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Ocean Acidification and Mollusc Settlement in *Posidonia oceanica* Meadows: Does the Seagrass Buffer Lower pH Effects at CO₂ Vents?

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Abstract: Ocean acidification has been broadly recognised to have effects on the structure and functioning of marine benthic communities. The selection of tolerant or vulnerable species can also occur during settlement phases, especially for calcifying organisms which are more vulnerable to low pH–high pCO₂ conditions. Here, we use three natural CO₂ vents (Castello Aragonese north and south sides, and Vullatura, Ischia, Italy) to assess the effect of a decrease of seawater pH on the settlement of Mollusca in *Posidonia oceanica* meadows, and to test the possible buffering effect provided by the seagrass. Artificial collectors were installed and collected after 33 days, during April–May 2019, in three different microhabitats within the meadow (canopy, bottom/rhizome level, and dead matte without plant cover), following a pH decreasing gradient from an extremely low pH zone (pH < 7.4), to ambient pH conditions (pH = 8.10). A total of 4659 specimens of Mollusca, belonging to 57 different taxa, were collected. The number of taxa was lower in low and extremely low pH conditions. Reduced mollusc assemblages were reported at the acidified stations, where few taxa accounted for a high number of individuals. Multivariate analyses revealed significant differences in mollusc assemblages among pH conditions, microhabitat, and the interaction of these two factors. *Acanthocardia echinata*, *Alvania lineata*, *Alvania* sp. juv, *Eatonina fulgida*, *Hiatella arctica*, *Mytilus galloprovincialis*, *Musculus subpictus*, *Phorcus* sp. juv, and *Rissoa variabilis* were the species mostly found in low and extremely low pH stations, and were all relatively robust to acidified conditions. Samples placed on the dead matte under acidified conditions at the Vullatura vent showed lower diversity and abundances if compared to canopy and bottom/rhizome samples, suggesting a possible buffering role of the *Posidonia* on mollusc settlement. Our study provides new evidence of shifts in marine benthic communities due to ocean acidification and evidence of how *P. oceanica* meadows could mitigate its effects on associated biota in light of future climate change.

Keywords: ocean acidification; climate change; *Posidonia oceanica*; seagrasses; settlement; Mollusca; Mediterranean Sea

1. Introduction

In the last two centuries, the atmospheric CO₂ concentration levels have steadily increased due to anthropic activities, rising from 280 ppm during the Industrial Revolution to 411.5 ppm today [1]. Oceans act as an essential carbon sink, absorbing part of anthropogenic CO₂ emissions; however, this is causing a rise in pCO₂ concentrations at the sea surface, leading to a decrease in the ocean pH level and change in carbonate chemistry, a process broadly known as “ocean acidification” (OA) [2]. The Intergovernmental Panel on

Climate Change models [3] predict that the pH of surface seawater will drop by 0.4 units by the year 2100, increasing the acidity levels by 170% compared to the pre-industrial ones. OA is an ongoing process expected to intensify in the future, which, together with the other aspects of Global Climate Change, drastically threatens the structure and functioning of marine ecosystems, especially in a semi-closed basin such as the Mediterranean Sea [4].

Changes in pH and carbonate water chemistry have a severe impact at both the individual and ecosystem levels. Several studies and some reviews and meta-analyses highlighted different biological responses to OA across a wide range of taxa with adverse effects on survival, metabolism, calcification, growth, and reproduction ([5,6] and reference therein), especially for calcifiers. The rise of CO₂ in ocean waters leads, in fact, to more corrosive conditions, as well as a depletion of calcium carbonate, that affect calcifying organisms hindering them from building and maintain their carbonate shells and skeletons [7,8].

In light of the aforementioned biological responses, the structure and functioning of marine communities will change under acidified conditions: several authors have reported the loss of both species richness and functional diversity in the study of Mediterranean benthic communities associated with CO₂ vents systems [9–13], as well as alteration of competitive interactions and an overall trophic simplification [11,14]. These vent systems, characterised by zones of intense CO₂ emissions from the seafloor, represent functional natural laboratories to study the effect of medium- and long-term exposure to naturally acidified conditions on the benthic biota, and up to date, several of such natural systems have been described and studied around the world [15–17]. From vent studies, changes in benthic community structure due to OA may also depend on the larval pelagic stages or settlement processes that characterise invertebrate recruitment [18,19].

Posidonia oceanica (L.) Delile is the endemic seagrass species in the Mediterranean Sea and the most widespread; it forms extensive meadows in the infralittoral zone, representing one of the most important coastal ecosystems, where it plays key ecological, physical, and economic roles [20–22]. *Posidonia oceanica* meadows host several benthic assemblages within the multitude of microhabitats and the stratified layers (i.e., leaf canopy, rhizomes, and sediment/matte) they provide [23], forming a complex food web [24]. They also represent an important nursery and shelter for many animals and seaweeds, such that Boudouresque and Meinesz [25] named it an “oasis of life”. *Posidonia oceanica* meadows are threatened all around the Mediterranean Sea by several anthropogenic impacts [24,26] and climate change, since the species is susceptible to the increase of the temperature and has been often impacted by summer heat waves [27,28].

On the other hand, *P. oceanica* meadows have a pivotal role in mitigating climate change, mainly through carbon sequestration, which is stored in the sediment and matte over millennia (around 27% of the carbon fixed by the plant) [26,29–31]. If considered at the scale of the whole Mediterranean, *P. oceanica* meadows adsorb 11–42% of the CO₂ emissions produced around the coasts of this basin [26]. A further aspect of climate mitigation is related to *Posidonia* and other seagrasses’ photosynthetic performance, which buffers the low pH and reduces the effect of OA on the local benthic communities [32–34]. A similar buffering effect has also been demonstrated for some large seaweeds, also in respect to protecting larval stages [35,36].

Mollusca were recognised as one of the most well-known and diverse taxonomic groups in Mediterranean Sea habitats, encompassing around 2000 species [37]: their diversity and abundance are relevant in peculiar and protected Mediterranean habitats [38]. Mollusc settlement, as well as that of other marine invertebrates, is a complex process determined by the interaction of biotic and abiotic factors. Settlement patterns are influenced by the environmental features and habitat type, hydrodynamic process, seasonality, proximity of adult populations, chemical cues, and presence of conspecific/prey or other organisms (e.g., filamentous algae) [39,40]. It is difficult to distinguish among the contribution of each of the factors mentioned above, due to the different spatial and temporal scales at which they act, and because settlement patterns are often inferred from recruitment. The

abundance and diversity of molluscs are particularly relevant in *P. oceanica* beds, where, due to the habitat complexity, they show a wide range of forms, functions, and adaptations. For these reasons, they have been widely investigated along Italian coasts [41–50]. Molluscs have also been studied comparing two well-distinguished habitats within a *P. oceanica* meadow: leaf canopy and rhizomes. These two layers present differences in light intensity, hydrodynamics, stability and dynamics, and habitat heterogeneity. These two layers host different mollusc assemblages and zonation patterns within the meadow [51,52].

Experiments and studies carried out at vents of the Castello at Ischia, and in other vent systems, contributed to recognising molluscs as particularly sensitive to OA, especially on the vegetated hard bottoms [11,12,53]. In contrast, they seem less impacted within the *Posidonia* meadows ([15,51] and reference therein). The decrease in pH affects both larvae and adults' calcium carbonate secretion to build shells; furthermore, OA threatens metabolic patterns, acid-base status, reproduction, immune response, and the survival of molluscs. Given the diversity and abundance of molluscs, their altered conditions are supposed to have several ecological and economic consequences for the structure and functioning of the entire *P. oceanica* meadow ecosystem.

This study aims to assess the effect of the pH variation, along a natural gradient of OA in three CO₂ vent's systems, on the settlement of invertebrate fauna associated with *P. oceanica*, focusing on early-settled stages of Mollusca. This group has been selected due to its high diversity in *Posidonia* meadows and its potential sensitivity to OA, since most species associated with seagrasses are calcareous. Furthermore, in one of these systems, we tested the hypothesis that there would be significant differences in mollusc' settlement among stations characterised by living *P. oceanica* and those with dead matte bottoms, to assess the possible buffering effect provided by the seagrass.

