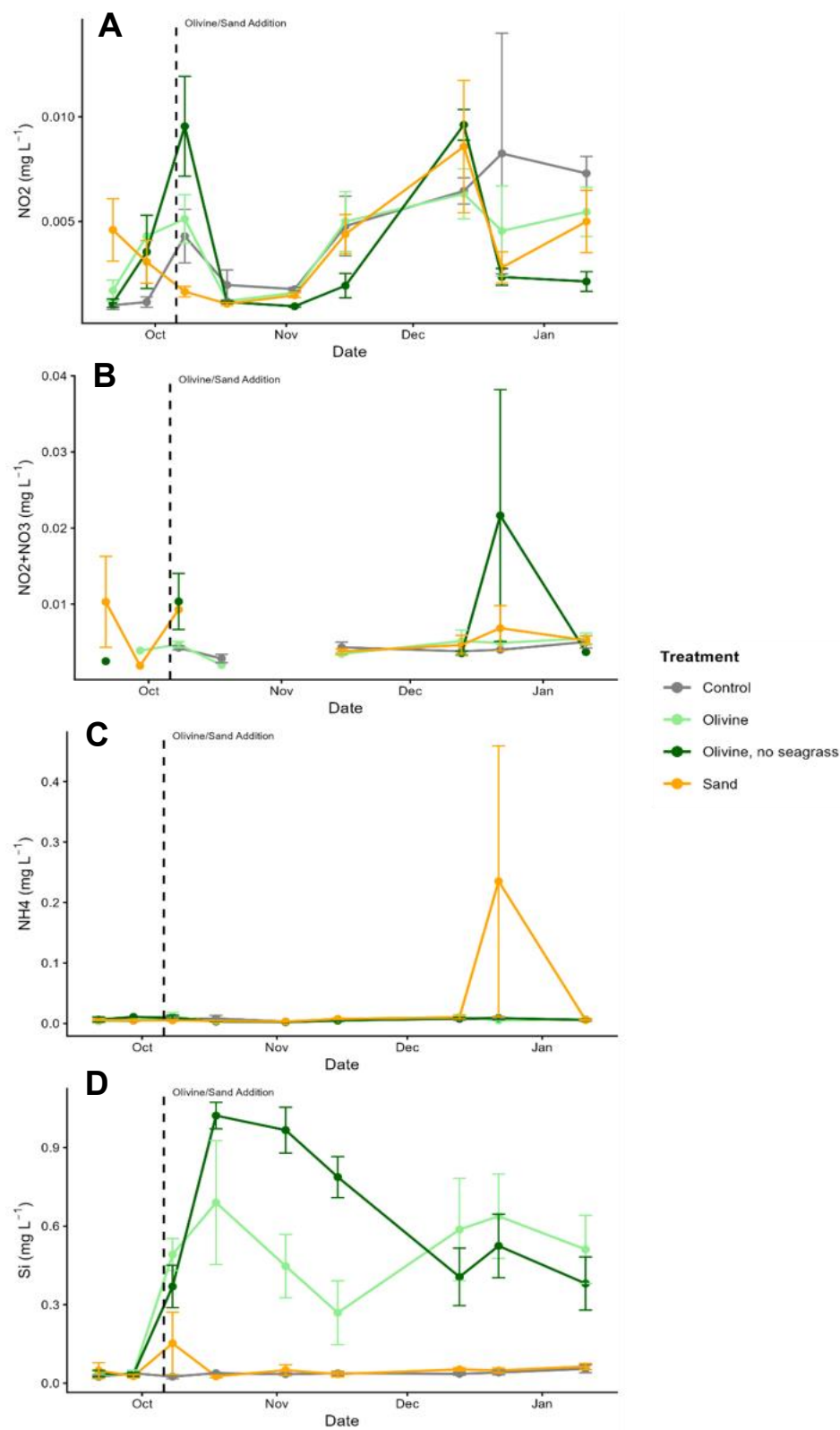


Figure S5: Dissolved nutrients: NO_2^- (A), $\text{NO}_2^- + \text{NO}_3^-$ (B), NH_4^+ (C), and Si (D) concentrations in seawater collected from tanks throughout the experiment.

