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Context-dependency of eelgrass–clam interactions: implications for coastal restoration

Lukas Meysick^{1,*}, Alf Norkko^{2,3}, Karine Gagnon¹, Max Gräfnings^{1,4},
Christoffer Boström¹

¹Environmental and Marine Biology, Åbo Akademi University, Tykistökatu 6, 20520 Turku, Finland

²Tvärminne Zoological Station, University of Helsinki, J.A. Palménin tie 260, 10900 Hanko, Finland

³Baltic Sea Centre, Stockholm University, 106 91 Stockholm, Sweden

⁴Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, 9700 Groningen, The Netherlands

ABSTRACT: Facilitative interactions between co-occurring species sustain diverse communities and constitute a vital functional component of coastal marine ecosystems. In seagrass ecosystems, facilitation ensures the survival and resilience of this important habitat. As seagrass meadows are in decline, innovative restoration strategies incorporating facilitative interactions could open new avenues in marine restoration. Here, we investigated the interactions between eelgrass *Zostera marina* and the Baltic clam *Macoma balthica*, and tested whether clams could enhance early survival and biomass increase of transplanted eelgrass shoots in the northern Baltic Sea. We measured eelgrass responses to differing densities of clams, as well as porewater ammonium (NH₄⁺) and phosphate (PO₄³⁻) concentrations in field and aquarium experiments. Overall, survival of transplanted plots was high, independent of clam density. Specifically, we found that clams facilitated eelgrass above- and below-ground biomass in low porewater nutrient conditions, potentially through nutrient release, but inhibited growth in high-nutrient conditions, particularly where clams were added at high densities. Our results show the important role of infaunal bivalves for nutrient fluxes within seagrass meadows. Most notably, we highlight the importance of considering and testing context- and density-dependency when studying interspecific interactions, as clams could both benefit and hamper *Zostera* biomass increase. This becomes particularly crucial when incorporating such interactions in a restoration context.

KEY WORDS: Facilitation · *Zostera marina* · *Macoma balthica* · Porewater nutrients · Species interactions · Ecosystem engineering · Density dependence · Restoration

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1. INTRODUCTION

Ecosystem engineering, i.e. the modification of the biotic and/or abiotic environment by one or multiple species, is often associated with facilitative processes (Jones et al. 1997). Facilitation is a crucial driver for the composition and persistence of natural communities, allowing organisms to live beyond their predicted fundamental niche (Bruno et al. 2003). Habitat amelioration by bioengineers can play an important

role especially in unstable habitats (Stachowicz 2001), such as soft-sediment coastal marine areas which are exposed to multiple natural and anthropogenic stressors (hydrodynamic forces, eutrophication, hypoxia and physical disturbances; Byers & Grabowski 2014). Here, a few dominant bioengineers including bivalves, seagrasses and mangroves can form complex biogenic habitats that host a variety of associated communities (Beck et al. 2001, Boström et al. 2011). Incorporating positive interactions into ecosystem

*Corresponding author: lukas.meyssick@abo.fi

restoration could improve success rates of restoration efforts by enhancing the survival and growth of habitat-forming species and the colonisation of associated species (Silliman et al. 2015). However, in contrast to terrestrial restoration programmes, facilitation processes are still understudied in marine ecosystem restoration programmes (Halpern et al. 2007, Zhang et al. 2018).

Seagrasses provide a multitude of important ecosystem services, including shoreline protection, carbon sequestration and nursery habitat for commercial fish and invertebrate species (Nordlund et al. 2016). Although some seagrass ecosystems are recovering from historic degradations (e.g. Lefcheck et al. 2018, de los Santos et al. 2019), globally they are declining at accelerating rates, with estimations of up to 30% of the known seagrass area lost since records began in 1879 (Waycott et al. 2009). The primary causes of seagrass loss are negative interactive effects of both global change (warming, sea level rise) and local anthropogenic stressors such as eutrophication, shoreline development, trawling and dredging (Grech et al. 2012, Dailianis et al. 2018). Over the past few decades, conservation and restoration efforts have increased, but long-term (>3 yr) success rates remain below 40% (Bayraktarov et al. 2016, van Katwijk et al. 2016).

Bivalves are commonly associated with seagrasses and can be important drivers of species interactions through ecosystem engineering (Peterson & Heck 2001, Wall et al. 2008, van der Heide et al. 2012, de Fouw et al. 2016, Meysick et al. 2019a). Based on a global review of 320 studies, facilitative interactions between seagrasses and bivalves are far more likely to occur (~50%) than negative interactions (~22%, Gagnon et al. 2020). However, the outcome of seagrass–bivalve interactions is assumed to be context-dependent, varying with *in situ* environmental conditions such as nutrient and food availability, temperature or the interacting species in question (Reusch 1998, Reusch & Williams 1998, Vinther & Holmer 2008, Sanmartí et al. 2018, Gagnon et al. 2020).

The Baltic clam *Macoma balthica* (hereafter *Macoma*) is an important component of soft-sediment communities in the North Atlantic and its marginal seas (including the Baltic Sea), with strong impacts on ecosystem functions such as bioturbation (Bernard et al. 2019) and nutrient release from sediment to water (Mortimer et al. 1999, Michaud et al. 2006, Norkko et al. 2013). Throughout the Baltic Sea, *Macoma* is the dominant infaunal bivalve, often associated with vegetated habitats including eelgrass,

Zostera marina, (hereafter *Zostera*) meadows. To date, 21 studies have investigated interactions between seagrasses and tellinid bivalves, of which only 9 addressed the interaction between *Zostera* and *Macoma* (Gagnon et al. 2020; our Fig. S1 and Text S1 in the Supplement at www.int-res.com/articles/suppl/m647p093_supp.pdf) and showed both positive and negative effects. Seagrass plays an important role in facilitating the settlement of tellinid larvae and juveniles in smaller patches and along edges by providing shelter from physical disturbance and predation (Boström & Bonsdorff 2000, Boström et al. 2010). Larger tellinids tend to prefer bare sediments, since seagrass rhizome mats might restrict movement in the sediment and prevent burrowing (Lohrer et al. 2016). Importantly, despite their natural co-occurrence and the potentially beneficial impacts of tellinid clams on sediment oxygenation and nutrient release, the effects of tellinids, and more specifically *Macoma*, on seagrass have not been experimentally tested (Fig. S1). While any interaction between species is likely to be conditional on the species densities *in situ*, density-dependent designs have been largely ignored in field experiments, but can reveal potential non-linear effects (e.g. Reusch & Williams 1998). As density-dependent interactions may take time to manifest, the study of potential 'legacy' effects may require multiple sampling seasons to detect these effects.

To explore the interactions between *Zostera*, *Macoma* and porewater nutrients, we conducted a field survey, a long-term (2 growing seasons) manipulative field experiment and a complementary aquarium experiment. Our specific research questions were as follows:

- (1) How are *Macoma* abundance and condition affected by the presence of *Zostera*?
- (2) Does *Macoma* facilitate early establishment and survival of *Zostera* by affecting nutrient pools in sediment porewater and the water column, and are there any density-dependent and/or long-term 'legacy' effects?
- (3) Do these species interactions have potential implications for coastal restoration?

2. MATERIALS AND METHODS

2.1. Field site

The field survey and experiment were carried out near the island of Fårö (59° 55' 20" N, 21° 47' 60" E) in the Finnish Archipelago Sea by SCUBA diving

(Fig. 1). The study site is semi-exposed and consists of sandy, organic-poor sediments (<0.5%, Gustafsson & Boström 2013). The unvegetated area is bordered by an extensive seagrass meadow starting at approximately 2 m depth, dominated by 3 angiosperms: *Zostera marina*, *Potamogeton perfoliatus* and *P. pectinatus* (see Gustafsson & Boström 2013 for details). Preliminary sampling showed that *Macoma*, the polychaetes *Nereis diversicolor* and *Pygospio elegans*, and *Hydrobia* spp. snails dominate the invertebrate community in both bare sediments and seagrass at the field site.