2. Materials and Methods

2.1. Study Area

Among the vent's system, the Castello Aragonese off the island of Ischia (Tyrrhenian Sea, Italy) represents model conditions to investigate how benthic invertebrates respond to OA in complex coastal systems, such as seagrass meadows. Another shallow system named "Vullatura", located near the Castello and recently investigated [13,54], is characterised by extended shallow *P. oceanica* meadows. These sites have been widely used, since they mimic future environmental conditions with a decrease in pH, even though they cannot be considered as perfect proxies of OA at a larger scale [15]. The study was carried out in three vent sites: two located in proximity to the Castello Aragonese (40°43'53.53" N, 13°57'50.98" E, north-eastern coast of Ischia, Gulf of Naples, Italy), on the north and south sides of the Castello (N and S), and a third site, named "Vullatura" (Vu) [55], located approx. 500 m north to the Castello (40°44'9.53" N; 13°57'28.19" E) (Figure 1). The gas emissions at the three vent systems are almost entirely composed of CO₂ (90.1–95.3%), and no differences in temperature and salinity occur if compared to surrounding waters, as well as no toxic sulphur compounds being associated with the emissions [9,15,55]. At the Castello, the vents cover about 5000 m², with differences in bubble emissions/m² among N and S sides: 0.7×10^6 L of gas emitted/day in the former and 1.4×10^6 L of gas emitted/day in the latter [9]. At the Vullatura, bubble emissions cover approximately 1200 m² with high densities of bubbles/m², as suggested by the site name, which means "boiling water" in the local vernacular [56]. These CO₂ flows modify pH and alkalinity of seawater at each of the sites mentioned above, creating a pH gradient developing parallel to the coastline.

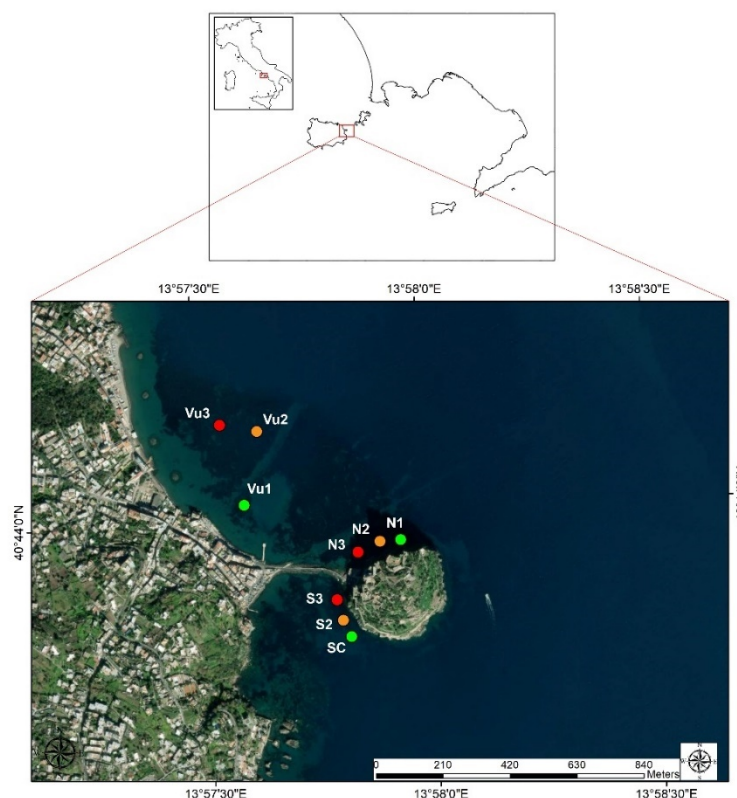


Figure 1. Location of the study area and disposition of the sampling stations along a pH gradient. Control/ambient conditions (Vu1, N1, and SC) are highlighted in green, low pH conditions (Vu2, N2, and S2) are indicated with orange, and extremely low pH conditions (Vu3, N3, and S3) are highlighted in red. Study sites were distinguished as follows: Vu-Vullatura; N-North of Castello Aragonese; S-South of Castello Aragonese. Basemap source: ESRI.

Dense *P. oceanica* beds develop between 1 and 6 m depth at Castello and Vullatura, with the highest shoot densities (up to 1000 shoots/m²) reported under the most acidified conditions at the Castello [5,9,57,58].

In all of the vent sites here considered, *P. oceanica* meadows settle on the sandy bottom. On the north and south sides of Castello, *Posidonia* forms a matte approx. 60 cm high, while at the Vullatura there are high pinnacles and ridges of matte up to 3 m high [13,56]. The shoot density at all stations have been measured in various previous studies, and show the highest values in the acidified stations both on the north and south sides of the Castello, and at the Vullatura (>1000 shoots m² at S3, 866 ± 148 shoots/m² at Vu3, and 858 ± 85 shoots m² at N3), while they are lower at the control stations (438 ± 88 shoots m² at N1; 494 ± 125 shoots m² at SC) [13,58].

At each of the sites, three stations characterised by different pH conditions were selected: control stations with ambient pH (8.2–8.0 pH units; N1, SC, and Vu1), low pH stations (7.8–7.4 pH units; N2, S2, and Vu2), and extremely low pH stations (less than 7.4 pH units; N3, S3, and Vu3) (Figure 1). The pH variations among and within the studied stations were assessed in previous studies, and here are reported graphically modified data available in the literature [13,53]. The pH data were measured in situ through the hourly acquisition of Sea-FET pH-meters, which was performed over more than 23 days at each of the investigated stations [59]. The SeaFET pH-meters were arranged close to the *P. oceanica* meadows: due to the size of the instrument and its mooring, and the canopy features as well, it was not possible to place them directly into the *Posidonia* canopy; therefore, the pH measurements refer to the meadow's surrounding waters.

2.2. Sampling and Data Collection

Artificial collectors are represented by 8 cm diameter rounded non-toxic nylon scouring pads; they were placed underwater (by scientific SCUBA divers) at each of the stations and collected after 33 days during the spring season of 2019 (from 28 April to 30 May). The same type of artificial collectors have been largely used in previous studies carried out at the Castello Aragonese vent systems [18,19,60], because they represent an effective tool for the collection of juvenile and adult invertebrate specimens. One month-long sampling period was chosen to allow sufficient time for invertebrate settlement. The April–May period was selected because it represents an active reproduction and settlement phase for several invertebrate species. At each vent and station, 4 scouring pad replicates were fixed to 1 m long bars and placed above the seagrass canopy (C), and an additional 4 replicates were fixed to 20 cm long bars and placed close to the bottom (B), at rhizome levels. Thus, a total of 8 artificial collectors were installed at each station. The replicate samples were placed at least 3 m distant from each other to avoid the pseudo-replicate related issue.

At the Vullatura, an additional 4 replicates of scouring pads for each station were placed on the dead matte substratum (M), without *Posidonia* cover, to assess *P. oceanica* buffering effects on mollusc settlement.

The artificial collectors were collected on the 30th of May 2019 and fixed in an alcohol 70% solution. The scouring pads were opened in the laboratory, and the material was washed in running water and filtered with a 120 µm mesh sieve size to retain all of the organisms and detritus collected. Organisms were sorted into large homogeneous taxonomic groups (i.e., Polychaeta, Gastropoda, Bivalvia, Amphipoda, Tanaidacea, Isopoda, Cumacea, Decapoda, Echinodermata) and counted under a stereomicroscope. As for Mollusca, all the specimens were identified to the lowest possible taxonomical level, and their names crosschecked with online resources (WORMS; www.marinespecies.org, accessed on 30 April 2021).