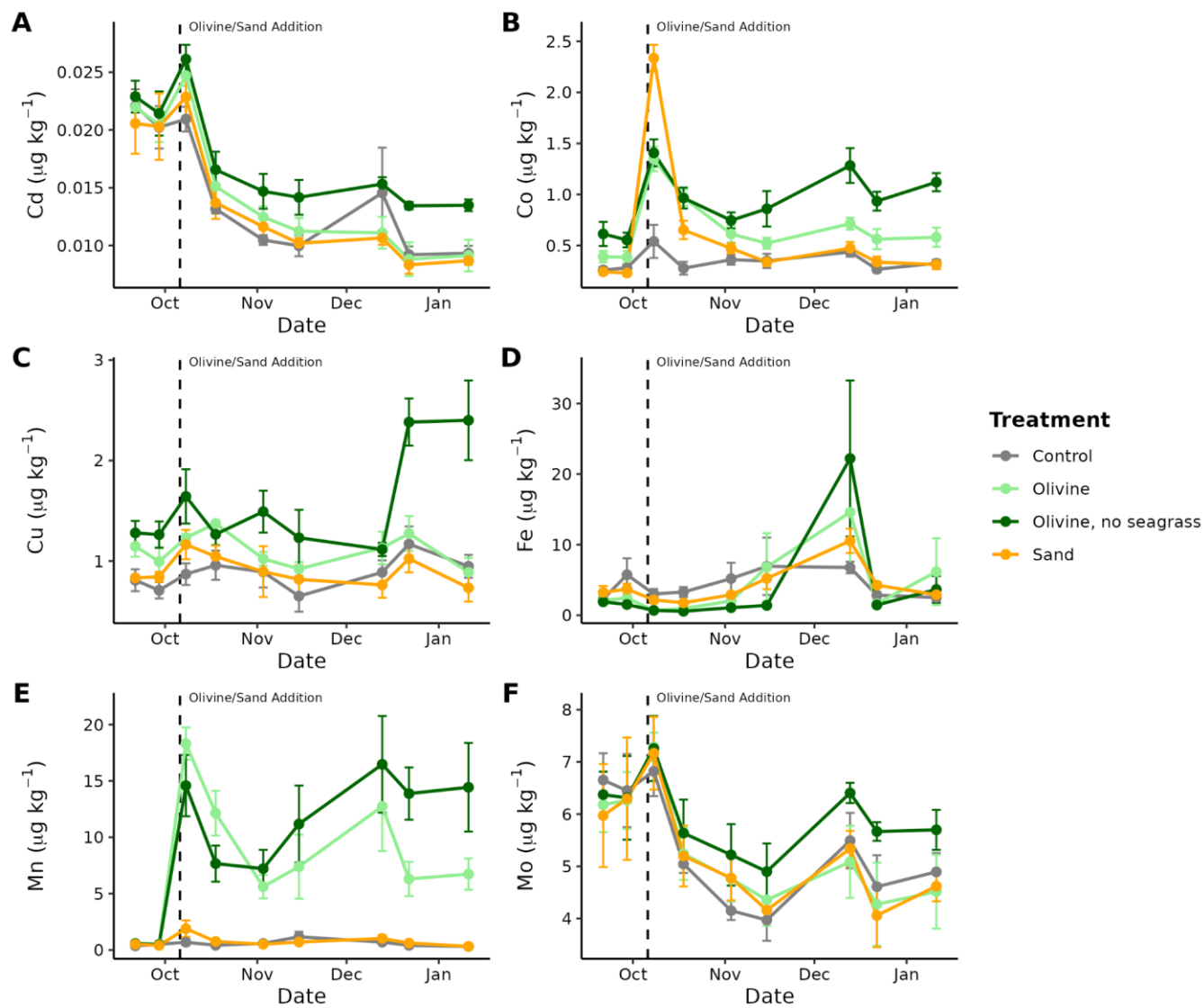


Figure S6: Seawater metals: Cd (A), Co (B), Cu (C), Fe (D), Mn (E) and Mo (F) concentrations in seawater collected from tanks throughout the experiment.