We collected *Macoma* for the field and aquarium experiments from bare sediments at 1.5–2 m depth at the field site. To efficiently collect large numbers of clams, we deployed impermeable plastic sheets (3 m × 1 m) on the seabed for 24 h (Norkko et al. 2010). This caused the clams to rise to the sediment surface as oxygen levels decreased, where they could be collected quickly and easily. Approximately 6000 individuals were collected and stored for 14 d in well-oxygenated, flow-through seawater aquaria with a 5 cm layer of sand from the field site (temperature

~11°C) and regularly fed with detritus. We collected *Zostera* shoots from the seagrass meadow at the field site and stored them in mesh bags deployed in seawater overnight until transplantation to the field and aquarium experiments.

2.2. Field survey

To assess natural abundances of *Macoma* at the field site, we collected infauna samples from bare sediments and the ambient *Zostera* meadow using a 10.3 cm diameter corer in June 2017 (n = 10) and September 2017 (n = 5). Adjacent to each infaunal core, we collected a sediment sample (2.1 cm diameter corer) to determine sediment organic content. Infaunal samples were sieved over a 0.5 mm sieve and preserved in 70% ethanol until *Macoma* were counted and measured for length in the laboratory. Based on size class histograms, we separated all *Macoma* into 2 size classes (<5 and ≥5 mm) (Fig. S2). We also determined condition index (CI) for all individuals ≥5 mm according to the ratio of meat to shell

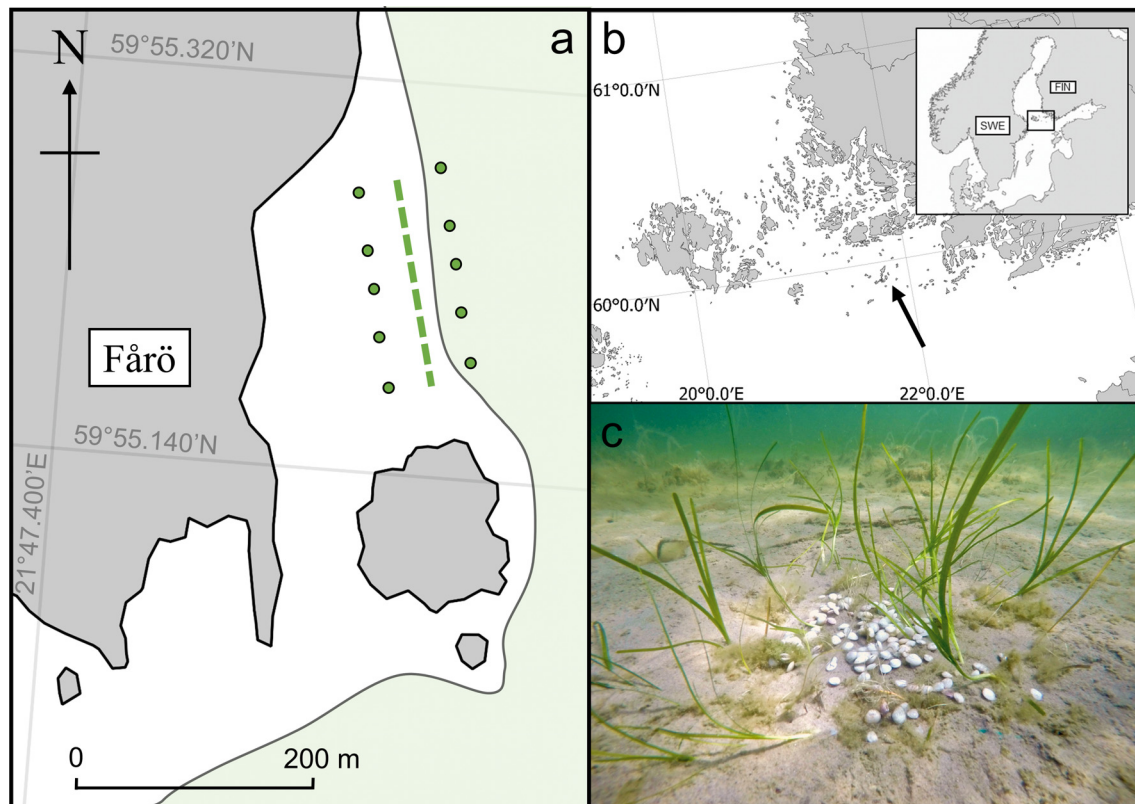


Fig. 1. (a) Location of the study site near the island Fårö within (b) the Finnish Archipelago Sea. The light green area indicates the extent of the local *Zostera* meadow. The green dashed line corresponds to the location of the field manipulation, while the green dots are the control samples from vegetated (light green) and unvegetated (white) areas. (c) Example of a manipulation plot right after clam addition

biomass after drying the meat and shell separately at 100°C until a constant weight was achieved (Walne 1976). To assess *Macoma* condition in 2 consecutive years, additional clams were sampled during the termination of the field experiment (September 2018). Sediment organic content was measured as loss on ignition in 6 h at 440°C, after drying the samples at 60°C to a constant weight.

2.3. Field experiment

To test for a potential density-dependent response of *Zostera* to *Macoma* addition, a field manipulation was initiated in June 2017 (T0 = start). In total, 60 *Zostera* plots were planted in the bare sediment along the edge of the seagrass meadow, approximately 3 m apart and 3 m from the edge of the meadow (Fig. 1). For each plot, we randomly selected 16 *Zostera* shoots and tied them gently to a 25 × 25 cm plastic mesh (mesh size 30 mm, see Gustafsson & Boström 2013), which was subsequently buried into the sediment and anchored with metal hooks. Shoot densities used for the transplantation experiment (256 shoots m⁻²) were lower than densities found in the adjacent natural seagrass meadow (722 ± 168 [SE] shoots m⁻²). We chose these densities to resemble early colonisation and densities relevant for restoration efforts. To each plot, we added 1 of 10 different *Macoma* densities, ranging from 0 to 2880 ind. m⁻² (0–180 ind. plot⁻¹), in intervals of 320 ind. m⁻² (20 ind. plot⁻¹). Since the experimental array (~200 m) was rather large, and to account for variability in environmental parameters such as porewater nutrient concentrations, temperature or light availability, the clams were added in a randomized block design with 3 replicate blocks. Within each block, all 10 clam densities were randomly added twice, allowing for 2 destructive sampling events with 3 replicates per treatment density. Prior to the experiment, *Macoma* were sorted by size. Since larger individuals are reported to be of greater functional importance than juveniles (Norkko et al. 2013), we selected only the largest individuals from the preliminary clam collection (≥8 mm). As removal of ambient clams in the field was unpractical and would have created severe disturbance to the seafloor, and variability of individuals ≥8 mm in bare sediments was low at the site (713 ± 106 (SE) ind. m⁻², n = 5), the manipulation was carried out as an addition to ambient clam densities. After releasing the clams on top of the *Zostera* plots, the majority buried into the sediment within minutes, and the remaining individuals were carefully pushed into the sediment by

hand, to avoid clams being washed away by currents and waves.

To measure potential longer-lasting effects of *Macoma* on *Zostera* growth and plot survival, we sampled the plots destructively at 2 time points: after 1 (T1 = 76 d; September 2017) and 2 growing seasons (T2 = 417 d; September 2018). Each time, we sampled 3 replicates per treatment (n = 30; one density treatment from each replicate block). Since only weak correlations between manipulated and realized *Macoma* densities were detected after T1, and transplantation plots demonstrated considerable spatial expansion during T0–T2, making it difficult to relate infauna and porewater nutrient samples to initial manipulations, the sampling effort at T2 focussed primarily on potential growth of *Zostera* as a legacy response to the initial density manipulation.