During both the installation and collection of the scouring pads, 15 orthotropic *P. oceanica* shoots were randomly collected at each station and then transported inside small plastic bags filled with seawater in a cooler box to the laboratory. For each shoot, the lengths of the longest leaf were measured. Furthermore, to characterise the epiphytic assemblages, we selected the external side of the two outermost adult leaves of each shoot (generally the oldest and therefore the most colonised by epiphytes). The percentage coverage of the main taxonomic groups was visually assessed: encrusting brown algae, encrusting red algae (mainly Corallinales), erect algae (both brown and green algae), bryozoans, hydrozoans, spirorbid worms, and foraminifers were taken into account. The percentage coverage of the main taxonomic groups was visually estimated through the semi-quantitative Braun–Blanquet categories: 0 = absent; 1 = occasionally present, <5%; 2 = 5–25%; 3 = 26–50%; 4 = 51–75%; 5 = >75%.

2.3. Statistical Analyses

Student's *t*-tests were carried out to test significant differences of *P. oceanica* mean leaf length according to pH conditions and sampling time (April–May). The number of taxa and number of specimens counted in each 8 artificial collector were used as primary descriptors of mollusc assemblages, and were analysed using Generalized Linear Mixed Effect Models (GLMMs) to assess their variation along the OA gradient. We selected a Poisson distribution with a logarithmic link (typical for count data), and the site was set as a random effect. Then, the number of taxa and the number of specimens were tested in relation to two fixed nested factors: pH conditions (3 levels: control pH, low pH, and extremely low pH) and Microhabitat (2 levels: Canopy and Bottom). Conversely, Generalized Linear Models (GLMs) were used to assess changes in the number of taxa and the number of individuals at the Vullatura in relation to the presence or the absence of the seagrass: pH conditions (3 levels) and Microhabitat [3 levels: C, B, and Matte(M)] were included in the model formulas as fixed and nested independent variables.

Multivariate permutational analyses of variance (PERMANOVA) based on Bray–Curtis similarity were used to test differences in the mollusc assemblages among sites, pH conditions, and microhabitats. Furthermore, PERMANOVA was applied to test differences in the mollusc assemblages among pH conditions and the three microhabitats sampled at the Vullatura. Furthermore, similarity percentage–species contribution analyses (SIMPER) were carried out to identify the taxa that mainly contributed to the dissimilarity among stations previously highlighted by PERMANOVA. In order to visualise dissimilarities among mollusc assemblages sampled at different pH conditions and microhabitats, Principal Coordinates Analyses (PCoA) based on Bray–Curtis similarity were applied.

For GLMMs, GLMs, and PERMANOVA, a significance level of 0.05 (p -value < 0.05) was chosen. All of the statistical analyses were performed in the R platform (version 3.5.2).

3. Results

3.1. pH Measures

The pH characterisation of the sites and stations, derived from previous measurements using in situ continuous SeaFET pH meters [13,53,59], showed a high daily variability at both low pH and extremely low pH stations if compared to the ambient pH condition of control stations (Supplementary Materials, Figure S2). In-situ pH measures highlighted severe acidification at the extremely low pH stations (N3: 7.2 ± 0.3 ; S3: 6.6 ± 0.4 ; Vu3: 7.2 ± 0.5), if compared to low pH stations (N2: 7.5 ± 0.2 ; S2: 7.7 ± 0.3 ; Vu2: 7.8 ± 0.3). Minimum pH values of 6.0 and 6.1 were recorded at stations S3 and Vu3, respectively.

3.2. *Posidonia Oceanica* Leaf Phenology and Epiphytic Assemblages

The mean length of *Posidonia* leaves was significantly higher in the control stations (t -test, p -value < 0.001), in respect to the acidified station, and no significant differences (t -test, p -value > 0.05) were reported between low and extremely low pH conditions (Supplementary Materials, Table S1 and Figure S3). The mean leaf length significantly differed among sampling months, with values in May higher than in April.

Bryozoans and hydrozoans mainly characterised the epiphytic assemblages in April 2019; erect fleshy algae were reported in the acidified stations (both low and extremely low pH), whereas encrusting red algae (Corallinales) and calcareous invertebrates (spirorbids and foraminifers) were found mainly in control stations (Supplementary Materials, Figure S4). In May 2019 as well, the percentage coverage of calcareous organisms was low in the acidified stations (Figure S4).

3.3. Effects of OA on Mollusc' Settlement

A total of 19,613 invertebrate specimens were collected and sorted at the three investigated sites and under different pH conditions. Invertebrate assemblages were quite similar among sites in the proportions of the different taxonomic groups. The structure of the assemblages found in the artificial collectors (Table 1) revealed a dominance of Polychaeta and Amphipoda: the former showed lower abundance at the acidified stations (from 37% to 33%) if compared to the ambient pH stations. On the other hand, the Amphipoda were more abundant at low and extremely low pH stations (from 19% to 31% and 25%). Mollusca (Gastropoda, Bivalvia, and Polyplacophora pooled together) accounted for 30% of the number of individuals at the three investigated sites. Each of the other taxonomic groups (i.e., Tanaidacea, Decapoda, Cumacea, Isopoda, and Echinodermata) never exceeded 10% of the total number of individuals.

Table 1. Composition of the benthic organisms collected in the samples in different sites and pH conditions and expressed as percentages (%) of each taxonomic group on the total number of individuals sampled.

| Class | Sites | | | pH Conditions | | |
|---------------|-------|----|----|---------------|--------|------------------|
| | Vu | N | S | Control | Low pH | Extremely Low pH |
| Polychaeta | 36 | 36 | 33 | 37 | 32 | 33 |
| Amphipoda | 24 | 22 | 26 | 19 | 31 | 25 |
| Tanaidacea | 9 | 7 | 4 | 5 | 5 | 6 |
| Gastropoda | 17 | 22 | 10 | 17 | 12 | 12 |
| Bivalvia | 7 | 8 | 20 | 15 | 13 | 18 |
| Decapoda | 0 | 0 | 0 | 0 | 0 | 0 |
| Cumacea | 1 | 1 | 1 | 2 | 1 | 1 |
| Isopoda | 1 | 2 | 5 | 4 | 4 | 3 |
| Echinodermata | 5 | 2 | 1 | 1 | 2 | 2 |

The mollusc specimens sorted and identified were 4659, belonging to 57 different taxa (15 Bivalvia, 41 Gastropoda, and 1 Polyplacophora; Table 2). Many of the collected specimens were juveniles, demonstrating the effectiveness of scouring pads in collecting these stages and assessing invertebrate settlement. *Mytilus galloprovincialis* juv. was the main representative species, accounting for 63% of Bivalvia and 33% among all Mollusca (1540 individuals); in extremely low pH stations, this percentage reached 45% of the total mollusc individuals. Other common taxa found in the samples were *Alvania lineata* (360 individuals: 7.7%) *Phorcus* sp. juv. (probably *Phorcus turbinatus*) (310, 6.6%), *Rissoa variabilis* (276, 5.9%), *Venerupis* sp. juv. (232, 5.0%), *Hiatella arctica* (191, 4.0%), *Eatonina fulgida* (147 specimens, 3.2%), and *Ecrobia ventrosa* (132 specimens, 2.9%) (Supplementary Materials, Figure S5).

The number of taxa showed higher values from extremely low to ambient pH at the investigated sites, while the number of individuals showed comparable values, except at the S extremely low pH station where a peak was observed (Figure 2). The mean values of the two descriptors were lower at N and Vu stations if compared with S ones, where variability among replicates was also wider. The maximum number of individuals (159.0 ± 161.0) was reached at the extremely low pH S station, whereas the minimum value (4.7 ± 3.0) was reported for low pH station at the Vu site. The mean number of taxa did not show any relevant difference between canopy (C) and bottom (B) layers, especially between low and extremely low pH conditions. The intercepts of the GLMMs carried out on both the number of taxa and individuals were deemed significant and were defined by ambient pH and microhabitat B (Table 3). The number of taxa was significantly lower under extremely low and low pH conditions; all of the factors considered, and their relative interactions, yielded significant p-values for the number of individuals. Mollusc abundance was significantly lower under low and extremely low pH, especially in microhabitat C. Conversely, the interaction between low and extremely low pH conditions and microhabitat C showed a significant increase in the number of individuals if compared to the combination defined by the intercept.