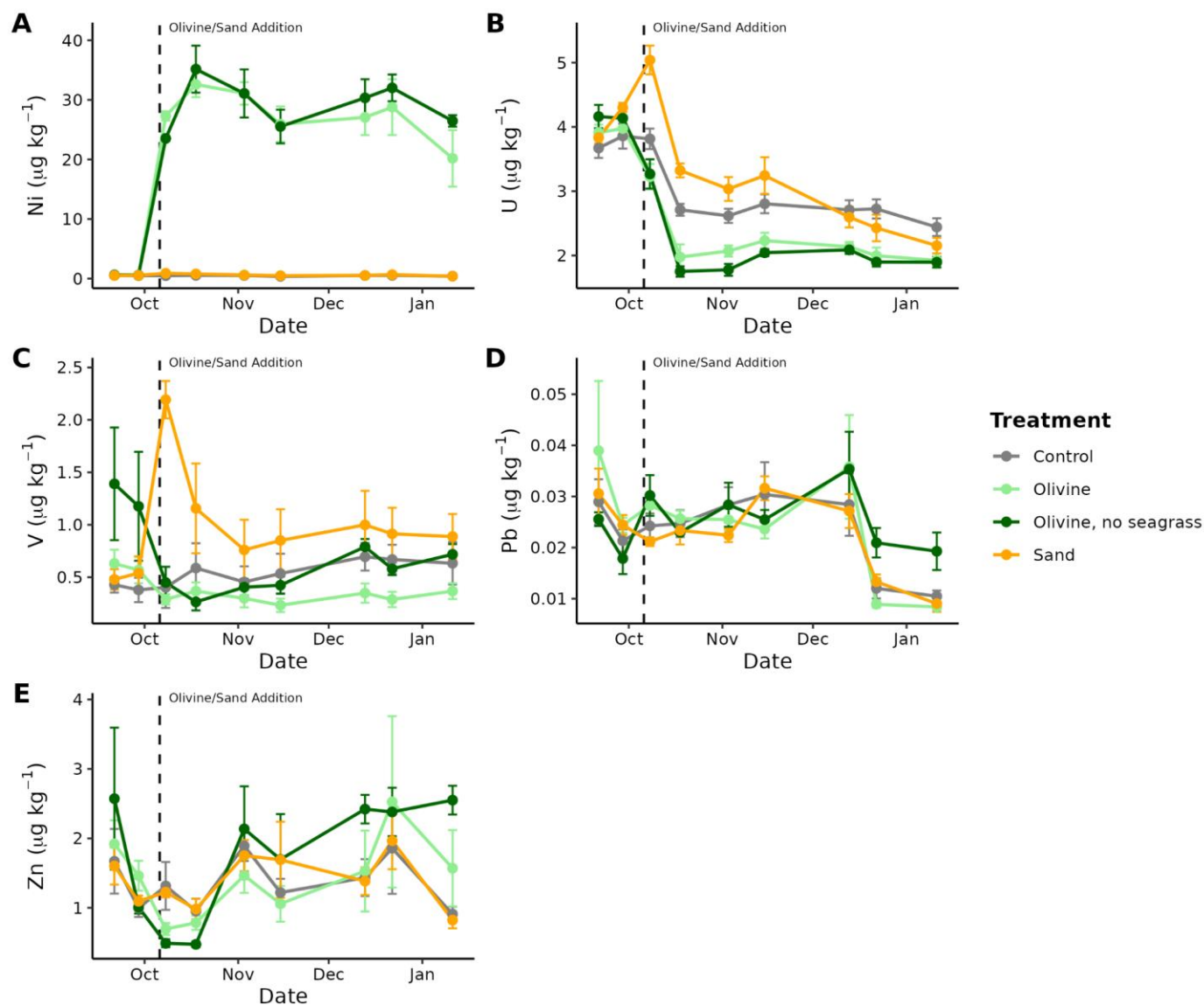


Figure S7: Seawater metals: Ni (A), U (B), V (C), Pb (D) and Zn (E) concentration in seawater collected from tanks throughout the experiment.