We considered any remaining and live plant material in a plot as plot survival. We first measured the spatial expansion of *Zostera* (distance from the centre of the grid to the furthest shoot), and then took a sediment sample (using a 2.1 cm diameter corer) from the centre of the plot, for sediment organic content analysis. To quantify nutrient conditions, we also took 1 porewater sample from each plot prior to collection at T1, using Rhizon 10 cm soil moisture samplers (Eijkelkamp Agrisearch Equipment) attached to vacuum containers. We collected the plots by quickly placing the plastic grid with the attached *Zostera* (all above- and below-ground plant parts) into a mesh bag (mesh size = 0.5 mm), including all plant material that had grown out of the plot. We then immediately sampled the infaunal community below each plot using a 10.3 cm diameter corer. Infauna samples were sieved over a 0.5 mm sieve and preserved in 70% ethanol until processing for counting *Macoma*. We measured *Zostera* production of 2 randomly chosen shoots per plot at T1, by pushing a syringe needle through the meristem 12 d before collection ('punching method', see Ziemann 1974). After collection, we separated these shoots from the rest of the plot, then calculated the amount of leaf tissue produced between the reference hole and the leaf scar. Ten additional random shoots from the adjacent *Zostera* meadow were marked and analysed for primary production to assess the performance of the transplantation plots compared to natural conditions at the field site.

After each destructive sampling, we rinsed the *Zostera* samples and separated the roots, rhizomes and above-ground material. We then counted the shoots from each plot and measured the length of 5 randomly chosen shoots. The biomass of each com-

ponent (roots, rhizomes, above-ground) was determined by weighing after drying to constant weight at 60°C. The marked shoots for leaf growth were processed individually after being identified, and the newly grown leaf material was separated and dried and weighed separately, to determine the growth rate (g dry weight [DW] d⁻¹). From each plot, we counted and measured the size of each *Macoma*, and determined CI (see Section 2.2). We determined sediment organic content for each plot according to the methods from the field survey. The porewater samples were analysed for ammonium (NH₄⁺) and phosphate (PO₄³⁻) concentrations using a single-cuvette spectrophotometer for ammonium (Koroleff 1976) and a nutrient auto-analyzer (Thermo Scientific Aquakem 250) for phosphate at Tvärminne Zoological Station.

2.4. Aquarium experiment

To complement the field experiment, we conducted an aquarium experiment under controlled conditions for 32 d in July–August 2017. Here, we tested the response of *Zostera* to 3 densities of *Macoma* (control = 0, low = 1000, high = 3000 ind. m⁻²) in 36 aquaria organised in a randomized block design with 6 replicate blocks. Each aquarium (28 cm length × 17 cm width × 50 cm height) had a drainage system in the corner, allowing for constant water exchange through water overflow. Sediment was collected at the field experiment site and was sieved at 0.5 mm to exclude any macrofauna. Each aquarium was filled with a 10 cm sediment layer to allow for *Zostera* roots and rhizomes to grow and for clams to bury and bioturbate. The aquaria were in a covered outdoor facility with natural light and ambient seawater pumped from the nearby harbour (~3 m depth), and the water exchange rate in each aquarium was adjusted to approximately 1 l min⁻¹ throughout the duration of the experiment.

In each aquarium, we planted 12 *Zostera* shoots corresponding to ~270 shoots m⁻². To reduce the effect of transplantation stress, we left the plants to acclimatize for 7 d prior to starting the experiment. To quantify individual morphological traits (shoot length and width) and to approximate biomass (DW) per aquarium at the beginning of the experiment based on 12 shoots (n = 4), we randomly selected 48 shoots from the collected shoot pool. We also took a sediment sample (2.1 cm diameter corer) from each aquarium to determine initial sediment organic content. We then started the experiment by adding 0 (control), 45

(low density) or 135 (high density) *Macoma* individuals to each aquarium. Twelve days before termination, we marked 2 shoots per aquarium to measure *Zostera* growth rate, and sampled porewater after 30 d (using the same methods as in the field experiment). The experiment was terminated after 32 d, at which point we took another sediment sample (2.1 cm diameter corer) from each aquarium to quantify changes in organic content and then carefully collected all plant material. The methods for determining plant traits (above-ground biomass, root biomass, rhizome biomass, shoot length, growth rate) and sediment parameters (organic content, porewater ammonium concentration, porewater phosphate concentration) were identical to the field experiment.

2.5. Statistical analysis

We analysed the data from the field survey using 2-way ANOVA to determine differences in *Macoma* abundance and sediment organic content between habitat (2 levels: *Zostera*, bare sediments) and time (2 levels: June, September) and to determine differences in *Macoma* CI between habitat (2 levels: *Zostera*, bare sediments) and time (2 levels: 2017, 2018). If significant habitat × time interactions were detected, pairwise differences for each month or year, respectively, were assessed using Holm-Šídák (abundance) and Tukey's HSD multiple comparison tests (CI). Since data on adult *Macoma* abundance showed heterogeneous variances, they were square root transformed before applying ANOVA.

In the field experiment, we used linear models (LMs) to quantify whether the density manipulation at T0 affected realized *Macoma* densities, *Macoma* CI, porewater ammonium and phosphate concentrations and sediment organic content in the plots at T1. We analysed changes in *Zostera* traits (above-ground biomass [AB], rhizome biomass, root biomass, shoot length, shoot count, spatial expansion) over time using a generalised linear mixed model (GLMM) with *Macoma* density and sampling time as fixed effects and replicate block as a random effect to minimize potential sources of variation in environmental parameters (temperature, light availability, porewater nutrient concentrations).

Data on porewater nutrient concentrations revealed a strong underlying spatial gradient at the field site. To explore potential mediation effects of sediment chemistry on *Zostera*–*Macoma* interactions, *Zostera* trait responses were separately re-analysed at the first sampling event (T1) where the data collec-

tion had been more extensive and included porewater nutrient sampling. Here, we used LMs with *Macoma* density treatment and porewater nutrient concentrations (ammonium/phosphate) as fixed effects on *Zostera* traits (shoot biomass, rhizome biomass, root biomass, shoot length, shoot count, spatial expansion). In our study region, both ammonium and N:P ratios are typically near or below *Zostera* growth requirements (Boström et al. 2004). Since ammonium can both facilitate *Zostera* growth, but also hamper it at certain threshold levels (ammonium toxicity, e.g. van Katwijk et al. 1997), which in the perspective of context-dependency and species interactions is an important fact to investigate, this study will primarily focus on ammonium concentrations. Porewater ammonium and phosphate concentrations were highly correlated ($r^2 = 0.86$), and models including phosphate instead of ammonium can be found in the supplementary material. Although the porewater nutrient concentrations were not manipulated in the experiment, we decided to include them as continuous explanatory variables, due to the use of replicate blocks. Moreover, *Macoma* manipulation and porewater ammonium ($r^2 < 0.01$, $p = 0.81$)/phosphate concentrations ($r^2 < 0.01$, $p > 0.95$) showed negligible correlations, indicating their suitability as independent variables.

In the aquarium experiment, we used GLMMs to test for differences in plant traits, organic content and porewater nutrient concentrations between *Macoma* treatments. *Macoma* density was included as a fixed factor with 3 levels (control, low, high, $n = 12$ for each level), while the 6 replicate blocks were included as a random factor.

Assumptions of homoscedasticity were tested through Spearman's rank correlation test (linear regression) and the Brown-Forsythe test (ANOVA). Normality was tested with the Shapiro-Wilk test. Visual assessment of Q-Q and residual plots was conducted for the LMs and GLMMs. Models on porewater nutrients in the aquarium experiment were adjusted by including a logarithmic link function, since assumptions of homoscedasticity were not met.

All data analyses were conducted in the R-environment version 3.4.0 (R Development Core Team 2017).

3. RESULTS

3.1. Distribution and condition of clams at the study site

The field survey showed that differences in *Macoma* abundance between the bare sediment and the

natural *Zostera* meadow were size and time dependent. Densities of large clams (≥ 5 mm) were almost twice as high, and thus significantly more abundant in vegetated than in unvegetated areas ($F = 35.06$, $p < 0.001$) with no effect of sampling time ($F = 0.37$, $p = 0.548$, Fig. 2a, Table S1a). Abundances of small individuals (< 5 mm), however, were more variable and showed a significant habitat \times time interaction ($F = 13.91$, $p < 0.001$). In late June 2017, there were higher abundances of small *Macoma* in *Zostera* than in bare sediments, whereas the opposite pattern was observed in September 2017 (Fig. 2b, Table S1b).