Table 2. Number of individuals divided per species in each site (Vu, N, S), pH gradient (control, low, extremely low) and microhabitat (C = “canopy”, B = “bottom”, M = “dead matte”).

| | Control/Ambient pH | | | | | | | Low pH | | | | | | | Extremely Low pH | | | | | | |
|--|--------------------|-----|-----|----|----|-----|-----|--------|-----|-----|----|----|-----|-----|------------------|-----|-----|----|----|-----|-----|
| | VuC | VuB | VuM | NC | NB | SC | SB | VuC | VuB | VuM | NC | NB | SC | SB | VuC | VuB | VuM | NC | NB | SC | SB |
| Bivalvia | | | | | | | | | | | | | | | | | | | | | |
| <i>Abra alba</i> (W. Wood, 1802) | | | | | | | | | | | | | | | 1 | | | | | 4 | 7 |
| <i>Acanthocardia echinata</i> (Linnaeus, 1758) | 11 | 2 | 5 | 3 | 4 | 18 | 14 | | | | 3 | 2 | 14 | 2 | 10 | | 1 | 2 | 2 | 8 | |
| <i>Anomia ephippium</i> (Linnaeus, 1758) | 9 | | 2 | 1 | | 3 | 6 | | | | | | | 3 | | | | | | 1 | 4 |
| <i>Arca noae</i> (Linnaeus, 1758) | 9 | 1 | | | 1 | | 5 | | | | | 1 | | 1 | | 2 | | | | | |
| <i>Gregariella semigranata</i> (Reeve, 1858) | 1 | 1 | | | | | | | | | | | | | | | 2 | | | | |
| <i>Haminoea</i> sp. | | | | | | | | | | | 1 | | | | | | | | | | |
| <i>Hiatella arctica</i> (Linnaeus, 1767) | 15 | 2 | 10 | 16 | | 21 | 29 | | | 4 | 28 | 1 | 9 | 9 | 9 | 1 | | 7 | | 28 | 2 |
| <i>Lima</i> sp. | 10 | 4 | 6 | 8 | 4 | 6 | 9 | 1 | | | 13 | | 5 | 3 | 5 | | | 3 | | | 1 |
| <i>Mimachlamys varia</i> (Linnaeus, 1758) | 4 | | | 2 | 1 | 7 | 5 | 2 | | | 1 | | 8 | 1 | | | | 1 | | 3 | |
| <i>Musculus subpictus</i> (Cantraine, 1835) | 12 | 3 | 2 | 12 | 16 | 2 | 14 | 1 | 2 | | 8 | 1 | 4 | 3 | | 3 | 1 | 1 | 3 | 2 | 4 |
| <i>Mytilus galloprovincialis</i> juv. (Lamarck, 1819) | 23 | 4 | 11 | 18 | 46 | 140 | 187 | | | | 12 | 3 | 222 | 231 | 2 | 1 | | 13 | 10 | 340 | 277 |
| <i>Parvicardium</i> sp. | | | | 1 | | 3 | | | | | | | 2 | 3 | | | | | | 5 | |
| <i>Pecten</i> sp. | | | | | | | 5 | | | | | | 2 | | | | | | | | 1 |
| <i>Venerupis</i> sp. | 3 | 2 | 4 | 2 | 14 | 30 | 50 | | | | 5 | | 25 | 34 | 12 | 1 | | | | 36 | 14 |
| Veneridae gen. sp. | 7 | | | | | 4 | | | | | | 2 | 12 | 13 | | | | 1 | 1 | 7 | 15 |
| Gastropoda | | | | | | | | | | | | | | | | | | | | | |
| <i>Alvania cimex</i> (Linnaeus, 1758) | | 7 | 6 | | | 2 | | | | | | | | | 6 | | | | | | |
| <i>Alvania discors</i> (T. Brown, 1818) | | | 3 | | | | | | | | | | 2 | | | | | | | 5 | |
| <i>Alvania lineata</i> (Risso, 1826) | 8 | 47 | 24 | 2 | 17 | 1 | 20 | 2 | 6 | 1 | 8 | 15 | | 62 | | 76 | 25 | | 18 | 2 | 26 |
| <i>Alvania pagodula</i> (Bucquoy, Dautzenberg & Dollfus, 1884) | | 2 | 20 | | | | | | | | | | | | | | | | | | |
| <i>Alvania piersmai</i> (Moolenbeek e Hoenselaar, 1989) | | | | | | 3 | 1 | | | | | | 2 | 2 | | | | | 8 | | |
| <i>Alvania scabra</i> (Filippi, 1844) | | | 5 | | 1 | | 9 | | | 3 | | | 4 | 8 | | | 1 | | | | 1 |
| <i>Alvania</i> sp. juv. | | | 1 | 2 | | | 13 | | 1 | | 3 | 9 | 16 | | | | | | | 4 | 87 |
| <i>Alvania subareolata</i> (Monterosato, 1869) | | | | | | | | | 1 | 3 | | | | | | 1 | | | | | 4 |
| <i>Ammonicera fischeriana</i> (Monterosato, 1869) | 2 | 1 | 1 | 5 | 1 | 2 | 3 | | | | 4 | | 8 | 1 | | | | 5 | 1 | | 1 |
| <i>Bittium latreillii</i> (Payraudeau, 1826) | | 4 | | | | | 2 | | 1 | | | | 1 | | | | 1 | | | | |
| <i>Bolma rugosa</i> (Linnaeus, 1767) | | | | | | | | | | | | | | | 1 | 5 | | | | | |
| <i>Cerithium vulgatum</i> (Bruguère, 1792) | | | | 5 | 2 | 1 | | | | | | 1 | 1 | 2 | | | | | | 2 | 1 |
| <i>Crisilla beniamina</i> (Monterosato, 1884) | 8 | | | | | | | | | | | | 3 | | | | | | | 2 | 7 |
| <i>Eatonina fulgida</i> (J. Adams, 1797) | | | 2 | 7 | | 11 | 3 | 1 | | 3 | 16 | 4 | 14 | 7 | | 4 | 1 | 19 | 22 | 9 | 24 |
| <i>Ecrobia ventrosa</i> (Montagu, 1803) | 3 | | | 54 | 13 | 13 | | 20 | | 1 | 10 | 1 | 5 | | 11 | | | 7 | 1 | | |
| <i>Gibberula philippii</i> (Monterosato, 1878) | | | | | | | 7 | | | | | | | 2 | | | | | | | 2 |
| <i>Gibbula</i> sp. | | | | 2 | 4 | 11 | | | 1 | | | 2 | 1 | 20 | | 3 | | 3 | 4 | 2 | 8 |
| <i>Hydrobia acuta neglecta</i> (Muus, 1963) | | | | | | | 2 | | | | | | | 2 | | 1 | | | | | |
| <i>Jujubinus exasperatus</i> (Pennant, 1777) | | | | | 1 | | 1 | | | | | | | | | | | | | | |
| <i>Jujubinus gravinae</i> (Dautzenberg, 1881) | | | | 4 | 2 | 19 | 23 | 1 | 1 | 1 | | 4 | | 22 | | 1 | | | 6 | | 2 |
| <i>Jujubinus striatus</i> (Linnaeus, 1758) | 1 | | | 3 | 4 | | 6 | 5 | 1 | 2 | 6 | | 4 | 4 | | 1 | 2 | 1 | 2 | | 1 |
| <i>Melanella petitiiana</i> (Brusina, 1869) | | 1 | | 1 | 18 | | | 2 | 1 | | | 1 | | | 1 | 15 | 3 | | | | |

