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Limnology and Oceanography

Volume 65, Issue 1

2020,

pp. 37-50

The publisher's version is available at:
https://doi.org/10.1002/lno.11246

Ocean acidification alters meiobenthic assemblage composition and organic matter degradation rates in seagrass sediments

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Running head: benthos responses to multiple stressors

Keywords: bacteria, climate change, enzymatic activity, fertilization, global-scale change, marine angiosperm, meiofauna, multiple stressors

Abstract

Seagrass meadows are an important organic matter (OM) reservoir, but are currently being lost due to global and regional stressors. Yet, there is limited research investigating the cumulative impacts of anthropogenic stressors on the structure and functioning of seagrass benthic assemblages, key drivers of OM mineralization and burial. Here, using a 16-months field experiment, we assessed how meiobenthic assemblages and extracellular enzymatic activities (as a proxy of OM degradation) in *Posidonia oceanica* sediments responded to ocean acidification (OA) and nutrient loadings, at CO_2 vents. *P. oceanica* meadows were exposed to three nutrient levels (control, moderate and high) at both ambient and low pH sites. OA altered meiobenthic assemblage structure, resulting in increased abundance of annelids and crustaceans, along with a decline in foraminifera. In addition, low pH enhanced OM degradation rates in seagrass sediments, by enhancing extracellular enzymatic activities, potentially decreasing the sediment carbon storage capacity of seagrasses. Nutrient enrichment had not effect on the response variables analysed, suggesting that, under nutrient concentration unlikely to cause N- or P- limitation, a moderate increase of dissolved nutrients in the water column had limited influence on meiobenthic assemblages. These findings show that OA can significantly alter meiobenthic assemblage structure and enhance OM degradation rates in seagrass sediments. Since meiofauna are ubiquitous key actors in the functioning of benthic ecosystems, we postulated that OA, altering the structure of meiobenthic assemblages and OM degradation, could affect organic carbon sequestration over large spatial scales.

Introduction

Seagrass meadows are among the most productive and valued ecosystems on Earth, as they sustain biodiversity and a range of ecosystem services, including enhanced water quality, coastline protection from erosion and productive fisheries (Larkum et al. 2007, Barbier et al. 2011). They also have a large influence on coastal biogeochemical processes, such as carbon storage and nutrient regeneration, at global scale (Fourqurean et al. 2012, Duarte et al. 2013, Macreadie et al. 2017). These biogeochemical processes occur mostly in the belowground sediments and are driven by interactions between fauna and heterotrophic prokaryotes, primary mediators of OM mineralization and burial (Danovaro 1996, Snelgrove et al. 2018, Trevathan-Tackett et al. 2018a).

Many seagrass beds, and the ecosystem functions and services they provide, have been degraded worldwide, with an estimated global decline of 7% annually since 1990 (Orth et al. 2006, Waycott et al. 2009). Coastal eutrophication is one of the major drivers of seagrass loss, either resulting in nitrogen toxicity for plants or reduced light availability on leaves due to epiphyte overgrowth (Ralph et al. 2006, Burkholder et al. 2007, Marbà et al. 2014). More recently, global scale stressors, such us seawater warming, ocean acidification and extreme events, have been shown to impair plant production and contribute to the decline and degradation of seagrasses (Marba and Duarte 2010, Jordà et al. 2012, Ravaglioli et al. 2017, Arias-Ortiz et al. 2018, Chefaoui et al. 2018). As seagrass meadows are key for organic carbon sequestration, their decline is raising concerns over the potential release in the atmosphere, as CO_2, of large amounts of the carbon immobilized by the belowground compartment, potentially exacerbating climate changes (Fourqurean et al. 2012, Pendleton et al. 2012). Nonetheless, the cumulative impacts of environmental stressors on the structure and functioning of benthic assemblages associated to seagrass systems, and their links to biogeochemical cycles, remain poorly understood.

Anthropogenic ocean acidification (OA), resulting from the global enhanced CO_2 emission, is one of the greatest threats to coastal habitats (IPCC 2014). While the responses of seagrasses and the associated epiphytic communities to low pH have been thoroughly assessed (Hall-Spencer et al. 2008, Martin et al. 2008, Campbell and Fourqurean 2014, Cox et al. 2015, Guilini et al. 2017, Ravaglioli et al. 2017), there is a dearth of studies dealing with the impacts of OA on meiofauna that inhabit seagrass sediments. This abundant and high diverse group of small invertebrates (< 1mm) plays key ecological roles in marine sediments, contributing to energy transfer to higher trophic levels (Schratzberger and Ingels 2018) and increasing OM remineralisation, through the stimulation of microbial activities (Nascimento et al. 2012, Bonaglia et al. 2014, Lacoste et al. 2018). OA can change meiobenthic assemblages, either directly, by altering metabolic processes or, indirectly, by modifying interactions among species and trophic groups (e.g. predation pressure) (Kurihara et al. 2004, Dashfield et al. 2008, Widdicombe and Spicer 2008, Kroeker et al. 2011, Meadows et al. 2015, Mevenkamp et al. 2018). Laboratory (generally short-term) studies have reported divergent responses to OA of different meiobenthic taxa, with the dominant one (typically nematodes and copepods) generally remaining unaffected or even increasing in abundance, while others, such as copepod naupli, gastrotrichs and foraminifera, showing opposing trends (Haynert et al. 2011, Meadows et al. 2015, Lee et al. 2017, Mevenkamp et al. 2018). In contrast, long-term exposure to low pH condition at submarine CO_2 vents led to a severe decline in meiofaunal density, suggesting limited capacities for several taxa to withstand or adapt to OA (Molari et al. 2018). The alteration or loss of meiofaunal biodiversity could ultimately result in a significant decline of important ecosystem functions, including prokaryote production and OM mineralization (Danovaro et al. 2008, Pusceddu et al. 2014b).

OA could further affect OM degradation in seagrass sediments, by altering microbial activities. Microbes mediate OM degradation by releasing extracellular enzymes, which catalyse the degradation of complex and refractory molecules to more labile forms of OM that, in turn, can be used by heterotrophs (Cunha et al. 2010). Several studies suggested that bacteria extracellular activities may increase under OA scenario, likely triggered by the higher availability of organic resources due to enhanced primary production (Cunha et al. 2010, Piontek et al. 2013, James et al.