The *Macoma* CI differed significantly between habitats ($F = 40.23$, $p < 0.001$) and sampling occasions ($F = 6.47$, $p < 0.012$) and also showed a habitat \times time interaction ($F = 13.42$, $p < 0.001$, Table S2). Overall, CI was higher in bare sediment than in the ambient *Zostera* meadow (Fig. 3). However, multiple comparison revealed that these differences were only statistically significant in September 2017. The food source for bivalves in terms of sediment organic content was significantly higher in the ambient *Zostera* meadow (0.46 ± 0.03 [SE] %) than in the bare sediment (0.35 ± 0.02 %, $F = 13.15$, $p = 0.002$), independent of sampling time ($F = 0.41$, $p = 0.529$).

3.2. Field experiment

Although variability was rather high, linear regression indicated that the density manipulation of *Macoma* in the field experiment had a significant positive effect on clam abundance after 76 d (T1) (Fig. 4, $r^2 = 0.26$, $p = 0.006$). *Macoma* CI was not affected by the density manipulation ($r^2 = 0.02$, $p = 0.498$). Sediment organic content in the plots (0.38 ± 0.01 [SE] %) matched the ambient low levels (see Section 3.1), and clam density had no effect on sediment organics ($r^2 = 0.01$, $p = 0.713$).

Survival of seagrass plots was 100% for both investigation periods (T1, T2, $n = 60$). Leaf growth rate of the transplanted plots measured at T1 (2.01 ± 0.12 [SE] mg DW d⁻¹) was similar to growth rates in the ambient seagrass meadow (2.21 ± 0.28 mg DW d⁻¹, $F = 0.45$, $p = 0.510$). *Zostera* AB increased significantly across all plots over time, with an average 2-fold increase at T1 and a 10-fold increase at T2 compared to T0 (Fig. 5, Table 1, $\chi^2 = 445.76$, $p < 0.001$). Similarly, rhizome biomass increased significantly over time by 3 times (T1) and 10 times (T2) (Table 1, $\chi^2 = 401.09$, $p < 0.001$). Root biomass increased significantly over time, although the increase

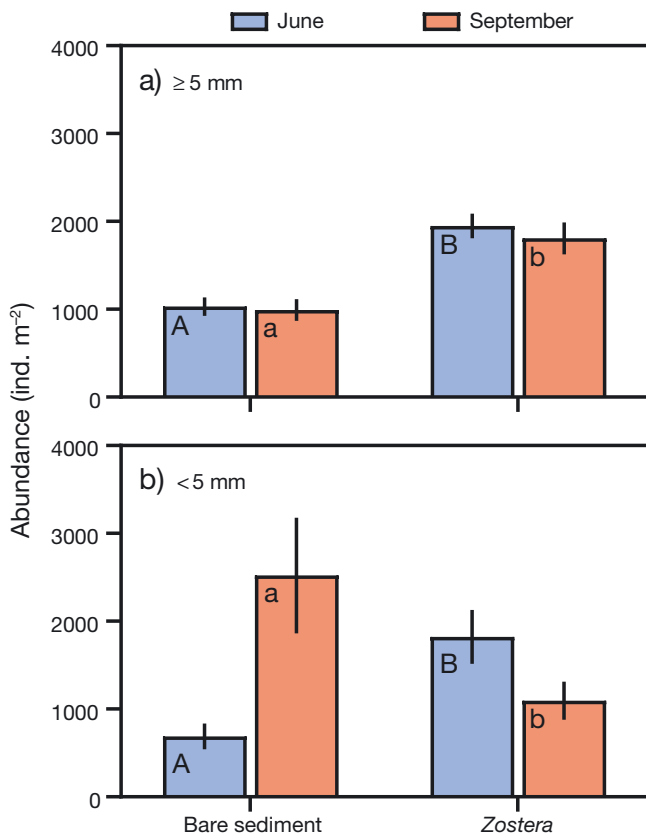


Fig. 2. *Macoma* abundance by size class in bare sediments and natural *Zostera* meadow: (a) clams ≥ 5 mm, (b) clams < 5 mm. Values are mean \pm SE ($n = 10$ for June, $n = 5$ for September). Significant differences ($p > 0.05$) between habitats are indicated by different uppercase (June 2017) and lowercase (September 2017) letters



Fig. 3. Condition index (CI) of *Macoma* in bare sediment and in the *Zostera* meadow for September 2017 and 2018. Values are mean \pm SE ($n = 30$ – 55). Significant differences ($p > 0.05$) between habitats are indicated by different uppercase (2017) and lowercase (2018) letters. Dashed line shows the mean CI of *Macoma* sampled from the field experiment as a reference

was lower than for AB and rhizomes (1.75 times at T1 and 4.5 times at T2, Table 1, $\chi^2 = 200.14$, $p < 0.001$). Additionally, the number of shoots per plot (Table 1, $\chi^2 = 664.45$, $p < 0.001$), mean shoot length (Table 1, $\chi^2 = 859.07$, $p < 0.001$) and spatial expansion (Fig. 5, Table 1, $\chi^2 = 1647.66$, $p < 0.001$) increased over time. The ratio of above- to below-ground biomass (AB:BB) slightly decreased from 0.83 to 0.74 at T1, before increasing to 1.23 at T2 (Fig. 5, Table 1, $\chi^2 = 154.38$, $p < 0.001$). Despite the average increase in most plant traits over time, there was no direct response to the clam density manipulation (Fig. 5, $\chi^2 < 1.96$ for all traits, $p > 0.162$ for all traits).

Porewater sampling at T1 revealed a strong spatial gradient in ammonium and phosphate concentrations at the field site, with the highest concentrations in the centre of the plot row, i.e. halfway along the experimental array. The linear model including both *Macoma* density manipulation and porewater ammonium concentrations at T1 highlighted a significant interaction between both variables on AB, and rhizome and root biomass as well as shoot count (Fig. 6, Table 2). Under low ammonium concentrations ($< 1200 \mu\text{g l}^{-1}$), clams had a positive effect on AB growth (measured as the proportion of DW biomass at T1 compared to T0), resulting in a 2.5 \times higher AB at plots with highest clam densities compared to plots without clam manipulation. Similarly, AB increased strongly with ammonium concentrations in plots without or with low *Macoma* densities ($< 1000 \text{ ind. m}^{-2}$).

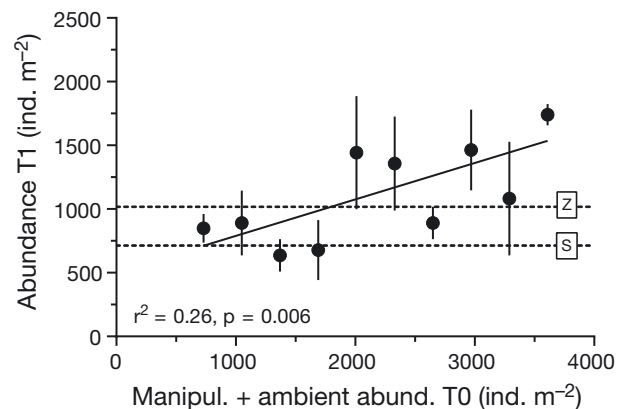


Fig. 4. Effect of *Macoma* manipulation at the beginning of the experiment, T0 (in addition to ambient abundances: $713 \pm 106 \text{ ind. m}^{-2}$) on overall abundance of clams ≥ 8 mm at the first sampling event, T1 (76 d). Values are averaged per treatment (\pm SE, $n = 3$). Black solid line corresponds to linear regression line (based on individual samples, $n = 27$; 3 samples were lost due to alcohol leakage during the storing period). Top and bottom dashed lines correspond to observed mean abundances (≥ 8 mm) in *Zostera* (Z) and bare sediment (S) habitats, respectively