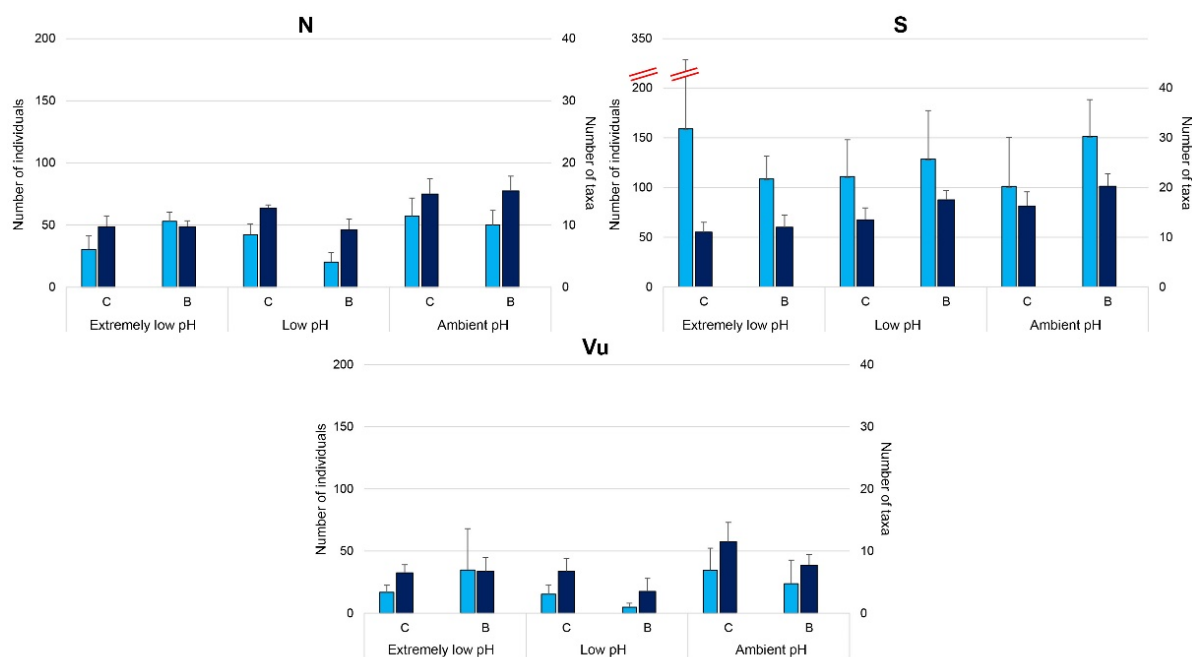


Figure 2. Mean number of individuals (cyan) and mean number of taxa (dark blue) with SD bars according to site (N, S, and Vu), pH stations, and microhabitats within the meadow (C = canopy; B = bottom). Number of individuals' axis break for the South (S) site is indicated in red.

Table 3. Outputs of the GLMMs carried out on the number of taxa and number of individuals. Significant values are highlighted in bold.

| Source of Variation | Value | SD Error | t-Value | p-Value |
|-------------------------------------|--------|----------|---------|------------------|
| Number of taxa | | | | |
| Intercept | 2.629 | 0.194 | 13.567 | <0.001 |
| Low pH | −0.355 | 0.118 | −3.009 | 0.002 |
| Extremely low pH | −0.414 | 0.120 | −3.449 | <0.001 |
| Microhabitat (C) | −0.017 | 0.107 | −0.162 | 0.871 |
| Low pH × Microhabitat (C) | 0.096 | 0.165 | 0.582 | 0.561 |
| Extremely low pH × Microhabitat (C) | −0.026 | 0.174 | −0.153 | 0.878 |
| Number of individuals | | | | |
| Intercept | 4.056 | 0.419 | 9.667 | <0.001 |
| Low pH | −0.382 | 0.052 | −7.307 | <0.001 |
| Extremely low pH | −0.136 | 0.048 | −2.800 | 0.005 |
| Microhabitat (C) | −0.154 | 0.049 | −3.153 | 0.001 |
| Low pH × Microhabitat (C) | 0.248 | 0.074 | 3.338 | <0.001 |
| Extremely low pH × Microhabitat (C) | 0.171 | 0.072 | 2.379 | 0.017 |

The PCoA ordination plots of the mollusc assemblages revealed distinct clusters according to pH conditions and microhabitats (Figure 3): the three sites (N, S, and Vu) were shown in separate plots to avoid site-related effects. The separation between acidified and ambient stations was appreciable in Vu and N plots, whereas the S plot showed replicate dispersion, indicating higher variability among assemblages. Furthermore, mollusc assemblages differed between C and B microhabitats, which revealed dissimilarity between the layers within the meadows. Significant differences were also highlighted through PERMANOVA (Table 4) when analysing mollusc assemblage composition for all the factors considered and their relative interactions.

The similarity percentages–species contribution analysis (SIMPER) indicated high dissimilarity among pH conditions at the three sites, ranging from 51.4% to 88.4%. The

taxa most responsible for the 60% dissimilarity among stations (SIMPER analyses) are shown in Table S2 (Supplementary Materials). *Alvania lineata*, *Alvania* sp. juv, *E. fulgida*, *H. arctica*, *M. galloprovincialis*, *Phorcus* sp. juv, and *R. variabilis* mostly contributed for among pH conditions dissimilarity and were mainly found in samples exposed to extremely low pH conditions.

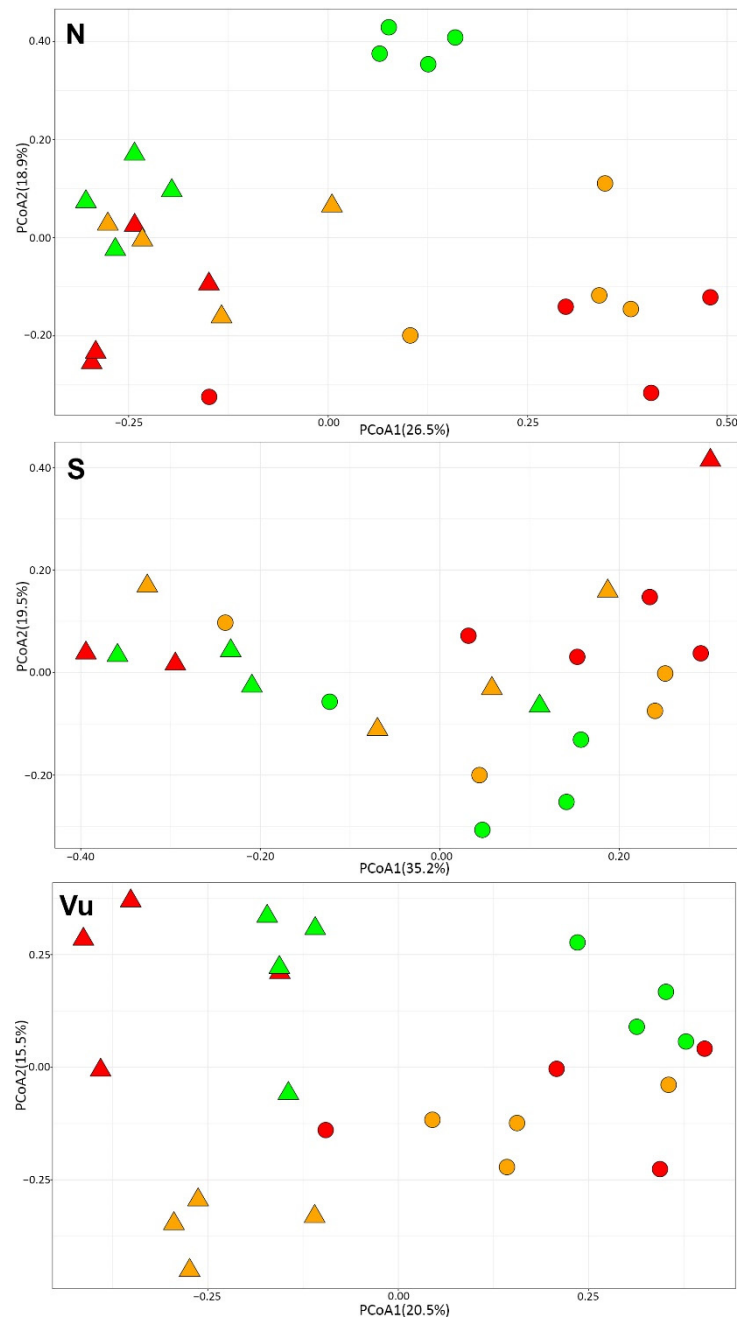


Figure 3. Principal Coordinates Analysis (PCoA) of the mollusc assemblages sampled in N, S, and Vu sites. Colours indicate pH conditions: green = ambient pH; orange = low pH; red = extremely low pH. Microhabitats are indicated through circles (Canopy) and triangles (Bottom).

