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Shifting microbial communities in acidified seawaters: insights from polychaetes living in the CO_2 vent of Ischia, Italy

Irene ARNOLDI,[1,2,3,4] Davide CARRARETTO,[3] Marco MUNARI,[5,7] Matteo NANNINI,[6] Maria Cristina GAMBI,[8] Antonio CANNAVACCIUOLO,[9] Camilla DELLA TORRE[1,7] and Paolo GABRIELI[1,2]

[1]Department of Biosciences, University of Milan, Milan, Italy, [2]Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan, Italy, [3]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy, [4]University School of Advanced Studies Pavia, IUSS, Pavia, Italy, [5]Department of Biology, Stazione Idrobiologica Umberto d'Ancona, University of Padova, Chioggia (Venice), Italy, [6]Department of Integrative Marine Ecology, Ischia Marine Centre, Stazione Zoologica Anton Dohrn, Ischia (Naples), Italy, [7]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy, [8]Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, Triest, Italy and [9]Department of Integrative Marine Ecology, Fano Marine Centre, Stazione Zoologica Anton Dohrn, Fano, Pesaro-Urbino, Italy

Abstract

Oceans' absorption of human-related CO_2 emissions leads to a process called ocean acidification (OA), consisting of the decrease of the seawater pH with negative consequences for many marine organisms. In this study, we investigate the microbial community of two species of polychaetes found in naturally acidified CO_2 vents: the nereid *Platynereis massiliensis* complex and the syllid *Syllis prolifera*. Animals were collected in the CO_2 vents of Castello Aragonese (Gulf of Naples, Ischia, Italy) in three zones at decreasing pH. For the analysis of the microbiome, the V3-V4 hypervariable region of the 16S ribosomal RNA gene of 40 worm samples was sequenced on an Illumina MiSeq platform. No difference in the microbial alpha diversity of both species was highlighted. On the contrary, the microbial composition of worms collected in the site at normal pH was different from that of the individuals obtained from the sites at lower pH. This effect was evident also in samples from the site with a slight, but relevant, degree of acidification. Amplicon sequence variants showing a significant variation among the groups of samples collected from different pH zones were reported for both polychaetes, but no common trend of variation was observed. The present study deepens our knowledge about the composition of polychaete microbiome in marine naturally acidified sites. Our results stress the importance of future investigations about the connection between the variation of environmental and polychaete microbial communities induced by OA and about the effect of these variations on polychaete key biological and ecological traits.

Key words: climate change, microbiome, ocean acidification, *Platynereis massiliensis* complex, *Syllis prolifera*

Correspondence: Paolo Gabrieli and Camilla Della Torre, Department of Biosciences University of Milan, 20133, Milan, Italy.

Email: paolo.gabrieli@unimi.it and camilla.dellatorre@unimi.it

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INTRODUCTION

Since the industrial revolution, the augmented concentration of carbon dioxide in the atmosphere has led to a rise in its absorption by oceans, driving a process called "ocean acidification" (OA), which has been associated with the reduction of seawater pH and with changes in the sea carbonate chemistry (Caldeira & Wickett 2003; Figuerola *et al.* 2021). Indeed, OA is potentially involved in the alteration of global biogeochemical cycles and marine biomineralization, determining an increase in the solubility and the dissolution of $CaCO_3$, a main component of sediments and the shells or skeletons of many marine calcifiers (Orr *et al.* 2005; Lebrato *et al.* 2016; Figuerola *et al.* 2021). This phenomenon is quick and extended: Seawater surface pH has decreased by 0.1 units since 1850 and is predicted to diminish by 0.3 units by the year 2100 (Caldeira & Wickett 2005; IPCC 2019).