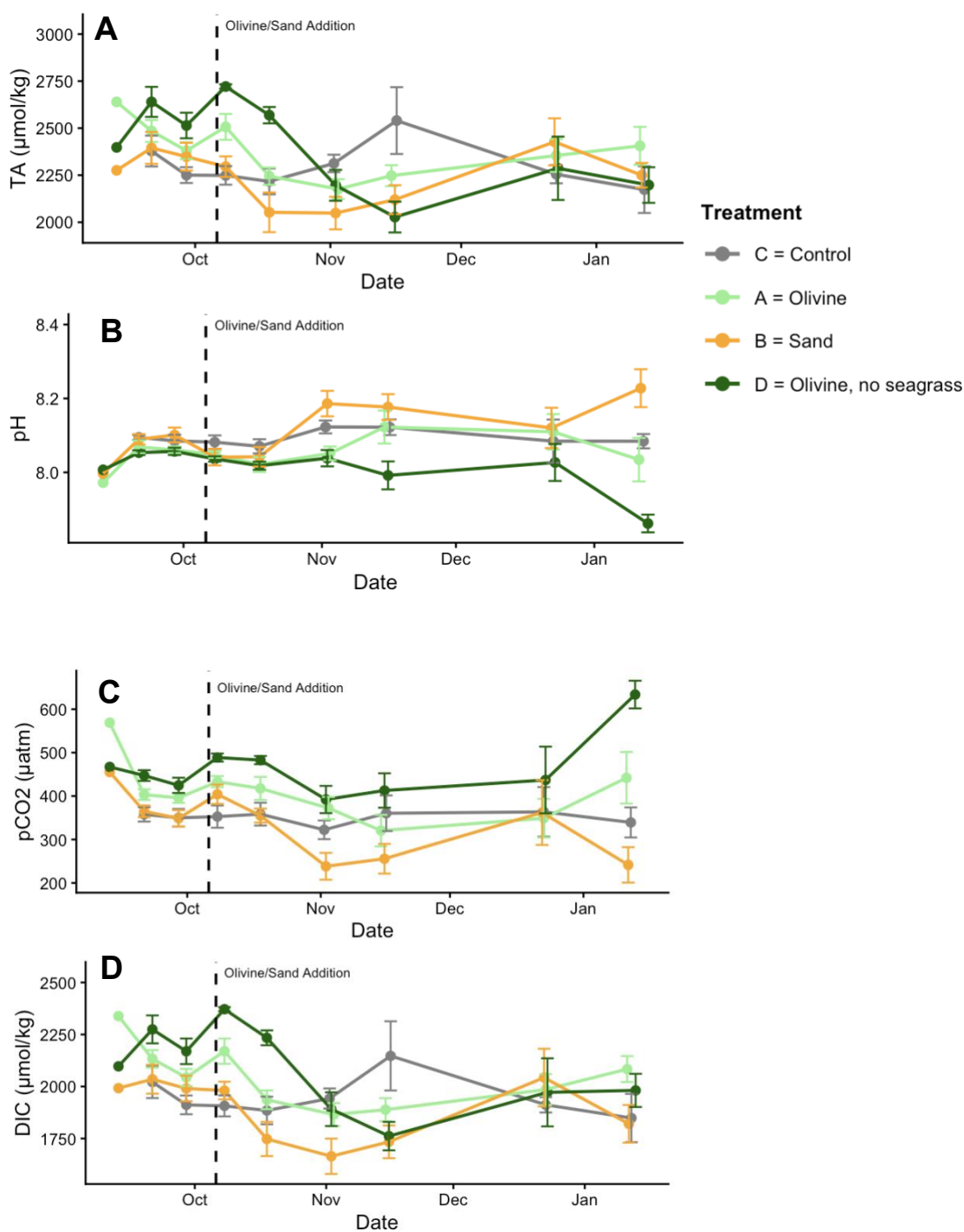


Figure S8: Seawater carbonate chemistry: TA (A), pH (total scale) (B), pCO₂ (C), and DIC (D) concentrations in seawater collected from tanks throughout the experiment.