Table 4. Results of permutational multivariate analysis of variance (PERMANOVA) on mollusc assemblages found on *P. oceanica* meadows. Significant values are highlighted in bold.

| Source of Variation | Df | SS | MS | F | <i>p</i> -Value |
|--------------------------|----|-------|-------|--------|-----------------|
| Site | 2 | 5.376 | 0.233 | 14.840 | 0.001 |
| pH | 2 | 1.065 | 0.046 | 2.932 | 0.001 |
| Microhabitat | 1 | 2.329 | 0.101 | 12.833 | 0.001 |
| Site × pH | 4 | 1.814 | 0.078 | 2.498 | 0.001 |
| Site × Microhabitat | 2 | 0.776 | 0.033 | 2.136 | 0.003 |
| pH × Microhabitat | 2 | 0.716 | 0.031 | 1.972 | 0.010 |
| Site × pH × Microhabitat | 4 | 1.353 | 0.058 | 1.863 | 0.002 |
| Residuals | 53 | 9.623 | 0.417 | | |

3.4. Does *Posidonia Oceanica* Buffer the Effect of OA on Mollusc Settlement?

The number of taxa and the number of individuals did not show a visible differentiation pattern among the three investigated microhabitats (C, B, and M) at Vu site (Figure 4). Both the descriptors, however, showed higher mean values in samples collected at control pH stations. The maximum value of the number of taxa was reported (11.5 ± 3.1) in C, whereas the maximum number of individuals was collected in M (40.2 ± 18.6), both under control pH conditions. The intercepts of both the GLMs were significant and defined by control pH conditions and B microhabitat (Table 5). The number of taxa significantly differed at low pH exclusively; the other factors and their combination analysed in the model did not yield significant *p*-values. The GLM carried out on the number of individuals found on artificial collectors showed significant changes according to pH conditions, microhabitats, and their combination. In particular, the highest significant reduction in mollusc abundance was reported for the combination defined by extremely low pH condition and microhabitat M.

The PCoA revealed diversification of the mollusc assemblages according to microhabitats (Figure 5). For example, the observations from the canopy (C) produced a separate cluster if compared to the samples of B and M. Within each of the considered microhabitats, the four replicates exposed to a given pH condition were closer to each other, although mollusc assemblages collected on dead matte (M) were characterised by higher dispersion in the Euclidean plane, reflecting considerable variability.

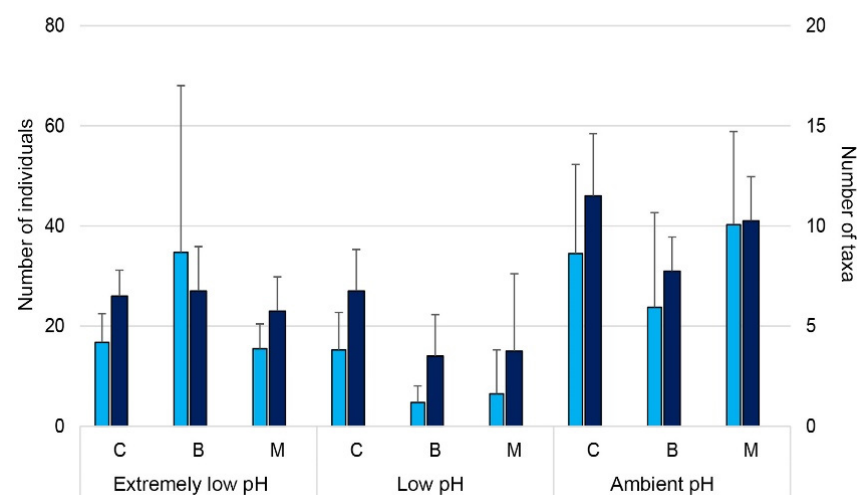
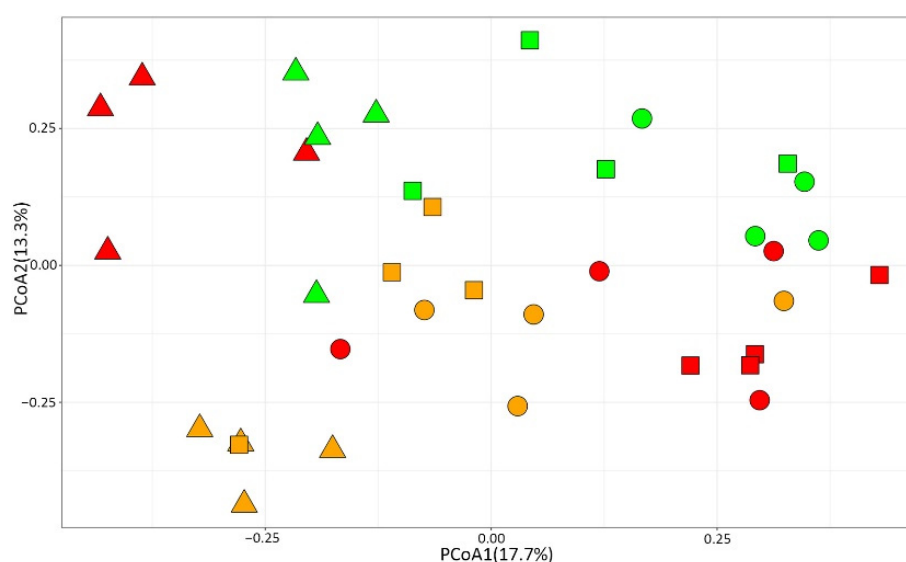
**Figure 4.** Mean value of number of individuals (cyan) and mean number of taxa (dark blue) with SD bars in the three pH conditions, and microhabitats within the meadow (C = canopy; B = bottom, M = dead matte) at the Vu site.

Table 5. Output of the GLMs fitted to the number of taxa and number of individuals in the three microhabitats at the Vu site. Significant values are highlighted in bold.

| Source of Variation | Estimate | SD Error | t-Value | p-Value |
|--|----------|----------|---------|------------------|
| Number of species | | | | |
| Intercept | 2.047 | 0.179 | 11.401 | <0.001 |
| Low pH | −0.795 | 0.322 | −2.469 | 0.013 |
| Extremely low pH | −0.138 | 0.263 | −0.525 | 0.599 |
| Microhabitat (C) | 0.395 | 0.232 | 1.698 | 0.089 |
| Microhabitat (M) | 0.279 | 0.238 | 1.175 | 0.24 |
| Low pH × Microhabitat (C) | 0.262 | 0.403 | 0.650 | 0.515 |
| Extremely low pH × Microhabitat (C) | −0.432 | 0.359 | −1.202 | 0.229 |
| Low pH × Microhabitat (M) | −0.211 | 0.441 | −0.477 | 0.633 |
| Extremely low pH × Microhabitat (M) | −0.439 | 0.370 | −1.188 | 0.235 |
| Null deviance: 62.86 on 35 df Residual deviance: 30.58 on 27 df | | | | |
| Number of individuals | | | | |
| Intercept | 3.167 | 0.102 | 30.874 | <0.001 |
| Low pH | −1.609 | 0.251 | −6.404 | <0.001 |
| Extremely low pH | 0.380 | 0.133 | 2.859 | 0.004 |
| Microhabitat (C) | 0.373 | 0.133 | 2.801 | 0.005 |
| Microhabitat (M) | 0.527 | 0.129 | 4.078 | <0.001 |
| Low pH × Microhabitat (C) | 0.793 | 0.294 | 2.692 | 0.007 |
| Extremely low pH × Microhabitat (C) | −1.118 | 0.200 | −5.583 | <0.001 |
| Low pH × Microhabitat (M) | −0.214 | 0.328 | −0.651 | 0.515 |
| Extremely low pH × Microhabitat (M) | −1.335 | 0.200 | −6.669 | <0.001 |
| Null deviance: 513.63 on 35 df Residual deviance: 251.88 on 27 df | | | | |