2017). This could ultimately result in the reduction of organic carbon sequestration in seagrass sediments (Trevathan-Tackett et al. 2018a).

In addition to the global threat of OA, the structure and functions of benthic assemblages associated to seagrass system can be further affected by local changes of dissolved inorganic nutrient concentrations in seawater. In seagrass sediments, a large amount of organic detritus is generally refractory and not readily available for consumers (Danovaro 1996, Pusceddu et al. 2003). Enhanced nutrient concentration might increase the abundance and diversity of meiofauna indirectly, by enhancing the nutritional quality of food (e.g. lower C/N ratio) (Antón et al. 2011), thus fostering feeding activities (Pascal et al. 2013). In contrast, excessive organic loadings, typical of eutrophic waters, may strongly alter sediment characteristics (e.g. sediment biochemical composition and oxygen availability) (Pusceddu et al. 2009, Pusceddu et al. 2011), which could affect negatively meiofaunal assemblages (La Rosa et al. 2001, Mirto et al. 2002, Gambi et al. 2009).

Nutrient enrichment has been further shown to enhance sediment microbial biomass and their enzymatic activities in seagrass sediments (López et al. 1998, La Rosa et al. 2001, Liu et al. 2017), potentially exacerbating the enhanced degradation rate of OM expected under OA scenario. However, to date, the compounded effects of OA and nutrient enrichment on community composition and OM processes associated to seagrass sediments remain largely unexplored, challenging our capacity to predict alterations in ecosystem functioning and services of seagrasses under future environmental conditions.

In this study, we investigated the effects of OA and enhanced nutrient availability on meiobenthic assemblages and OM degradation rates in *Posidonia oceanica* sediments, at CO_2 vents along the coast of Ischia Island (Italy). We exposed *P. oceanica* meadows, at both ambient and low pH, to different levels of nutrient enrichment (control, moderate and high) for 16 months. Under OA scenario, food availability seems to play a critical role for marine invertebrates, by providing the energy required to support physiological responses to pH stress (Thomsen et al. 2013, Queiros et al. 2015, Ramajo et al. 2016). Under these circumstances, a moderate increase in nutrient availability could have positive effects on meiobenthos at low pH, possibly increasing the consumption of more bioavailable food (Danovaro 1996, Antón et al. 2011). By contrast, excessive nutrient loadings could worsen the impacts of OA on meiobenthos, by causing severe OM accumulation and lowering sediment oxygen concentration (Gambi et al. 2009). In addition, the combined effects of OA and enhanced nutrient concentration were expected to significantly increase OM degradation, by fostering extracellular enzymatic activities of bacteria (López et al. 1998, Piontek et al. 2013).

Materials and methods

Study site and experimental design

This study was carried out between April 2014 and July 2015 in shallow *P. oceanica* meadows at CO_2 vents off the Castello Aragonese isle (Ischia Island, 40°43’51.01’’N, 13°57’48.07’’E; Tyrrhenian Sea, Italy). Submarine vents have been extensively used to assess the effects of naturally acidified seawater on biological communities as they are characterized by the emission into seawater of gases, predominantly CO_2, that create gradients in pH and carbonate chemistry, without confounding gradients of other environmental variables, such as temperature, salinity, hydrodynamic conditions and toxic hydrogen sulphide (Hall-Spencer et al. 2008, Fabricius et al. 2011, Russell et al. 2013, Milazzo et al. 2016, Doubleday et al. 2019). In particular, in the last decade, previous studies carried out at Ischia Island vents have shown that areas exposed to CO_2 bubbling do not differ from control areas in terms of salinity (38 ‰), temperature (seasonal fluctuations of 14-25 °C), light (~7500 lx d^{-1}) and total alkalinity (2.5 mequiv. kg^{-1}), due to the fact that they are just 10s of m apart, at about 2-3 m water depth (Hall-Spencer et al. 2008, Martin et al. 2008, Cigliano et al. 2010, Kroeker et al. 2011, Garrard et al. 2014, Scartazza et al. 2017).

The effects of OA (ambient and low pH) and nutrient enrichment (control, moderate and high) on meiobenthic assemblages and microbial OM degradation were evaluated through a manipulative experiment. We identified two pH levels in dense and continuous meadows: ambient pH site and low pH site, the latter reflecting the pH value predicted by the end of the century. In order to measure the relative changes in pH between sites, water samples were taken from the water column using a 125 ml bottle at 11 and 10 dates, at ambient and low pH sites respectively, randomly chosen between May 2014 and March 2015. Measurements were made using a Mettler Toledo SG2 pH meter (accuracy ± 0.01 pH units) equipped with an InLab 413 electrode and calibrated regularly using NIST-traceable buffers. Although this approach does not measure the total hydrogen ion concentration, it provides an estimate of the relative change in pH between sites. The average pH (NBS scale) at ambient and low pH sites was 8.11 ± 0.007 and 7.78 ± 0.05 respectively (±SE, n=55 and n=50). In addition, in situ seawater pH measurements were recorded from June to July 2015, at the low pH site, using a SeaFET pH sensor, which records pH hourly. The average pH (total scale) was 7.74 ± 0.014 (±SE, n=464), with 42% of the hourly pH values below 7.8 (the predicted mean seawater pH value for the year 2100), in line with the results of (Kroeker et al. 2011).

An HOBO data logger was positioned between the two sites in order to monitor continuously (every 15 minutes) seawater temperature throughout the experiment. Temperature matched ambient season fluctuations, with warmest water occurring in August (26.3 ± 0.008) and coldest water in February-March (14.95 ± 0.006). Temperature was not expected to vary between sites at a depth of 2.5-3.5 m.