Biological responses to OA are highly variable among taxa, and the influence of this process on organism biology is exacerbated when evaluated in experiments with multi-species assemblages (Kroeker *et al.* 2013). OA seems to drive an overall decrease in diversity, biomass, and trophic complexity and functionality of the benthic marine communities (Kroeker *et al.* 2011; Teixido *et al.* 2018, 2024) and has a generally negative effect on survival, calcification, growth, and development, particularly on heavily calcified and sessile organisms (Kroeker *et al.* 2013; Range *et al.* 2014; Bressan *et al.* 2014; Simonetti *et al.* 2022; Palombo *et al.* 2023). On the contrary, more motile groups, including crustaceans and fishes, are less sensitive to acidification (Kroeker *et al.* 2013; Mirasole *et al.* 2021), and increased abundance and coverage in seagrass and some groups of macroalgae was predicted to be associated with high pCO_2 (Sunday *et al.* 2017).

A higher abundance of small-bodied, non-calcifying invertebrates, including some polychaetes, has been observed in naturally acidified sites (Kroeker *et al.* 2011; González-Delgado & Hernandez 2018). Polychaetes are one of the most abundant benthonic taxa (Giangrande *et al.* 2005) and show a heterogeneous species-specific response to environmental stress, probably due to their high morpho-functional diversity (Jumars *et al.* 2015). The influence of OA on the composition of the polychaete community has been examined in previous studies, by *in situ* observations of peculiar habitats, which are naturally acidified by the CO_2 surplus emitted from the seafloor, such as CO_2 vents. The ecological and life history traits that seem to be favored in these organisms under lower pH-high pCO_2 conditions have been studied (Gambi *et al.* 2016), and the effects of OA on metabolic rates (Calosi *et al.* 2013) and cellular pathways (Signorini *et al.* 2023), antioxidant capacity (Valvassori *et al.* 2019; Munari *et al.* 2022), and growth (Del Pasqua *et al.* 2019) have been described in different polychaete species.

It is well known that microbial communities play an important role in key biological processes of most multicellular eukaryotes (McFall-Ngai *et al.* 2013) and might contribute to developing tolerance of the holobiont to environmental disturbances. Some studies investigated the effects of OA on microbial composition of corals, sponges, foraminifera, oyster, and crustose algae both under laboratory-controlled conditions as well as upon natural gradient of pH variations, showing species-specific modifications and/or no variation of the microbiome (Webster *et al.* 2013, 2016; Meron *et al.* 2011, 2012; Morrow *et al.* 2015; Biagi *et al.* 2020; Barreto *et al.* 2021; Kong *et al.* 2022). Evidence provided by laboratory research highlighted that the microbial community composition might have a key role in shaping the performance of host organisms under OA (Palladino *et al.* 2022): This topic deserves a broad and deep investigation in the case of central (from a trophic point of view) benthic animals such as polychaetes, whose microbiome role in a naturally acidified environment has never been studied (Tangherlini *et al.* 2021). In the present study, the volcanic CO_2 vents of the Castello Aragonese of Ischia Island (Gulf of Naples, Italy, 40°43.84′N, 13°57.08′E) were selected as a well-characterized natural laboratory for OA studies (Fig. 1; Foo *et al.* 2018; Teixido *et al.* 2024). The vents do not present any environmental perturbing factor other than pH, which varies following a gradient from 8.1 to less than 7.4 (Kroeker *et al.* 2011). The polychaetes *Syllis prolifera* Krohn, 1852 and *Platynereis* spp. have been previously reported to be tolerant to OA and abundant even in the most acidified zones of this area (Calosi *et al.* 2013; Gambi *et al.* 2016).

Platynereis spp. and *S. prolifera* belong to two different families of Polychaeta (respectively, Nereididae and Syllidae). Individuals of the *Platynereis* genus have been proposed to belong to two main species complexes, the *Platynereis massiliensis* and *Platynereis dumerilii* complexes based on a phylogenetic analysis of the cytochrome oxidase I gene of individuals collected into acidified and nonacidified sites of the Mediterranean Sea (Wäge *et al.* 2017). Individuals of these species are not morphologically distinguishable in the adult immature stage, but they display specific biological features: *P. massiliensis* is a hermaphrodite and it is a brooding species, which deposits the lecitothrophic eggs into the