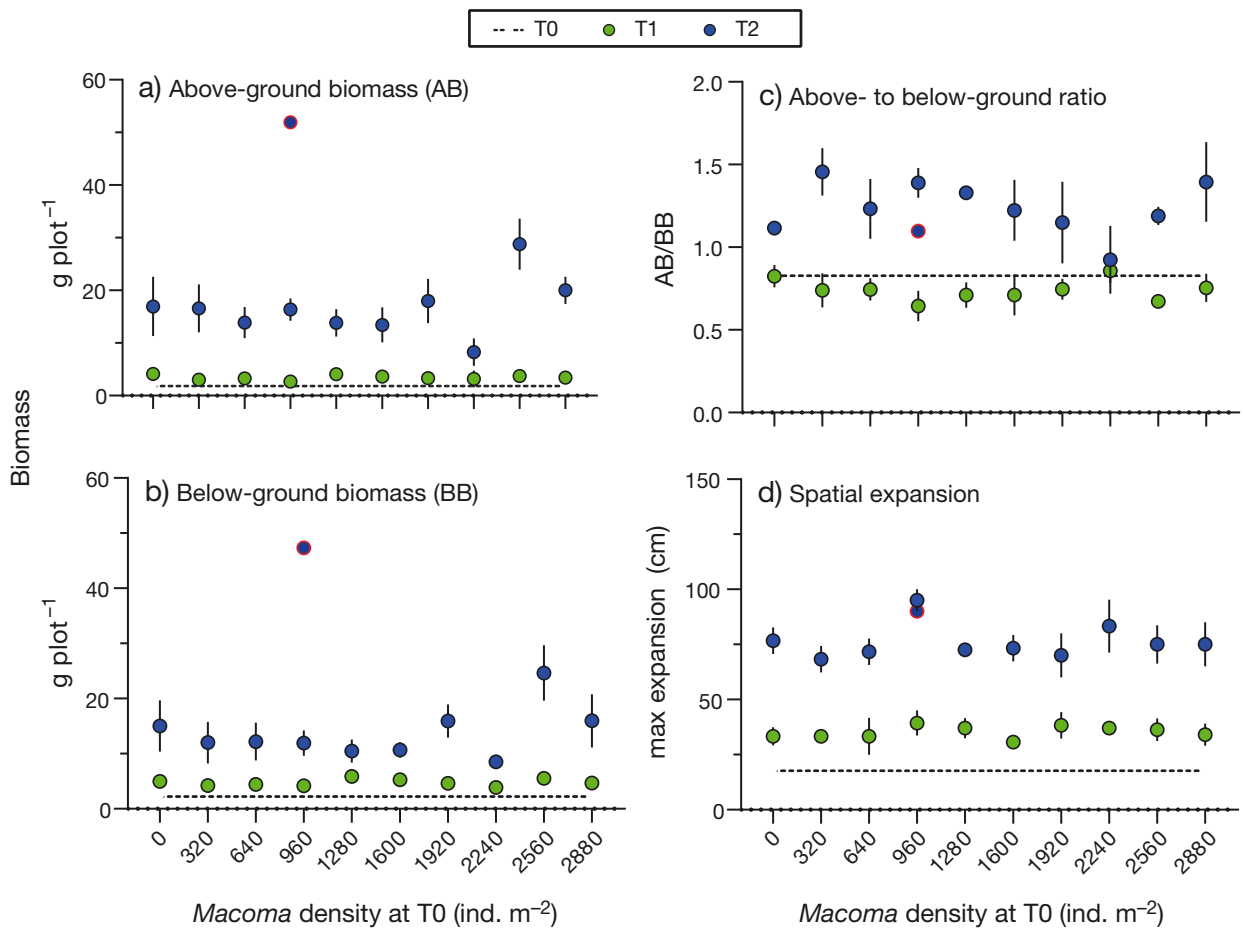


Fig. 5. Plant traits after the first sampling event (green dots, T1 = 76 d) and second sampling event (blue dots, T2 = 417 d) in response to *Macoma* density manipulations in the field experiment. Values are mean \pm SE ($n = 3$). Dashed lines correspond to initial trait values at T0 = 0 d. BB: sum of rhizome and root biomass. Note: one outlier at T2 was excluded from mean values after performing an outlier test (blue dot with red border)

Table 1. Average plant traits examined across all treatments for different sampling times (T0 = initial conditions, T1 = 76 d, T2 = 417 d). AB: above-ground biomass; BB: below-ground biomass; shoots: number of shoots; max. exp.: maximal spatial expansion; DW: dry weight. Values are means \pm SE. Note: for T0, mean and SE are based on randomly picked shoots from the collected shoot pool ($n = 16$), while for T1 and T2, mean and SE are based on the plots ($n = 30$)

	AB (g DW)	Roots (g DW)	Rhizomes (g DW)	AB:BB	Shoots (n)	Max exp. (cm)	Shoot length (cm)
T0	1.83 \pm 0.24	1.19 \pm 0.17	1.01 \pm 0.13	0.83 \pm 0.04	16	17.68	19.41 \pm 0.60
T1	3.45 \pm 0.15	1.75 \pm 0.09	3.02 \pm 0.14	0.74 \pm 0.02	53.63 \pm 2.16	35.27 \pm 0.89	28.87 \pm 0.51
T2	17.79 \pm 1.79	4.57 \pm 0.52	10.35 \pm 1.16	1.23 \pm 0.05	273.30 \pm 26.30	76.30 \pm 2.41	34.51 \pm 0.69

Here, AB was 3.5 times higher at the highest nutrient concentrations compared to the transplanted AB at T0. A combination of high *Macoma* densities and high ammonium concentrations, however, resulted in an inhibition and eventually negative AB growth (Fig. 6a, *Macoma* \times ammonium, $p = 0.027$, Table 2) and thus a decrease in biomass by $\sim 50\%$ after transplantation. Similar patterns were detected

for *Zostera* rhizome and root biomass (Fig. 6b,c, *Macoma* \times ammonium, $p = 0.038$ and $p = 0.006$, respectively, Table 2) and number of shoots (Table 2). Shoot length and spatial expansion were not affected by *Macoma* or porewater ammonium (Table 2). When including phosphate instead of ammonium porewater concentrations in the model, results were comparable (Table S3).

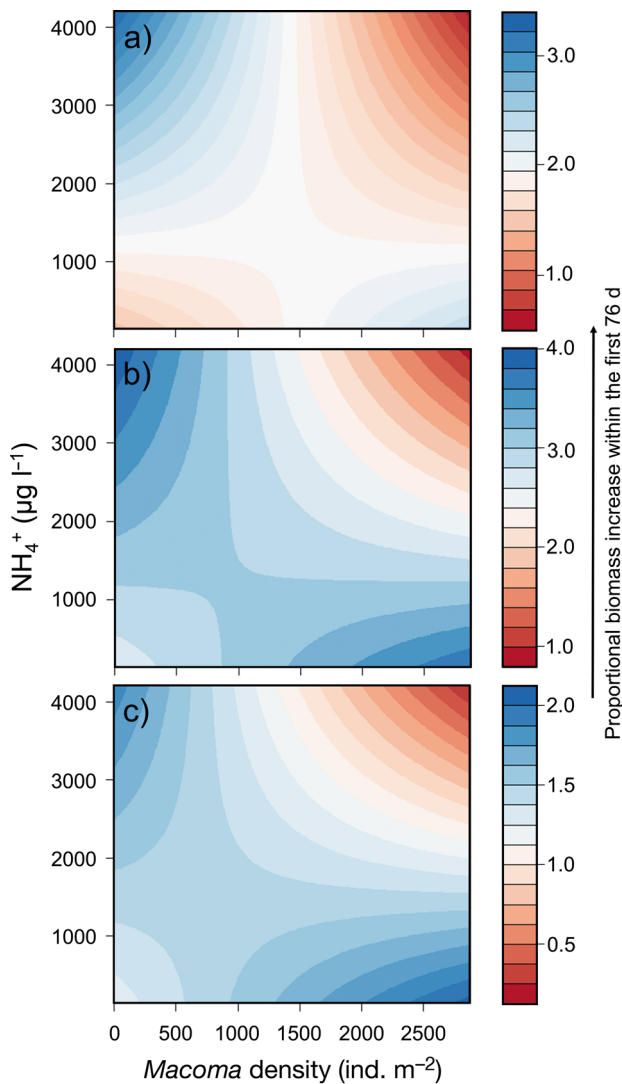


Fig. 6. Contour plots of *Zostera* biomass response (proportion T1:T0, where T1 is the first sampling event at 76 d, and T0 represents the initial values) of (a) above-ground, (b) rhizome and (c) root biomass to porewater NH_4^+ concentrations and *Macoma* density manipulation from the field experiment