In April 2014, at each site, nine experimental plots (50 x50 cm) were established at a depth of about 3 m within *P. oceanica* meadow and marked at their corners using iron rebars. Three plots were then randomly assigned to each nutrient level (control, moderate and high), for a total of 18 replicate plots. Nutrients (Osmocote slow release fertilizer pellets, 17:11:10 N:P:K) were added in three plastic net bags (1-mm mesh size) per plot, fixed by means of plastic cable ties to a iron bar hammered in the middle of each plot. Nutrient bags were, thus, suspended at a distance of about 10 cm from the bottom, within seagrass canopy. This method has been widely used in previous manipulative experiments to assess the impacts of elevated nutrient concentration in marine systems (Worm et al. 2000, Bulleri et al. 2012, Tuya et al. 2015). The amount of fertilizer used to generate the high and moderate nutrient levels were, respectively, 400 g (three bags containing 133 g each) and 200 g (three bags containing 67 g each). Nutrient bags were replaced every two months, ensuring their effectiveness in releasing nutrients. Fertilizer weight in each nutrient bag was measured at the third decimal by means of a precision scale before deployment. Upon retrieval, nutrient bags were dried in a muffle for 28 hours at 60 °C and the amount of fertilizer that had not dissolved was re-weighted in order to estimate the total average nutrient released over the duration of the experiment. The amount of fertilizer released was significantly higher at high than moderate nutrient supply, while the amount of nutrient released did not different between pH levels ($F_{1,8}$=101.32; $P < 0.001$). In addition, in order to estimate water nutrient concentration, two water samples were taken from the water column in each experimental plot, using a 60 ml syringe, at three random dates during the experiment (May 2014, June and July 2015). Higher concentration of total dissolved inorganic nitrogen and phosphate were achieved under enhanced nutrient treatments compared to control level (Fig. S1).

Meiobenthic assemblage structure

At the end of the experiment (July 2015), meiobenthos (i.e. metazoan meiofauna plus foraminifera) abundance and taxa diversity were assessed in two sediment samples, randomly collected in each experimental plot, for a total of 36 replicates. Sediment cores were hand sampled by divers, by inserting Plexiglas cores (30 mm internal diameter and 270 mm length) at least 5-10 cm into *P. oceanica* matte. Once collected, each sediment sample was transferred in net bags and preserved in 70% ethanol solution until analysis. In laboratory, the meiobenthos was extracted using the decantation method. The samples were sieved through a 500-µm mesh (upper limit) and 50-µm mesh size (lower limit) to retain the meiobenthic organisms (Pusceddu et al. 2014b, Bertocci et al. 2019). The extraction procedure was repeated five times, and at each time, sediment was carefully checked in order to collect all the animals. All animals were then counted and classified per taxon under stereomicroscope. The invertebrates were classified to the taxonomic resolution varying from phylum to order. Due to variations in volume and composition (sandy sediment, plant detritus and gravel) among cores, sediment samples were left to dry in the laboratory for two weeks and, then, the total weight of each sample and that of its sandy, gravel and plant detritus fractions were measured at the third decimal by means of a precision scale. Grain size analysis was carried out by dry sieving sediment through a 1-mm mesh to separate sandy sediment from gravel and plant detritus fractions. Although meiofaunal abundance is typically expressed as number of individuals 10 cm^{-2}, due to different volume of sediment in each core, the abundance of individuals of each taxon was standardized to the sediment weight (g DW) for each sample. Taxonomic diversity was measured using the Shannon index.

Extracellular enzymatic activities in the sediment

Samples for extracellular enzymatic activities (aminopeptidase and ß-glucosidase) were collected at the end of the experiment. Two aliquots (topmost 2 cm) of sediment were collected from each experimental plot, using Plexiglas tubes, for a total of 36 replicates. For the determination of the extracellular enzymatic activities, 2.5 mL of sediment subsamples were incubated at 20°C in the dark for 2 h with 2.5 mL of filtered, sterile seawater containing 200 μM L-leucine-4-methylcumarinyl-7-amide and 75 μM 4-methylumbelliferyl β-D-glucopyranoside separately for aminopeptidase and β-glucosidase activities, respectively. After incubation, the slurries were centrifuged and supernatants were analysed fluorometrically (at 365 nm excitation, 455 nm emission for b-glucosidase, and 380 nm excitation, 440 nm emission for aminopeptidase). Data were normalized to sediment dry weight (60 °C, 24 h) and reported as μmol substrate degraded g^{-1} h^{-1} (Pusceddu et al. 2003). The aminopeptidase and β-glucosidase activities were converted into C degradation rates (μgrams of C per gram per hour), using 72 μg of C per μmole of substrate as the conversion factor (Pusceddu et al. 2014a).

Statistical analyses

Effects of OA and nutrient enrichment on meiobenthic assemblages were tested by means of a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) performed on a Bray-Curtis dissimilarity matrix of untransformed data. The model included two factors: OA (fixed, with two levels: ambient and low pH) and nutrient enrichment (fixed, with three levels: control, moderate and high). To visualize patterns of variations in the meiobenthic assemblages between ambient and low pH and among nutrient enrichment levels an MDS plot after ordination of untransformed data was obtained from Bray-Curtis dissimilarities. A SIMPER analysis was applied to determine which groups were responsible for the dissimilarities among experimental treatments. A principal component analysis (PCA) was used to assess differences in the sediment composition (in term of sandy, gravel and plant detritus fractions) of plots under different experimental conditions. A two-way analysis of variance (ANOVA), with OA and nutrient enrichment as fixed orthogonal factors, was carried out on univariate data (meiobenthos abundance, taxa diversity and extracellular enzymatic activities). Cochran's C-test was used to check for homogeneity of variances and, when necessary, data were log- or square-root transformed. PERMANOVA, MDS, SIMPER, PCA and ANOVA were performed using the software R.

Results

Composition of sediment cores

The PCA on the sediment composition of sampling cores showed a substantial separation between ambient and low pH conditions (Fig. 1). Along PC1 axis, which explains 97.42 % of the total variance, there was one cluster including ambient pH on the left side of the plot and a second one on the right side, represented by low pH treatment. Sandy sediment component was the most negatively correlated to PC1, while gravel component contributes most to PC2 axis, which, however, explains only 2.29 % of the total variance. Sandy sediment content was then included as covariate in the PERMANOVA analysis assessing variations in the structure of the meiobenthic assemblage.