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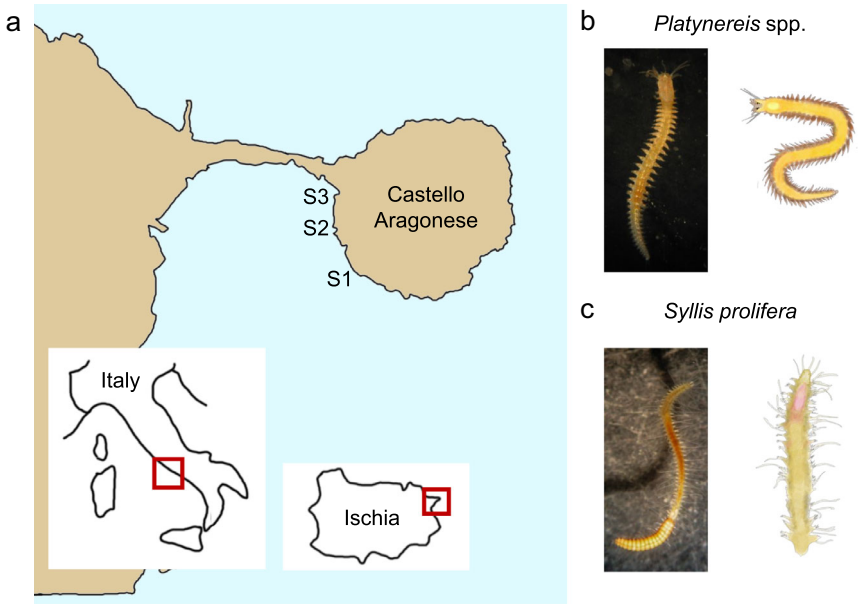

Figure 1 Study area and collected organisms. Three zones at decreasing pH (S3 < S2 < S1) were selected on the southern sides of CO_2 vents of Castello Aragonese (Ischia Island, Gulf of Naples, Italian Peninsula) (a). *Platynereis* spp. polychaetes (b) were collected in S1 and S2 zones, while *Syllis prolifera* polychaetes (c) in S1, S2, and S3 zones. *Platynereis* spp. and *Syllis prolifera* photos were taken by Maria Cristina Gambi.

tubes and shows a direct development with juveniles hatching directly from the eggs (Wäge *et al.* 2017); *P. massiliensis* species complex is probably predominant in the acidified areas of Ischia vents if compared to *P. dumerilii* (Lucey *et al.* 2015; Wäge *et al.* 2017) and its individuals are meso-herbivore, preferentially feeding on macroalgae. Individuals of the *P. dumerilii* complex are gonochoric and reproduce by epitoky transformation into a pelagic mature individual and free spawning with the death of the individual (Wäge *et al.* 2017)

S. prolifera is a gonochoric species and it reproduces mainly by the formation of stolons, defined as sexual satellites, produced from the pygidial zone during maturation and full of gametes (Fig. 1). These organisms show external fertilization and a short pelagic phase (Gambi *et al.* 2017). The species feeds preferentially on diatoms and small invertebrates. Interestingly, a recent work provided evidence about the pseudocrypticism of *S. prolifera* in the Mediterranean Sea (Del Olmo *et al.* 2024).

These polychaetes were sampled in three sites at decreasing pH of the Castello Aragonese vent to evaluate the differences in their microbial communities by sequencing the V3-V4 hypervariable region of the 16S rRNA gene on an Illumina MiSeq platform.

MATERIALS AND METHODS

Sample collection

Vents of Castello Aragonese of the Ischia Island (Tyrrhenian Sea, Italy) are generated by a subterranean source of CO_2 (and other gases in trace amounts, in the absence of sulfur), which opens into a shallow stretch of a volcanic islet with sloping rocky reefs (Kroeker *et al.* 2011). We specifically selected the CO_2 vents of Castello Aragonese because in this location, the pH is the main variable changing along a well-characterized gradient. Thus, sites showing a different pH range are very close to each other (along a distance of approximately 300 m) and host a similar habitat. Adult polychaetes samples were collected in three distinct zones associated with different vent activity/intensity and characterized by decreasing values of pH (Gambi *et al.* 2016). Particularly, the three zones on the southern side of the islet were selected: ambient or S1 (no venting, pH 8.1 ± 0.1), low or S2 (moderate venting, pH 7.8 ± 0.3), and extremely low or S3 (intense venting, pH 7.4 ± 0.5) pH zones (Fig. 1).