**Figure 5.** Principal Coordinates Analysis (PCoA) ordination plot of the mollusc assemblages sampled at the Vu site. Colours indicate pH conditions: green = control pH; orange = low pH; red = extremely low pH. Microhabitats are indicated through circles (Canopy), triangles (Bottom), and squares (Matte).

The observed differences in the composition of mollusc assemblages and relative abundance of the taxa were significant according to the pH condition, microhabitat, and pH × microhabitat interaction (PERMANOVA analysis; Table 6). *Alvania lineata*, *R. variabilis*, *E. ventrosa*, and *H. arctica* were the species most responsible for dissimilarity among pH conditions within each microhabitat, and those characterised by higher abundances under acidified conditions (SIMPER, Supplementary Materials, Table S3).

Table 6. Results of PERMANOVA on mollusc assemblages found on *P. oceanica* meadow at the Vu site. Significant values are highlighted in bold.

| Source of Variation | Df | SS | MS | F | <i>p</i> -Value |
|---------------------|----|-------|-------|-------|-----------------|
| pH | 2 | 1.601 | 0.122 | 2.943 | 0.001 |
| Microhabitat | 2 | 1.978 | 0.151 | 3.638 | 0.001 |
| pH × Microhabitat | 4 | 2.147 | 0.164 | 1.974 | 0.010 |
| Residual | 27 | 7.341 | 0.561 | | |

4. Discussion

Ocean acidification modifies the structure and composition of marine benthic communities; these aspects and processes can be addressed in studying naturally acidified systems, such as CO₂ vents. Although vents are not perfect predictors of changes that will occur at larger scale in future oceans, they provide information of the possible effects on local marine communities [61], otherwise impossible to achieve through mesocosms or Free Ocean Carbon Dioxide Enrichment (FOCE) systems [62]. Vents acidify surrounding waters and, as open systems, allow habitat connectivity through natural larval exchange, allowing researchers to study ecosystem processes, such as benthic community dynamics and changes under OA conditions [9].

In the three systems studied here (Castello north and south, and Vullatura), the pH exhibited wide daily fluctuations, especially at low and extremely low pH stations. This variability reflects local conditions at a small–medium spatial scale and in time ([15] and references therein), influenced by hydrodynamics, vents emissions, and biological activity (i.e., photosynthesis and respiration rates).

Our results show that settlement of mollusc taxa in *P. oceanica* meadows subjected to OA is highly influenced by the decrease of seawater pH at the three vents systems studied, although some species are less affected by these environmental conditions. Furthermore, this study highlights the role of *P. oceanica* meadows as a nursery area, buffering the effects of OA on the associated biota.

Posidonia oceanica leaf length showed significant variations among pH conditions, with lower values reported for low and extremely low pH stations. This pattern can be linked to the greater vulnerability of the seagrass under acidified conditions, due to the biological erosion performed by *Sarpa salpa* (Linnaeus, 1758) through grazing [13,58]. In fact, recent studies reported a higher abundance of the herbivorous fish *S. salpa* in the vents area if compared to adjacent ambient pH stations [63,64]. *Posidonia oceanica* leaves seem more palatable under acidified conditions, due to their nutritional quality [65–67] and the reduction or disappearance of calcareous epiphytes [13]. The alterations in the epiphytic community structure were evident at the acidified stations at the three studied sites. Calcareous algae and invertebrate taxa (i.e., encrusting red algae, spirorbids, and foraminifers) were the dominant groups in control stations, whereas they strongly reduced their abundance at acidified stations. At low and extremely low pH stations, the epiphytic communities shifted towards encrusting and erect non-calcareous algae and non-calcareous invertebrates. The results here provided are consistent with similar studies carried out in the same area, highlighting the reduction of the diversity of the *P. oceanica* epiphytic communities exposed to OA [13,58].

The scouring pads, used as artificial collectors, were effective tools in collecting both juvenile and adult invertebrate stages, and therefore assessing settlement in *P. oceanica* meadows. The artificial collectors provided the different composition of the assemblages according to the pH gradient, and the structure of the assemblages. As for the main taxonomic group collected and their relative abundance, this was consistent with results reported by Ricevuto et al. [60], a study that was performed on the Castello south and north side vents.

The molluscs sampled through artificial collectors showed a relatively high number of species, especially considering the relatively shallow depth examined and compared with other *Posidonia* beds studied around Ischia [42,50]. Molluscs sampled in several studies,

and different habitats around the Castello vents (including control areas outside the CO₂ bubbling), accounted for 87 species [15,68], while molluscs collected in a previous study at the Castello *Posidonia* meadows accounted for 51 species [54,69]. Therefore, the diversity observed in our artificial collectors is comparable to the natural habitat surrounding the vents. Most of the species found are typical of *P. oceanica* meadows, especially among the gastropods [70], while many have already been recorded in the Castello area, associated with both *Posidonia* and macroalgae bottoms [15,54,71]. Most of the individuals collected were composed mainly of juvenile stages. *Posidonia oceanica* meadows are considered nursery areas for many invertebrates and fishes [72]; however, this role has not been well evaluated and supported in specific studies, especially for mobile macro-invertebrates. *Posidonia* vents systems have been recently indicated as nursery grounds by Mirasole et al. [64] for the fish fauna of the Castello vents, while juveniles of invertebrates have been largely sampled by similar artificial collectors placed next to hard vegetated bottoms off the Castello vents [19,60].

Our results provide evidence that, although molluscs actively recruit in all of the *Posidonia* stations here studied, they exhibited a significant reduction in species settlement under OA conditions, showing sensitivity to pH reduction. The lower number of taxa and number of individuals were observed according to pH gradient, although at the Vullatura and North Castello site, extremely low pH conditions showed slightly higher values of both parameters if compared to low pH stations. As for the number of individuals, this could be due to the reduced competition among species under more severe OA conditions. In addition, the high number of individuals recorded on the South Castello side could be related to a storm (SE wind direction) that occurred in May 2019, and which may have contributed to the resuspension of organisms from the bottom. However, the south side of the Castello, which is more sheltered to the dominant winds on the area (N–W), showed the highest *Posidonia* shoot density at the vents area [54], and it was characterised by a higher number of individuals collected in previous studies in the hard bottoms [53], *Posidonia* meadow [54] and artificial collectors [19,60]. In general, such between-site differences in species diversity and abundance, although showing a similar distributional pattern across pH conditions, suggest that various local factors may have as much of an effect on mollusc settlement. However, mollusc settlement changed according to decreasing pH, although with different magnitudes according to site. The general pattern indicates that the number of individuals differed by 0% to 25%, and the number of taxa differed by 10% to 40% from ambient to extremely low pH. In particular, the mollusc diversity reduction according to OA conditions was evaluated in previous studies, considering all habitats and data available up to 2018 [15,73]. Data are consistent with those reported in this study: on the north side, mollusc's diversity changed by 39% between ambient and low pH, and by 48% between ambient and extremely low pH conditions, while on the south side the number of mollusc taxa was reduced by 47% between ambient and low pH, and by 53% between ambient and extremely low pH conditions.