Meiobenthic assemblage structure

The results of PERMANOVA showed significant differences in the structure of meiobenthic assemblages between ambient and low pH, regardless of nutrient treatments, which emerged also in the MDS ordination (Table 1, Fig. 2). There was no significant effect of the covariate (sandy sediment component). The SIMPER analysis showed a 28.6% contribution of nematodes to the dissimilarities between ambient and low pH, annelids (7%), which include polychaetes and oligochaetes, foraminifera (12.4%) and crustaceans (1.9%), which include copepods, cumaceans, amphipods, isopods and tanaids. All other taxa (including molluscs, ophiuroids, acarines, pantopods) contributed less than 0.5% to the overall dissimilarity (Table S1). ANOVA analyses were performed on meiobenthos groups mainly responsible for observed community changes. Since polychaetes and oligocheates, as well as, copepods, cumaceans, amphipods, isopods, tanaids responded similarly to low pH (Table S2; Fig. S2), they were pooled into two broad taxonomic groups of annelids and crustaceans, respectively.

There were no significant differences in the abundance of nematodes under different experimental conditions (Table 2, Fig. 3a). The abundance of annelids was higher at low than at ambient pH, but was unaffected by nutrient enrichment (Table 2, Fig. 3b). In contrast, foraminifera significantly decreased at low pH (Table 2, Fig. 3c). Although the effects of OA were not statistically significant, there was a trend for crustacean abundance to increase at low pH compared to ambient pH, regardless of nutrient treatments (Table 2, Fig. 3d). Finally, we did not detect significant effects of OA and nutrient enrichment on total meiobenthic abundance and taxa diversity (Table 2).

Extracellular enzymatic activities

The aminopeptidase and ß-glucosidase activities, used as proxies of protein and carbohydrate degradation rates, varied according to pH conditions, but were unaffected by nutrient enrichments (Table 3). Both extracellular enzymatic activities were higher at low than at ambient pH (Fig. 4 a,b).

Discussion

At our study site, long-term OA altered the composition of meiobenthic assemblages as well as OM degradation rates in seagrass sediments. Changes in meiobenthic assemblages were mostly due to an increase in the abundance of annelids and, to some extent, of crustaceans, whilst foraminifera abundance significantly decreased at low pH. In addition, OA appears to stimulate the microbial degradation of OM in seagrass sediments, potentially weakening the carbon storage capacity of seagrass meadows. In contrast to our predictions, enhanced nutrient levels had no effects on meiobenthic assemblages and OM degradation rates, and interactions between nutrient enrichment and OA were not detected.

Previous studies have already shown that OA can shift meiobenthic community composition, as a result of differential sensitivity of the different taxa (Hale et al. 2011, Schade et al. 2016, Mevenkamp et al. 2018). In accordance with the literature, nematodes, the dominant meiobenthic taxon at our study site, were unaffected by low pH. Results from previous studies, though mostly conducted under controlled laboratory settings, suggest that nematodes can be highly tolerant to low pH, as their densities were often unaffected or even increased under the OA scenario predicted for the end of this century (Dashfield et al. 2008, Widdicombe et al. 2009). Negative effects on nematode survivorship have been documented only at extremely low pH levels ($\sim \leq 6$). However, a recent study using a staining technique, found an increase in nematode mortality under OA, while nematode density was unaffected, likely due to a reduced degradation rate of dead nematode bodies at low pH (Mevenkamp et al. 2018). These results stress the importance of assessing nematode mortality in OA studies, as stable or even increased densities of these animals could be an artefact of reduced body decomposition, potentially hiding more severe impacts of OA on this dominant group.

We documented an increase in the abundance of annelids at low pH, in line with reports of previous studies on epibenthic fauna at submarine CO_2 vents (Kroeker et al. 2011, Ricevuto et al. 2012, Garrard et al. 2014). In this regard, it has been reported that, around CO_2 vents of Ischia, polychaetes maintain high density along pH gradients, suggesting that some species may be tolerant to OA due to their high physiological plasticity or local adaptation (Calosi et al. 2013). However, responses to low pH vary among different groups of polychaetes, with filter feeder and herbivore species generally favoured at expenses of deposit feeders, omnivores and carnivores (Gambi et al. 2016, Molari et al. 2018). Thus, a more detailed analysis on polychaete species composition or functional traits could provide further insights on the sensitivity of this taxonomic group to long-term low pH exposure. Furthermore, there was a tendency ($P= 0.08$) in the abundance of crustaceans to increase at low pH. Crustaceans are considered quite robust to OA due to their internal acid-base regulation and external organic layer that protect skeleton from corrosive low pH water (Melzner et al. 2009, Ries 2009). Although some studies found negative effects of low pH on reproduction or larval development of copepods (Kurihara et al. 2004, Fitzer et al. 2012), studies testing the effects of OA at the community level showed no changes or even an increase in crustacean abundance at low pH, possibly due to decreased predation rate or increased food availability (Kroeker et al. 2011, Garrard et al. 2014).

The abundance of foraminifera significantly decreased at low pH site. Previous studies reported a substantial vulnerability of benthic foraminifera to OA (Hall-Spencer et al. 2008, Fabricius et al. 2011, McIntyre-Wressnig et al. 2013, Martinez et al. 2018), likely because many of them build shells of calcium carbonate. Accordingly, decreases in the diversity of foraminifera community and changes in their community composition from calcifying to non-calcareous forms has been reported in the Mediterranean Sea (around CO_2 vents of Ischia; Dias et al. 2010) and Pacific Ocean, around CO_2 vents of Papua New Guinea (Fabricius et al. 2011). These unicellular organisms are a key benthic component in coastal systems, since they serve as food source together with the rest of meiofauna for higher trophic levels, and are important contributors of the annual carbonate production and denitrification process in coastal areas (Risgaard-Petersen et al. 2006, Høgslund et al. 2008, Langer 2008). Although our study cannot discern if foraminifera were alive, the significant reduction in their abundance detected at low pH suggest that OA could have negative cascading effects on carbon and nutrient cycles within seagrass meadows.