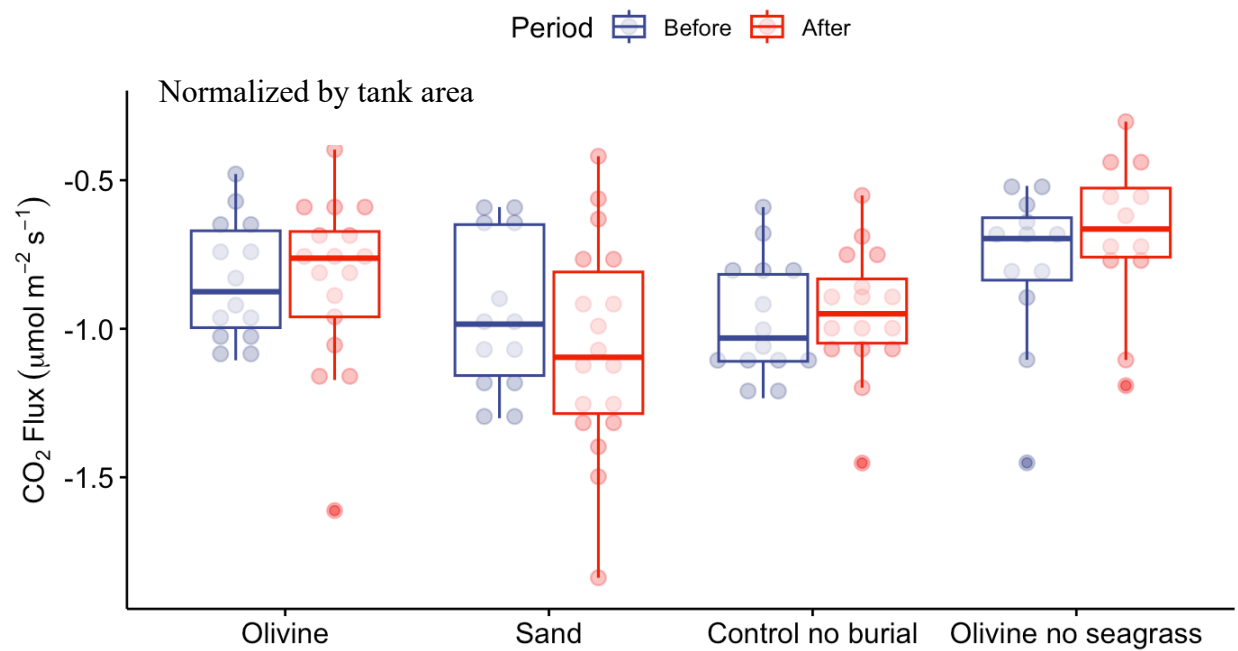


Figure S9: Water-air flux of CO₂ measured in the tank head space normalized by tank area (μmol m⁻² s⁻¹) across all treatments, including the no seagrass, + olivine. Points are individual flux measurements and colors indicate measurement period (before or after olivine/sand addition). Mean (bold line), 25th and 75th percentile (box), minimum and maximum value (vertical line) and outliers (filled dots) are presented.

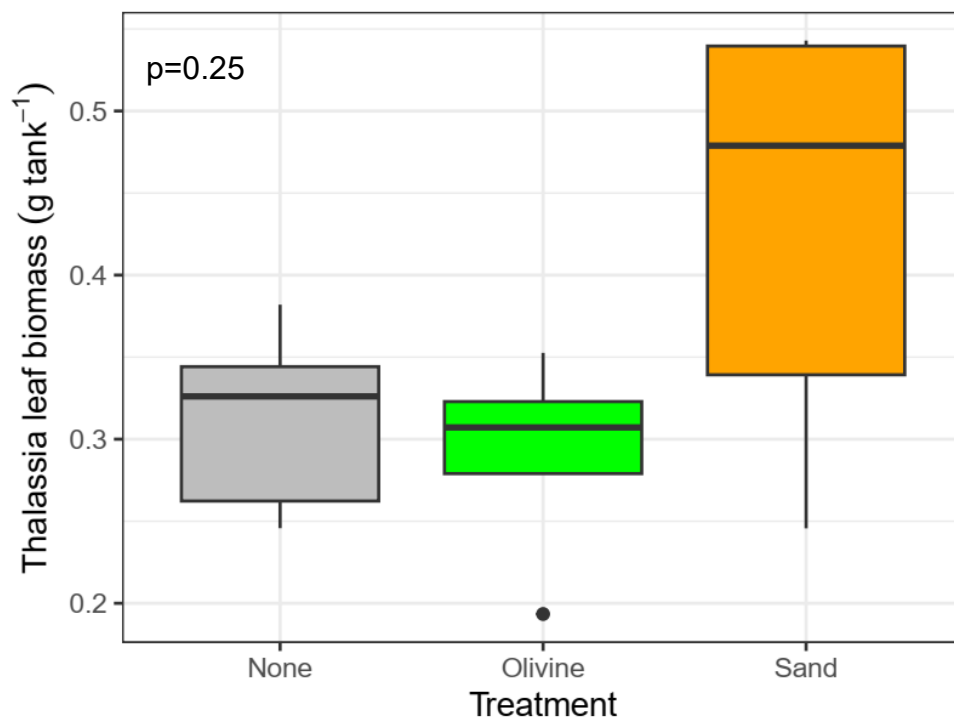


Figure S10: Thalassia leaf biomass prior to olivine / sand addition