Individuals from *Platynereis* spp. (*P. massiliensis* and *P. dumerilii* complexes) and *S. prolifera* were harvested

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in June 2021. Since the species live in close association with macroalgae, the brown macroalga *Halopteris scoparia* (Linnaeus) Sauvageau, 1904 was collected by hand by scuba divers at 1–2 m depth in all collection sites using fabric bags. After sorting, each individual was isolated, identified morphologically under a stereomicroscope, and stored in 95% ethanol at 4°C until genomic DNA (gDNA) extraction. For each site, eight individuals were collected for each species, apart from *Platynereis* spp., which were not found in the S3 zone.

Genomic DNA extraction and molecular identification of the species

Samples were hydrated overnight in phosphate buffer saline, and gDNA was extracted from the whole body of the organisms following the manufacturer's instructions (Monarch® Genomic DNA Purification Kit, New England BioLabs). This preparation did not enable us to distinguish between gut-residing and epibiotic bacteria during the analysis of the polychaetae microbiome. Amplification of the mitochondrial locus cytochrome oxidase I (COI) was performed as previously described using LCO and HCO primers (Folmer *et al.* 1994; Arnoldi *et al.* 2022) to carry out identification at the species level. PCR products were sequenced (Eurofins Genomics, Ebersberg, Germany) and compared with those annotated in the GenBank database using the nucleotide Basic Local Alignment Search Tool (Altschul *et al.* 1990). Sequences were edited with AliView software (Larsson 2014) and submitted to the GenBank database under accession numbers OQ673176-OQ673212.

rRNA amplicon sequencing

gDNA from eight individuals from each species for each pH zone (besides *Platynereis* spp. from S3) were processed for the amplification of the V3-V4 hypervariable region of the 16S rRNA gene by PCR using Pro341F and Pro805R primers (Takahashi *et al.* 2014) and GoTaq® DNA Polymerase (Promega) (modified thermal profile: 94°C 30″; 40 cycles 94°C 15″, 60°C 30″, 72°C 1′; 72°C 5′, 4°C 15′). The hypervariable region V3-V4 has been extensively used in the past to characterize microbial communities (Takahashi *et al.* 2014).

Dual-indexing Next Generation Sequencing (NGS) was performed on an Illumina MiSeq platform (300 Pair End) after the linkage of Nextera XT barcoded sequencing adapters to the amplicons (BMR Genomics, Padova, Italy). The dataset resulting from the NGS sequencing was submitted to GenBank under the accession number PRJNA947580.

Sequence data processing and analysis

Sequence data processing and analysis were done in R software 4.2.0. Quality assessment of the sequencing reads was done using the *ShortRead* package (Morgan *et al.* 2009) and reported in Supporting Information S1. Raw data were processed using the *dada2* package as follows (Callahan *et al.* 2016): Sequences were trimmed at 250 bp, quality-filtered (quality score > 2), and dereplicated. To reveal the composition of the sequenced communities, error rates were learned by alternating between sample inference and error rate estimation until convergence. Forward and reverse sequences were merged into contigs, and chimera sequences were removed. All sequences kept for the analysis were 425 bp long. Sequences were classified into amplicon sequence variants (ASVs) using the Silva (version 138.1) reference library (Quast et *al*. 2013) with the assignTaxonomy function (minimum bootstrap = 50).

Data were represented and analyzed using the *phyloseq* package (McMurdie & Holmes 2013). Two datasets were generated: one for *S. prolifera* (SY) and one for *P. massiliensis* complex (PL) samples. Datasets were refined by excluding samples showing less than 30 000 reads before quality filtering or displaying an uncertain molecular identification (Table 1). At this point, uncharacterized ASVs were removed from each sample retrieved in the analysis. Additionally, a prevalence threshold was defined and applied to maintain only the ASVs that were present in at least three samples. Sequences per sample were rarefied to the minimum number of reads observed in a sample in each dataset to eliminate the variability due to the number of sequences per sample.