The divergent responses of taxonomic groups to low pH lead to no differences in term of total meiofaunal abundance and taxa diversity. These results are in contrast with those of Molari et al. (2018), who found a decrease in the biomass and density of meiofauna in sandy areas near CO_2 vents. Such discrepancy could be due to the different habitat characteristics (bare sandy sediment *versus P. oceanica* sediments in Molari et al. (2018) and our study site, respectively). In fact, seagrass sediments are generally characterized by higher supply of organic matter, derived from both seagrass production and the trapping of other organic particles (Kennedy et al. 2010). Although we did not measure the amount of organic matter in *P. oceanica* sediments, the supposed higher food availability may have mediated the susceptibility of marine invertebrates to low pH at our study site. The lack of detectable effects of low pH on taxa diversity could also be influenced by the taxonomic aggregation used in this study. Thus, the use of a fine taxonomic resolution (e.g. genus or species levels) could provide a deeper insight into the changes in community diversity and composition under future climate scenario (Bevilacqua et al. 2012).

Low pH fostered extracellular enzymatic activities in seagrass sediments. Extracellular enzymes play a crucial role in benthic systems as they break down high molecular weight organic compounds into low molecular weight compounds that can then be readily used by heterotrophs (Cunha et al. 2010). Contrary to intracellular enzymes that are buffered by the cell's cytoplasm, extracellular enzymatic activities are directly impacted by external changes in pH. An increase in the H^+ concentration, due to lower seawater pH value, may modify the three-dimensional protein structure of the active site of the enzyme, thus affecting enzymatic activities (Cunha et al. 2010). At the same time, changes in the meiobenthic assemblage composition at low pH, with an increase in the abundance of annelids and a reduction of foraminifera, could have entailed cascading effects on microbial-mediated OM degradation rate (Piot et al. 2014, Lacoste et al. 2018). For instance, polychaetes are known to enhance bacterial activities, either directly, by consuming bacteria and thus stimulating their growth (Montagna 1984), or, indirectly, through particle reworking and solute transport due to bioturbation activity (Aller and Aller 1992). In addition, an increase in extracellular enzymes under OA could also be related with enhanced availability of organic matter as a consequence of higher primary productivity (Piontek et al. 2013). Regardless of the specific mechanisms stimulating microbial extracellular enzymatic activities, our results suggest that long-term OA may lead to increased degradation of carbohydrates and proteins in seagrass surface sediments. Our findings can be generalized as previous results from benthic (Molari et al. 2018) and pelagic (Grossart et al. 2006, Piontek et al. 2013) systems found an increase in the extracellular enzymatic activity at low pH. A further decline in pH could, however, result in a decreased rate of enzymatic activity (Cunha et al. 2010). For instance, in a mesocosm experiment, (Rastelli et al. 2016) reported that very low pH value (< 7), associated to high CO_2 leakages, can result in a significant reduction of the aminopeptidase and ß-glucosidase activities and an increase in sediment protein accumulation. Finally, variable effects of OA on OM degradation rates could also depend upon the different edaphic conditions (i.e. grain size and mineralogy) in different sediment typologies.

None of the response variables analysed was affected by enhanced nutrient loading. We hypothesized that a moderate nutrient enrichment would have been able to mediate meiobenthos responses to low pH indirectly, by increasing food quality. However, at our study site, background N P concentrations were comparable to those observed in urbanized coastal areas in the NW Mediterranean (Balata et al. 2008, Balata et al. 2010), and, therefore, unlikely to be limiting for benthic invertebrates. Furthermore, a previous work has documented low C/N ratio of organic detritus at CO_2 vents of Ischia, suggesting no nitrogen deficiency in invertebrate diets at low pH

(Ricevuto et al. 2015). In contrast, severe nutrient enrichment may negatively affect meiobenthic assemblages and foster bacterial activity in seagrass sediments as a consequence of the severe modifications caused to sediment chemistry (e.g. high biopolymeric carbon content and reduced O_2 availability) (López et al. 1998, Gambi et al. 2009, Pusceddu et al. 2011). In our experiment, the simulation of heavy nutrient enrichment of the water column was not effective in generating concentrations high enough to cause severe organic matter accumulation in the sediments, as observed in eutrophic systems (Dell'Anno et al. 2002, Pusceddu et al. 2009). Indeed, signals of seagrass meadow degradation are often reported in coastal systems characterized by dissolved nutrient concentrations considerably higher than those generated in our experiment (Cardoso et al. 2010, Hughes et al. 2013). Also, it has been repeatedly observed that the impacts of eutrophication in terms of inorganic nutrient concentration in the water column (Burkholder et al. 2007) could not be automatically detected in the benthic environment (López et al. 1998, Dell'Anno et al. 2002, Pusceddu et al. 2009, Pusceddu et al. 2011). Moreover, the lack of detectable effects of enhanced nutrient availability in the water column on the benthos could also be explained considering that we tested our hypotheses in *P. oceanica* sediments, where, because of the high background loads of OM in seagrass sediments, effects of benthic eutrophication could be not clearly detected (Pusceddu et al. 2007).

Overall, the results of our experiment show that long-term OA can significantly alter meiobenthic assemblage composition and foster microbial OM degradation in *P. oceanica* sediments. In contrast, nutrient enrichment did not affect seagrass benthic assemblages, probably because our experiment was not effective in generating eutrophic conditions. This could explain the lack of interactive effects between OA and nutrient loading on all the response variables analysed. Therefore, the response of seagrass assemblages to OA under eutrophic conditions is yet to be explored. To the best of our knowledge, this is the first study investigating the combined effects of a global and a local stressor on meiobenthic communities and ecosystem functioning in seagrass sediments. Meiofauna have been recently shown to have important effects on benthic ecosystem processes, such as OM mineralization and nitrogen cycling, likely by stimulating microbial activity (Nascimento et al. 2012, Bonaglia et al. 2014). Thus, further studies are warranted to assess how changes in meiobenthic assemblage in response to OA could entail cascading effects on microbial communities, ultimately altering ecosystem functioning.