3.3. Aquarium experiment

In the aquarium experiment, AB (Fig. 7a, $\chi^2 = 0.74$, $p = 0.690$), root biomass (Fig. 7b, $\chi^2 = 1.22$, $p = 0.544$), daily leaf growth rate (Fig. 7c, $\chi^2 = 0.72$, $p = 0.699$), number of shoots (Fig. 7e, $\chi^2 = 4.78$, $p = 0.092$) and sediment organic content (Fig. 7g, $\chi^2 = 2.86$, $p = 0.239$) did not differ between *Macoma* treatments (Table S4). However, there was a significant treatment effect on rhizome biomass (Fig. 7f, Table S4, $\chi^2 = 6.81$, $p = 0.033$), which was highest at low *Macoma* density, but there were no differences between high *Macoma* and control treatments. There were also significant dif-

Table 2. Linear model results from the field experiment at T1 (76 d) on the response of *Zostera* traits to *Macoma* density manipulation and underlying porewater ammonium concentrations. AB: above-ground biomass. ** $p < 0.01$, * $p < 0.05$

	df	Sum sq	F	p(>F)
AB				
<i>Macoma</i>	1	0.002	0.009	0.927
NH_4^+	1	0.004	0.024	0.878
<i>Macoma</i> × NH_4^+	1	1.870	10.956	0.003**
Residuals	23	3.926		
Rhizomes				
<i>Macoma</i>	1	0.006	0.014	0.909
NH_4^+	1	1.641	4.009	0.057
<i>Macoma</i> × NH_4^+	1	2.427	5.927	0.023*
Residuals	23	9.418		
Roots				
<i>Macoma</i>	1	0.079	0.673	0.421
NH_4^+	1	0.955	8.130	0.009**
<i>Macoma</i> × NH_4^+	1	0.840	7.145	0.014*
Residuals	23	2.703		
Shoot count				
<i>Macoma</i>	1	0.188	0.481	0.495
NH_4^+	1	0.049	0.125	0.727
<i>Macoma</i> × NH_4^+	1	3.414	8.731	0.007**
Residuals	23	8.993		
Shoot length				
<i>Macoma</i>	1	0.002	0.099	0.756
NH_4^+	1	0.013	0.556	0.463
<i>Macoma</i> × NH_4^+	1	0.027	1.170	0.291
Residuals	23	0.543		
Spatial expansion				
<i>Macoma</i>	1	0.007	0.345	0.563
NH_4^+	1	0.003	0.168	0.686
<i>Macoma</i> × NH_4^+	1	0.013	0.677	0.419
Residuals	23	0.442		

ferences between treatments for both porewater ammonium and phosphate concentrations (ammonium: Fig. 7d, $\chi^2 = 26.07$, $p < 0.001$; phosphate: Fig. 7h, $\chi^2 = 31.96$, $p < 0.001$; Table S4). Ammonium concentrations were more than 3 times lower in *Macoma* treatments compared to the control. Similarly, phosphate concentrations were more than 5 times lower in *Macoma* treatments. For both ammonium and phosphate, this effect was independent of *Macoma* density.

4. DISCUSSION

4.1. Summary of main findings

We conducted a 2 yr density-manipulation experiment and explored interactions and potential legacy effects between 2 key ecosystem engineers; eelgrass

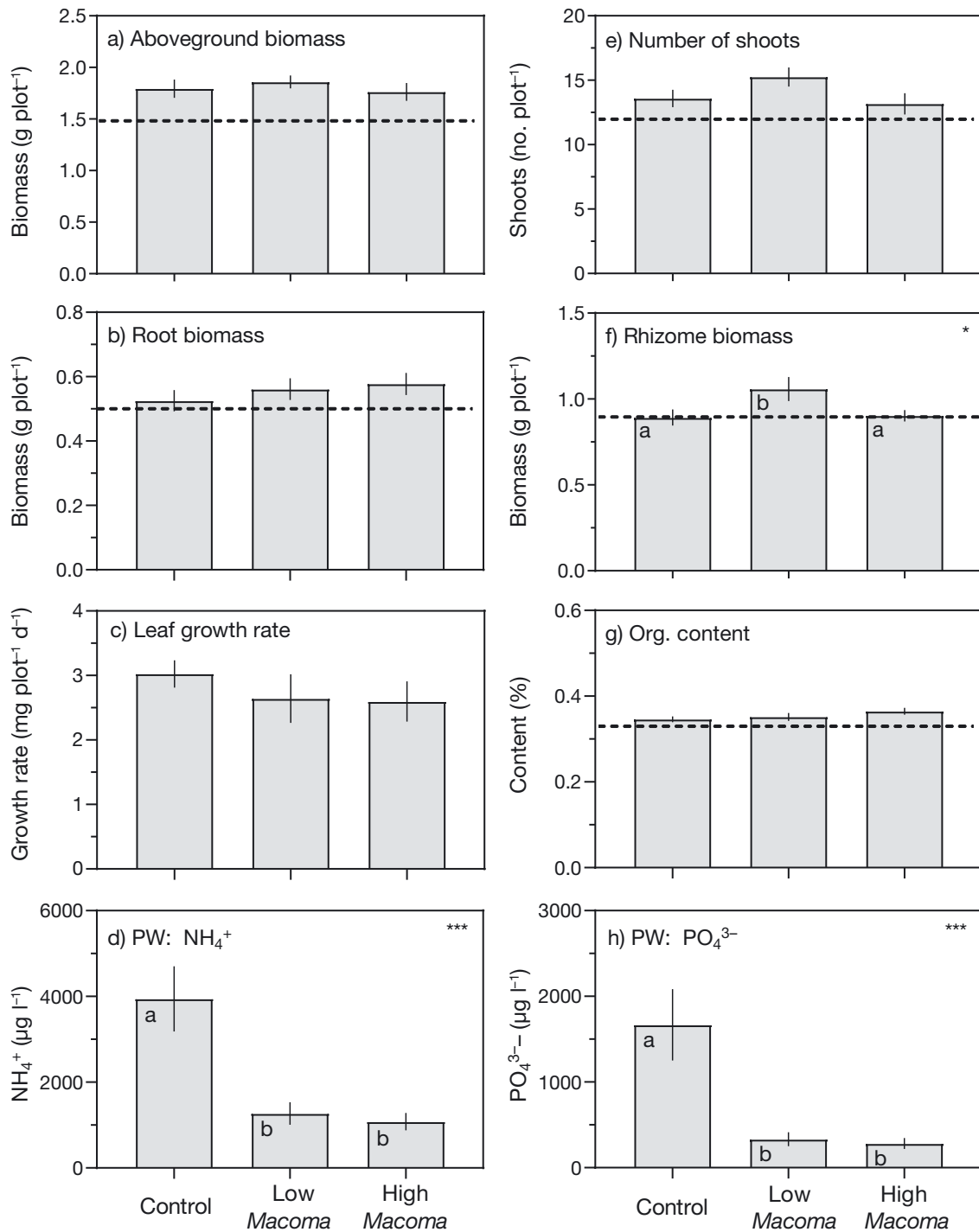


Fig. 7. Response of plant traits, sediment organic content and porewater (PW) nutrient concentrations to *Macoma* manipulations from the aquarium experiment. Values are mean \pm SE ($n = 12$). Dashed lines indicate levels at the start of the experiment. Overall statistically significant effects of treatments are indicated by *** $p < 0.001$, * $p < 0.05$. Different letters indicate significant differences ($p < 0.05$) between levels, based on Tukey multiple comparison tests

Zostera marina and the Baltic clam *Macoma balthica*. We further aimed to investigate whether these interspecific interactions could be useful for seagrass ecosystem restoration. Our results indicate that, while

Macoma often has a strong association with local seagrass meadows in the study area, its effects on *Zostera* traits are complex and context-dependent, and vary with porewater nutrient concentrations.