This general pattern reflects what was previously observed in the same area in studies where molluscs and other calcifying invertebrates were considered, and it is related to the sensitivity of calcareous organisms to OA [9,18,53,60]. Furthermore, our results are consistent with similar studies carried out in Kueishan Island (Taiwan) vent systems [74,75], where water physio-chemical features, modified by vent emissions (although different from those characterising our Ischia vents), shape the composition and structure of mollusc assemblages.

However, some species showed variable trends according to pH reduction, with the gastropods *A. lineata*, *E. fulgida*, *Phorcus* sp. juv, *R. variabilis*, and the bivalves *A. echinata*, *H. arctica*, *M. subpictus*, and *M. galloprovincialis*, as the most frequent species occurring at low and extremely low pH. *Mytilus galloprovincialis* juvenile stages (2–3 mm length) showed a discrete tolerance to acidified conditions and accounted for 45% of individuals sampled in extremely low pH stations. High *M. galloprovincialis* settlement under acidified conditions was described previously at the Castello north and south sides using artificial collectors

placed next to hard vegetated bottoms [60]. Although the juveniles of *M. galloprovincialis* were dominant in samples at the acidified stations, adults of the species are absent in the extremely low pH zones and very scarce at low pH, and occur mainly on the control areas far from the CO₂ emissions [71]. The absence of adults of several taxa in the adjacent areas suggests that selection produced by OA happens subsequently, during the adult phase rather than in the settlement and post-settlement stage. As evidence of a post-settlement selection, the comparison of the adult mollusc assemblages found in the Castello Aragonese vent areas are provided as Supplementary Materials (Table S4; [15,54,69]); in our acidified stations, 30 taxa (52.6%), over the total of 57 taxa identified in this study, had been reported in adult stages in *Posidonia* meadows or in the vegetated hard bottoms inside or near the vent's area. Also, natural/control stations showed a high rate of post-settlement selection (54.9% of the total identified taxa). However, several natural factors, besides OA, cause the mortality of early juvenile invertebrates, such as biological and physical disturbance, delay in metamorphosis, predation, and competition [76]. Likely, OA increases the early life stages and adult mortality rate, acting as a steady physiological stress inducing the loss of functional and taxonomic diversity at the vent's sites [10].

The occurrence of *A. echinata*, *H. arctica*, and *M. subpictus* individuals at acidified stations seems to confirm the robustness of heavily calcified bivalves to a decrease of pH [54]. No visible sign of shell erosion, or differences in shell thickness among control and acidified conditions, were reported for the mollusc specimens found in the artificial collectors exposed to OA conditions. Likely, the short period of exposure to low/extremely low pH (33 days), as well as the natural fluctuation of pH values at the studied sites, can explain the observed pattern. Shell erosion has been observed in different adult mollusc species, such as *Patella* spp., *Phorcus turbinatus* (Born, 1778) and *Columbella rustica* (Linnaeus, 1758), in both vegetated hard bottoms and *Posidonia* meadows of the Castello vents [9,69].

Among gastropods, many herbivorous taxa can benefit from CO₂ increases at vent sites through the provision of higher habitat complexity and food, as an effect of the increase in primary production [77]. In particular, *R. variabilis* deserves particular mention, since this species showed sensitivity to pH reduction in laboratory trials, with alteration in its ability to perceive chemical cues from prey [78]; however, *R. variabilis* did not show changes in settlement along the pH gradient.

No relevant differences in species composition, richness, and abundance were reported between canopy (C) and bottom (B) samples, except for stations at low and extremely low pH on the south side. At this site, the bottom samples hosted a higher number of taxa if compared to the canopy ones, while abundance showed a peak in the canopy at extremely low pH. This pattern may be related to high shoot density values reported in the south side vents (>1000 shoots/m²). In this zone, a similar pattern has been observed on the invertebrate community associated with *Posidonia* by Garrard et al. [54], where the abundance recorded in the acidified zone (low pH) was doubled if compared to the control zone.

The mollusc species showing a certain degree of robustness to OA may take advantage of the absence of the other species selected against water acidification, and, therefore, reduced competition. A similar mechanism of community compensation has been observed for the benthic community associated with the vegetated hard bottom of both sides of the Castello vents [53].

The samplings performed, especially at the Vullatura vent and control stations, considering three different microhabitats, offered an interesting perspective about the buffering effect provided by the *P. oceanica* cover. Mollusc assemblages showed qualitative and quantitative differences among microhabitats, due to the change of multiple environmental parameters, such as hydrodynamics and light, and the plant cover, which occurs from the canopy to the bottom of the meadow and in the dead matte. In our samples, and in all three of the sites considered, canopy and bottom samples always are separated (see PCoA models), likely suggesting a certain degree of buffering of the canopy. At the Vullatura, samples placed on the dead matte were more similar to those collected at the bottom level (B, among rhizomes). They showed lower diversity and abundances in the acidified

stations if compared to the bottom and canopy samples. At the same time, these two descriptors were similar to those reported for the canopy level and even higher than the bottom samples in the Vullatura control station. The mollusc assemblage settled on the dead matte also showed less consistency in the PCoA plane according to pH gradient. This pattern suggests that when living *Posidonia* shoots are present, they can buffer the effect of pH reduction on associated fauna, likely through photosynthesis processes, oxygen production, and carbon dioxide removal [62,79,80]. Buffering effects on the local pH have been highlighted recently in *Posidonia* and other seagrasses by an increasing number of studies [32–34]; however, the direct or indirect effects on the local fauna have been poorly considered. Also, in the study carried out by Garrard et al. [54], the highest abundances and richness recorded in *Posidonia* under acidified conditions have been attributed more to the indirect effect of the local increase of the habitat complexity (i.e., shoot density) than to the direct effect of buffering low pH. Especially for small invertebrates with cryptic life habits, as most of the invertebrates associated with *Posidonia* are, dense seagrass and macroalgal cover may represent a “chemical” refuge from local pH fluctuations and other stressing conditions. Therefore, even calcifying organisms, as molluscs, could be less disadvantaged from the effect of the alteration of carbonate chemistry within the canopy of this seagrass.

Although preliminary to properly considering this aspect, the present study suggests that *P. oceanica* plays a pivotal role in the survival of several associated species, both as a nursery area and also as a refuge, via the mitigation of future climate change through contrasting OA, by oxygen production, CO₂ removal, and by carbon storage. Therefore, both high habitat complexity and climate mitigation effects contribute to making *P. oceanica* meadows an increasingly essential marine ecosystem for the future of the Mediterranean Sea, so that protection and conservation measures, such as the EU Biodiversity Strategy for 2030, should be encouraged to maintain this habitat to be as healthy, diverse, and productive as possible.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13070311/s1>, Figure S1: picture of *P. oceanica* subject to CO₂ bubbling at the S3 station (Castello south side vents), Figure S2: pH trend at the studied stations, Table S1: leaves length values and *t*-test pairwise comparisons, Figure S3: leaf length of *P. oceanica* measured during the two sampling periods, Figure S4: composition of epiphytic assemblages on *P. oceanica* leaves, Figure S5: common taxa found in the artificial collectors, Table S2: results of the SIMPER analyses performed among pH conditions at the investigated vent sites, Table S3: results of the SIMPER analyses performed among the three investigated microhabitats at the Vullatura vent site, Table S4: comparison among post-settlement molluscs described in the present study and the checklist of the mollusc taxa recorded in the CO₂ vent sites of the Castello Aragonese.

Author Contributions: Conceptualisation, M.C.G. and E.C.; methodology, M.C.G. and E.C.; formal analysis, A.B. and L.C.; data curation, A.B., L.C. and E.C.; writing—original draft preparation, A.B., L.C., M.C.G. and E.C.; writing—review and editing, G.A., M.C.G. and E.C.; supervision, G.A., M.C.G. and E.C. All authors have read and agreed to the published version of the manuscript.

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