Seagrass meadows are recognized hotspots of sediment organic matter sequestration (Fourqurean et al. 2012), due to their high primary production and leaf ability to trap allochthonous suspended particles (Kennedy et al. 2010). In addition, the low nutrient (nitrogen and phosphate) content of seagrass litter and sediment hypoxic condition slow organic matter decomposition, thus resulting in the immobilization of organic carbon in the belowground compartments for millennia (Mateo et al. 2006, Duarte et al. 2013, Trevathan-Tackett et al. 2017). In particular, *P. oceanica*, with its long-lived rizhomes and slow growth rate, is among the most efficient seagrasses in accumulating carbon in sediments (Fourqurean et al. 2012). Nonetheless, seagrasses are declining worldwide, raising concerns over a weakening of their ability to buffer climate changes through carbon sequestration (Fourqurean et al. 2012, Duarte et al. 2013, Lovelock et al. 2017, Bulleri et al. 2018, Chefaoui et al. 2018). Previous studies have shown how climate changes (e.g. seawater warming, heat waves) and local stressors (e.g. water quality degradation, mechanical disturbance) may reduce seagrass carbon storage capacity (Jordà et al. 2012, Serrano et al. 2016, Arias-Ortiz et al. 2018, Trevathan-Tackett et al. 2018b). Our results indicate that OA predicted by the end of this century could trigger OM degradation in seagrass sediments, reducing their carbon storage capacity and enhancing CO_2 release. While seagrass productivity is generally expected to increase in response to low pH, under nutrient concentration unlikely to cause N-limitation (Stitt and Krapp 1999, Alexandre et al. 2012, Russell et al. 2013, Sunday et al. 2016, Ravaglioli et al. 2017), our study highlights the need of assessing belowground processes to understand the mechanisms underpinning the net carbon budget in seagrass meadows.

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Acknowledgments

We sincerely thank Maria Cristina Buia and the staff of Villa Dohrn (Ischia, NA, Italy) of the Stazione Zoologica Anton Dohrn for their support in the fieldwork and data collection. The authors gratefully acknowledge financial supports from MIUR through the PRIN 2010 project TETRIS and from the University of Pisa through the PRA 2015 project.

Figure legends

Fig. 1. PCA analysis conducted on the sediment components of cores (sandy sediment, gravel and plant detritus) at ambient and low pH (respectively grey and black symbols) and among nutrient enrichment levels (circle =control nutrient, quadrat = moderate nutrient enrichment and triangle up = high nutrient enrichment).

Fig. 2. MDS ordination on untransformed data obtained from Bray-Curtis dissimilarities showing differences in meiobenthic assemblages between ambient and low pH (respectively grey and black symbols) and among nutrient enrichment levels (circle control nutrient, triangle down = moderate nutrient enrichment and triangle up = high nutrient enrichment).

Fig. 3. Abundance (mean ± SE, n=6) of **a)** nematodes, **b)** annelids, **c)** foraminifera and **d)** crustaceans in ambient and low pH conditions under different levels of nutrient enrichment (control, moderate and high). The inserts in **b)** and **c)** indicate the mean abundance (± SE) of annelids and foraminifera at ambient and low pH level (n= 18; data pooled across nutrient treatments).

Fig. 4. a) Aminopeptidase and b) ß-glucosidase (μmol g^{-1} h^{-1}, mean ± SE, n=6) in ambient and low pH conditions under different levels of nutrient enrichment (control, moderate and high). The inserts in a) and b) indicate the mean concentration (± SE) of aminopeptidase and ß-glucosidase at ambient and low pH level (n= 18; data pooled across nutrient treatments).

Table 1. PERMANOVA on the effects of OA (ambient and low pH) and nutrient enrichment (control, moderate and high) on the meiobenthic assemblage. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Source of variation	**df**	**MS**	**Pseudo-*F***
Covariate	1	0.020	0.202
OA	1	0.517	5.311**
Nutrient (Nu)	2	0.096	0.988
OA x Nu	2	0.171	1.752
Residual	11	0.097	
Total	17		

Table 2. ANOVA on the effects of OA (ambient and low pH) and nutrient enrichment (control, moderate and high) on the abundance of nematodes, annelids, foraminifera, crustaceans, total meiobenthic abundance and taxa diversity. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

		Nematodes		**Annelids**		**Foraminifera**	
Source of variation	**df**	**MS**	***F***	**MS**	***F***	**MS**	***F***
OA	1	3.372	2.216	3.075	32.379***	43.572	20.400***
Nutrient (Nu)	2	2.204	1.448	0.181	1.909	3.373	1.579
OA x Nu	2	2.539	1.668	0.138	1.456	2.223	1.041
Residual	12	1.522		0.095		2.136	
Transformation		Sqrt (x+1)		Log (x+1)		None	
Cochran’s test		ns		ns		ns	

		Crustaceans		**Total abundance**		**Diversity**	
Source of variation	**df**	**MS**	***F***	**MS**	***F***	**MS**	***F***
OA	1	0.906	3.539	1.878	1.392	0.064	1.054
Nutrient (Nu)	2	0.116	0.453	1.170	0.868	0.086	1.410
OA x Nu	2	0.015	0.058	2.512	1.862	0.140	2.210
Residual	12	0.256		1.349		0.061	
Transformation		None		Sqrt (x+1)		None	
C test		ns		ns		ns	

Table 3. ANOVA on the effects of OA (ambient and low pH) and nutrient enrichment (control, moderate and high) on aminopeptidase and ß-glucosidase activities in the sediment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

		Aminopeptidase		**ß-glucosidase**	
Source of variation	**df**	**MS**	***F***	**MS**	***F***
OA	1	389.0	6.617*	1.663	6.093*
Nutrient (Nu)	2	69.30	0.118	0.024	0.087
OA x Nu	2	140.2	0.238	0.036	0.131
Residual	12	588.8		0.273	
Transformation		None		Log (x+1)	
Cochran's test		ns		$P < 0.05$	