The strong impact of *Macoma* on nutrient release from the sediment to the water column, as shown in the aquarium experiment, seems to benefit *Zostera* only at low porewater ammonium concentrations ($<1500 \mu\text{g l}^{-1} \text{NH}_4^+$), but is potentially detrimental when ammonium concentrations are elevated.

4.2. Response of *Macoma* to *Zostera*

Zostera can promote higher abundance and diversity of associated invertebrates through provision of food sources or shelter from physical disturbance or predation (Boström & Mattila 1999, Meysick et al. 2019b). Consistent with Boström & Bonsdorff (1997), we found elevated *Macoma* abundances in *Zostera* compared to adjacent bare sediments. However, this was only true for large individuals ($\geq 5 \text{ mm}$). In contrast, the relative abundance of small clams ($< 5 \text{ mm}$) in different habitats was temporally variable: higher in *Zostera* in June, but higher in bare sediments in September. The peak settlement of *Macoma* larvae in the northern Baltic Sea typically occurs during July (Bonsdorff et al. 1995, i.e. between our 2 sampling times), indicating the presence of a settlement shadow (Orth 1992) in which larval settlement is reduced towards the interior of the *Zostera* meadow, confirming previous findings on *Macoma*–*Zostera* interactions (Boström et al. 2010). Also, the presence of algal mats, which often cover seagrass meadows in this region during summer (Gagnon et al. 2017), could explain the high spat mortality through filtering and short-term hypoxia (Bonsdorff et al. 1995). In 2017, the *Macoma* CI was higher in bare sediment compared to the ambient seagrass meadow, suggesting that food supply can be reduced towards the meadow interior, e.g. through flow reduction (Reusch 1998, Carroll & Peterson 2013). The generally low CI in 2018 might be related to a heatwave occurring in this area that year (Fig. S3). Although *Macoma* is a facultative deposit-feeding species when environmental conditions allow for it (e.g. in muddy, organic-rich sediments), the elevated organic matter concentrations within the ambient *Zostera* meadow might be a negligible food source at such low concentrations ($< 0.5\%$, Ólafsson 1986). In the field experiment, *Macoma* treatments experienced considerable loss ($> 50\%$) in clam abundance over time (T0–T1), potentially through migration and mortality. Yet, the significant correlation between manipulated and realized *Macoma* densities indicates generally favourable conditions for *Macoma* in *Zostera* patches for the investigated period, even above natural densities. Several

other studies have demonstrated opposite patterns, with lower or similar *Macoma* abundance in *Zostera* compared to adjacent bare sediments (Lappalainen et al. 1977, Boström & Bonsdorff 2000, Fredriksen et al. 2010, Dąbrowska et al. 2016, Meysick et al. 2019b), indicating that the association of *Macoma* to *Zostera* might be very site-specific and dependent on biotic and abiotic drivers, including predator abundance, wave exposure and overall food availability.

4.3. Response of *Zostera* to *Macoma*

At the termination of the field experiment, the highest above- and below-ground biomass of *Zostera* were found at the 2 highest *Macoma* density treatments. Although this may have been a potential threshold effect, we did not find any direct linear responses of *Zostera* to *Macoma* density over time. Rather, our results indicated that the response of *Zostera* to *Macoma* is mediated by the background nutrient availability in the porewater. Under low porewater ammonium concentrations, an increase in *Macoma* density seemed to promote above- and below-ground (roots and rhizomes) growth, while at higher ammonium concentrations, we observed the opposite patterns. Similarly, in the aquarium experiment, where porewater nutrient concentrations were initially high (NH_4^+ in control: $\sim 4000 \mu\text{g l}^{-1}$, see Fig. 7d), *Zostera* rhizome biomass increased at low, but not at high, *Macoma* densities relative to controls. While nutrient availability influencing the outcome of *Zostera*–*Macoma* interactions is a novel finding, it further stresses the importance of considering context-dependency to biotic or abiotic conditions in species interactions. Suspended sediment, for instance, can mediate or hamper the facilitative effect of a suspension-feeding bivalve on its associated macrofaunal community (Norkko et al. 2006). Organic matter content has been shown to negatively affect facilitation between lucinid bivalves and seagrass (Sanmartí et al. 2018), and invertebrate communities might rely more on shelter by seagrass meadows under physically harsh than benign conditions (Meysick et al. 2019b).

Nutrient cycling at the sediment–water interface is highly complex and depends on multiple covariates including macrofaunal community or sediment characteristics (Gammal et al. 2019). *Macoma* can have strong effects on the release of ammonium, nitrate and phosphate from the sediment through bioturbation and excretion (Mortimer et al. 1999, Michaud et al. 2006, Norkko et al. 2013). The burrowing activities also facilitate oxygenation of deeper sediments

by sediment mixing and increasing the sediment–water interface for solute exchange, further stimulating nitrification of ammonium to nitrate (Mayer et al. 1995, Volkenborn et al. 2012). While we did not measure nitrate concentrations in the water column, we confirmed that in the aquarium experiment, where porewater nutrient concentrations were initially high, but limited and bounded from external input, both phosphate and ammonium concentrations strongly decreased with *Macoma* presence over time, compared to controls without *Macoma*, indicating their mobilization and release into the water column. Sediment organic content was not affected by *Macoma* biodeposition or deposit feeding, neither in the field nor in the aquarium experiment, potentially since organic content was overall very low at the field site (~0.35%).