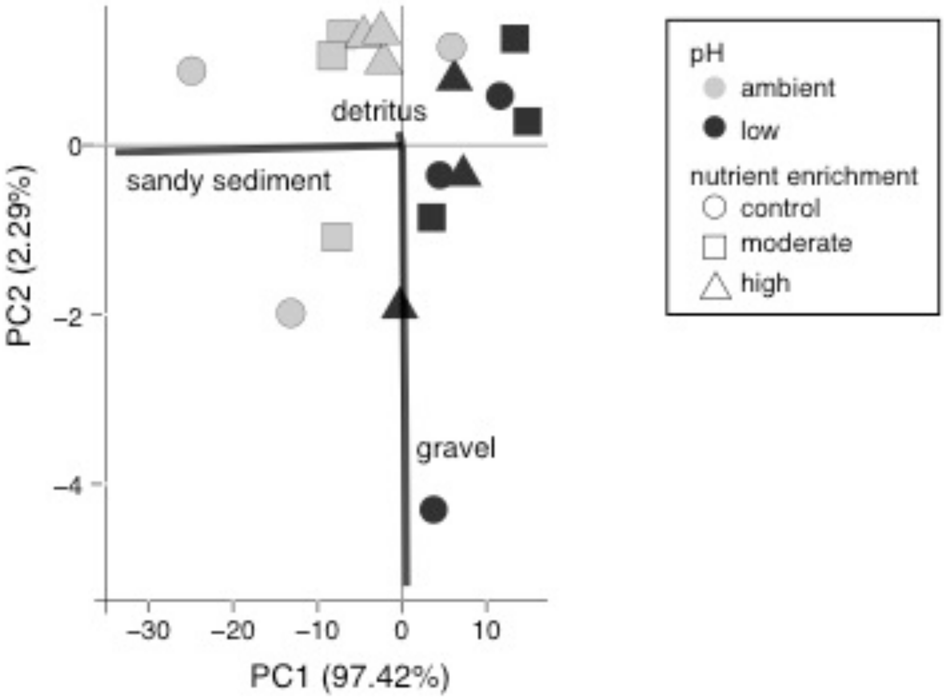
PC2 (2.29%)
0
−2
−4
PC1 (97.42%)
−30
−20
−10
0
10
detritus
sandy sediment
gravel
pH
ambient
low
nutrient enrichment
control
moderate
high

Figure 1

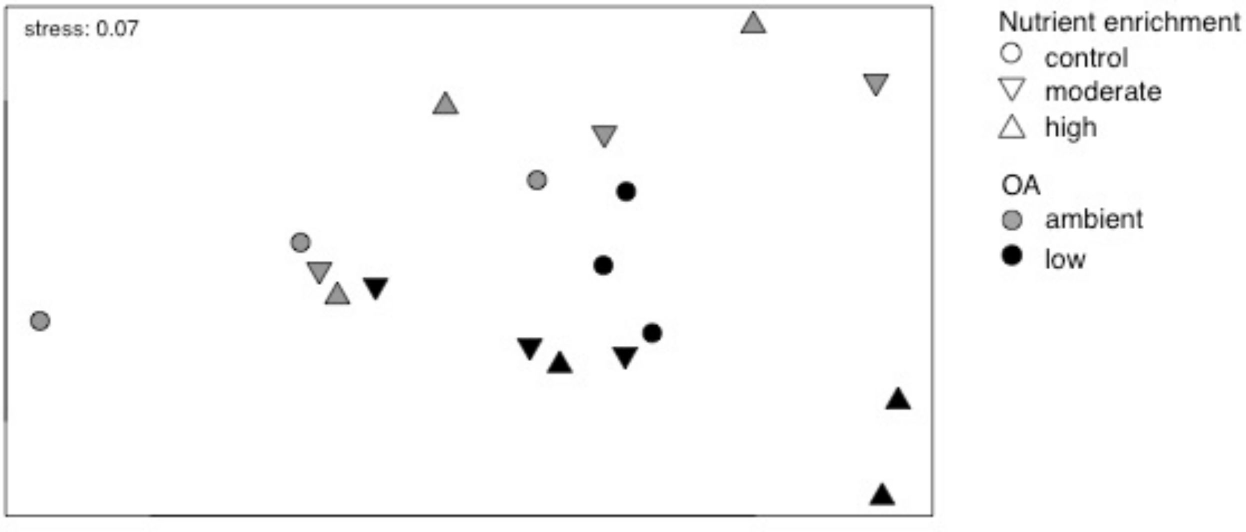
stress: 0.07
Nutrient enrichment
control
moderate
high
OA
ambient
low

Figure 2

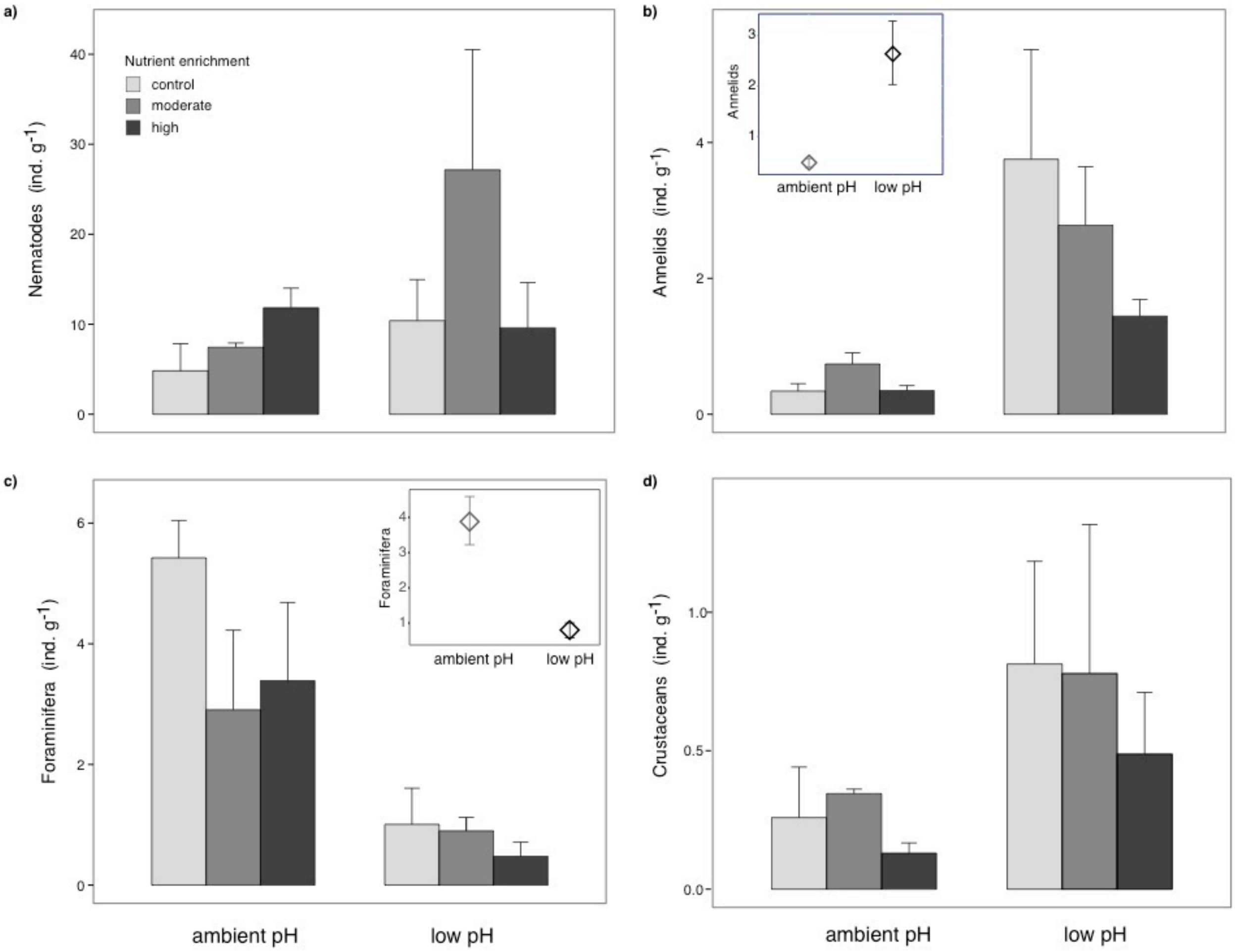
a)
Nematodes (ind. g-1)
0
10
20
30
40
Nutrient enrichment
control
moderate
high
b)
Annelids (ind. g-1)
0
2
4
Annelids
1
2
3
ambient pH
low pH
c)
Foraminifera (ind. g-1)
0
2
4
6
Foraminifera
1
2
3
4
ambient pH
low pH
d)
Crustaceans (ind. g-1)
0.0
0.5
1.0
ambient pH
low pH
ambient pH
low pH

Figure 3

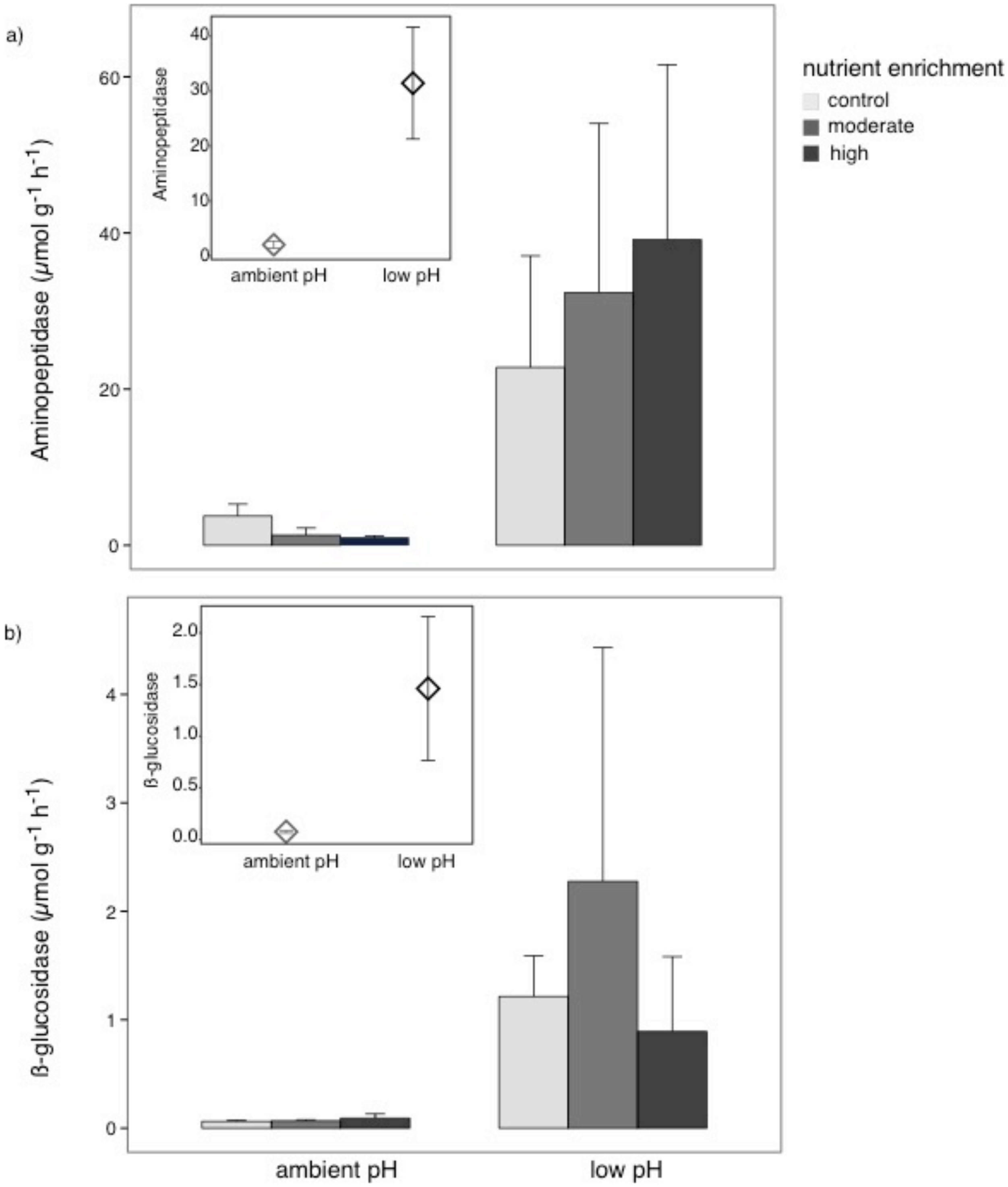
a)
Aminopeptidase (µmol g-1 h-1)
0
20
40
60
Aminopeptidase
0
10
20
30
40
ambient pH
low pH
nutrient enrichment
control
moderate
high
b)
ß-glucosidase (µmol g-1 h-1)
0
1
2
3
4
ß-glucosidase
0.0
0.5
1.0
1.5
2.0
ambient pH
low pH
ambient pH
low pH

Figure 4