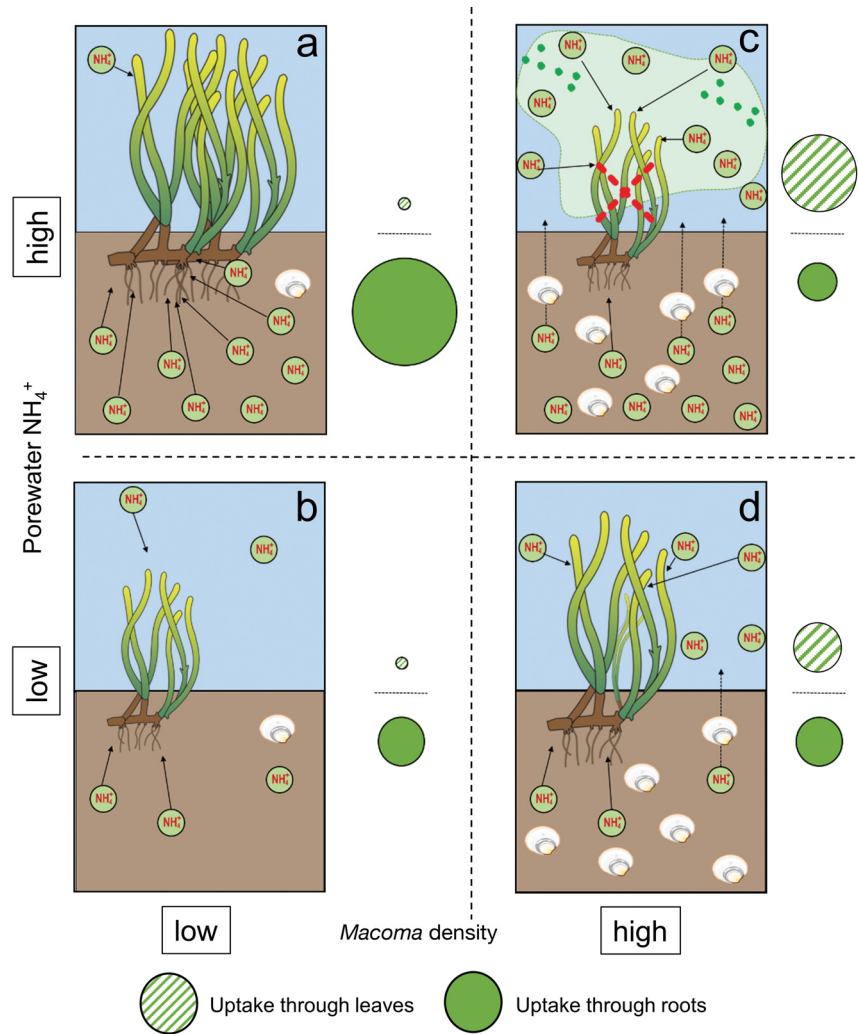
To understand the response of *Zostera* traits to *Macoma* manipulation and how this is potentially mediated by underlying porewater nutrient concentrations, it is important to first discuss potential *Zostera* nutrient sources and requirements. Since seagrasses are able to recycle a large part of their nutrients (Pedersen & Borum 1993), they have generally low requirements. Growth is suggested to be saturated at ammonium porewater concentrations above $100 \mu\text{mol l}^{-1}$, corresponding to $\sim 1800 \mu\text{g l}^{-1}$ (Dennison et al. 1987). In the southern Baltic Sea, *Zostera* does not seem to suffer from porewater nutrient limitations in early life stages; rather, intraspecific positive feedbacks (i.e. shoot density) drive growth during patch colonisation (Worm & Reusch 2000). However, in the northern Baltic Sea, sediment porewater concentration of ammonium can be much lower ($<10 \mu\text{mol l}^{-1}$), indicating that here *Zostera* occasionally suffers from nutrient limitations (Boström et al. 2004). Nutrient uptake in *Zostera* can occur both in the sediment through roots as well as in the water column through leaves (Thursby & Harlin 1982, Short & McRoy 1984, Pedersen & Borum 1993), but since ammonium (typically higher in the sediment than in the water column) is the preferred nitrogen compound, it is likely that sediment porewater is the major nitrogen source for *Zostera* (Short & McRoy 1984). However, nitrogen uptake rates in leaves and roots seem to be highly dependent on the relative ammonium concentrations in the water column and porewater. Thursby & Harlin (1982) indicated that under low ammonium concentrations in the water column, the ammonium uptake by roots follows Michaelis–Menten kinetics, i.e. increases with porewater ammonium concentrations until saturation, but root uptake was reduced by up to 80% when ammonium was added to the water column, while leaf uptake remained steady.

This mechanism might explain why we found that under low porewater nutrient concentrations in the field *Macoma* had a positive effect on *Zostera* biomass. Since *Macoma* promotes ammonium release to the water column, it could increase the total nutrient uptake of *Zostera*, by providing an additional nitrogen source without affecting the in any case low uptake by roots. At high porewater nutrient concentrations and high clam abundance, however, the excess ammonium in the water column might have caused inhibition of uptake through roots, resulting in an overall lower nutrient uptake. Other studies suggest that elevated ammonium concentrations in the water column can also cause necrosis and impair carbohydrate metabolism of seagrasses (Burkholder et al. 1992, 1994, van Katwijk et al. 1997). Simultaneously, phytoplankton and epiphytic growth on *Zostera* leaves can be facilitated when nutrient concentrations increase (Dennison et al. 1989, Sand-Jensen & Borum 1991, Neckles et al. 1993). This can eventually lead to a shift from slow-growing seagrasses to a system dominated by phytoplankton (Duarte 1995, Krause-Jensen et al. 2012). Fig. 8 summarizes these conditional interactions in a conceptual model. However, since we did not specifically manipulate the nutrient conditions in the field, but rather included an underlying nutrient gradient into the model, our results presented here have to be interpreted carefully. We therefore encourage testing the effect of *Macoma* on growth of *Zostera* in controlled lab and/or field conditions at sufficient time scales and under the hypothesis that nutrient availability mediates these interactions

4.4. Implications for (co-)restoration

In the last few decades, *Zostera* has been lost in northern European seas (Boström et al. 2014, Moksnes et al. 2018, de los Santos et al. 2019) due to eutrophication, diseases and heat waves, with calls for increased restoration efforts. Indeed, the heat wave in 2018 (Fig. S3) caused a strong decline in seagrass cover at the field site used in this study (L. Meysick pers. obs.), highlighting the necessity for successful and easily applicable restoration methods. Since restoration techniques tested in the Kattegat–Skagerrak region, e.g. the use of seed transplantation (Eriander et al. 2016, Infantes et al. 2016), cannot be used in the low-salinity northern Baltic, novel approaches are needed (Gagnon et al. 2020). Here, we evaluated our results from field and aquarium experiments in terms of restoration implications:

Fig. 8. Conceptual model of nutrient uptake by *Zostera* and how it is potentially mediated by *Macoma*. (a) When porewater ammonium concentrations are high, uptake through roots is high. (b) When porewater ammonium concentrations are low, uptake through roots is low. (c) *Macoma* increases nutrient release from the porewater to the water column through bioturbation and excretion. At high *Macoma* densities and high porewater nutrient concentration, this leads to increased water column nutrient concentrations, which can inhibit nutrient uptake through roots (Thursby & Harlin 1982), cause necrosis (Burkholder et al. 1992, van Katwijk et al. 1997) and increase turbidity through phytoplankton (symbolized as light green area and green dots) (e.g. Neckles et al. 1993), potentially leading to die back (red cross) of *Zostera*. (d) At low nutrient concentrations, however, nutrient release from the porewater by bioturbation can provide an alternative nutrient source in the water column, below toxic levels, and thus facilitate total nutrient uptake. Seagrass image courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/)



the transplantation via attachment of shoots to a plastic mesh was successful with a 100% survival rate and a mean 8-fold increase in total biomass 14 mo (T2) after transplantation. From a sustainability perspective, however, future restoration actions should consider biodegradable materials for attachment of shoots, such as coconut fibre (Sousa et al. 2017) or Hessian (burlap) bags (Unsworth et al. 2019). The initial decrease in the AB:BB ratio (T1) indicates that anchoring and nutrient uptake via roots plays a prioritised role during establishment. Thereafter, AB increased relatively quickly, while growth of below-ground biomass was comparably low (indicated by a high increase in the AB:BB ratio from 0.74 ± 0.02 at T1 to 1.23 ± 0.05 at T2).

Incorporating positive interactions has been shown to increase success rates in coastal ecosystem restoration projects (e.g. Silliman et al. 2015, Angelini et al. 2016, Gagnon et al. 2020 and references therein). Specifically, bivalves such as blue mussels *Mytilus*

edulis (Worm & Reusch 2000, Bos & van Katwijk 2007) or eastern oysters *Crassostrea virginica* (Grizzle et al. 2018) can support seagrass restoration, as mussels enhance nutrient availability through deposition and protection from hydrodynamics, while oysters increase light availability through particle filtering. However, we recommend careful consideration of environmental conditions, especially porewater nutrient concentrations, and informed site selection prior to restoration actions that include infaunal tellinid clams, since our results indicated variable outcomes of *Macoma*–*Zostera* interactions. While at sites with low porewater nutrient concentrations, *Macoma* could potentially facilitate *Zostera* restoration by stimulating nutrient uptake, it might have an opposite, potentially negative effect when nutrient concentrations are high, counteracting restoration efforts. Similarly, Reusch & Williams (1998) indicated that plant–bivalve restoration can be context-dependent. They showed how intermediate Asian date mussel *Musculista sen-*

housia biomasses supported leaf growth by increasing ammonium concentrations, whereas high mussel biomasses had a negative effect. Given the small response of *Zostera* to *Macoma* addition in the controlled laboratory experiment and the variable outcome in the field, based on this study we do not recommend the use of *Macoma* for *Zostera* restoration in the Baltic Sea. However, we acknowledge its importance for nutrient fluxes in these habitats. Our observations provide valuable mechanistic insights that can help guide future restoration efforts that incorporate interspecies interactions.

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Editorial responsibility: Adriana Vergés,
Sydney, New South Wales, Australia

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