At this point, the abundance of phyla and the differences in alpha and beta diversity were investigated. Depending on the dataset, samples were grouped by membership to a given site (S1, S2, S3) or pH zone, that is, control (corresponding to S1) or low (including S2 and S3).

Abundance of bacterial phyla was retrieved for each dataset in each group. Venn diagrams showing the exclusive or shared ASVs between groups were generated (*VennDiagram* package).

Alpha diversity was evaluated using the estimate_richness function and considering multiple indexes (Ferrari 2010; Thukral 2017). For species richness, which quantifies the number of ASVs present in a sample, we used the following indexes: observed species (the number

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Table 1 Overview of the polychaete samples analyzed in the present study

Sample ID	Miseq read count	Morphological identification	GenBank accession number	Excluded samples
1094535F1072701	122 972	*Syllis prolifera*	OQ673176	
1094536F1072702	107 974	*Syllis prolifera*	OQ673177	
1094537F1072703	98 328	*Syllis prolifera*	OQ673178	
1094538F1072704	73 596	*Syllis prolifera*	OQ673179	
1094539F1072705	72 018	*Syllis prolifera*	OQ673180	
1094540F1072706	65 135	*Syllis prolifera*	OQ673181	
1094541F1072707	68 557	*Syllis prolifera*	OQ673182	
1094542F1072708	139 231	*Syllis prolifera*	OQ673183	
1094543F1072709	68 346	*Syllis prolifera*	OQ673184	
1094544F1072710	72 975	*Syllis prolifera*	OQ673185	
1094545F1072711	64 279	*Syllis prolifera*	OQ673186	
1094546F1072712	30 034	*Syllis prolifera*	OQ673187	
1094547F1072713	34 749	*Syllis prolifera*	OQ673188	
1094548F1072714	47 765	*Syllis prolifera*	OQ673189	
1094549F1072715	47 491	*Syllis prolifera*	OQ673190	
1094550F1072716	52 963	*Syllis prolifera*	OQ673191	
1094551F1072717	81 859	*Syllis prolifera*	OQ673192	
1094552F1072718	95 703	*Syllis prolifera*	OQ673193	
1094553F1072719	84 949	*Syllis prolifera*	OQ673194	
1094554F1072720	97 670	*Syllis prolifera*	OQ673195	
1094555F1072721	101 142	*Syllis prolifera*	OQ673196	
1094556F1072722	92 146	*Syllis prolifera*		X[†]
1094557F1072723	96 320	*Syllis prolifera*	OQ673197	
1094558F1072724	81 919	*Syllis prolifera*	OQ673198	
1094559F1072725	53 824	*Platynereis* spp.	OQ673199	
1094560F1072726	47 143	*Platynereis* spp.	OQ673200	
1094561F1072727	39 821	*Platynereis* spp.	OQ673201	
1094562F1072728	31 950	*Platynereis* spp.		X[†]
1094563F1072729	20 576	*Platynereis* spp.		X[‡]
1094564F1072730	81 843	*Platynereis* spp.	OQ673202	
1094565F1072731	33 617	*Platynereis* spp.	OQ673203	
1094566F1072732	47 773	*Platynereis* spp.	OQ673204	
1094567F1072733	45 175	*Platynereis* spp.	OQ673205	
1094568F1072734	60 953	*Platynereis* spp.	OQ673206	
1094569F1072735	59 362	*Platynereis* spp.	OQ673207	
1094570F1072736	68 417	*Platynereis* spp.	OQ673208	
1094571F1072737	61 175	*Platynereis* spp.	OQ673209	
1094572F1072738	85 421	*Platynereis* spp.	OQ673210	
1094573F1072739	54 315	*Platynereis* spp.	OQ673211	X[§]

(*Continued*)

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Table 1 (Continued)

Sample ID	Miseq read count	Morphological identification	GenBank accession number	Excluded samples
1094574F1072740	50 198	*Platynereis* spp.	OQ673212	

For each sample, the Miseq read count after alignment and filtering of raw sequences is provided. Each sample was identified morphologically and molecularly, based on the Sanger sequencing of the Cytochrome Oxidase I (COI) gene. The GenBank accession numbers of the COI sequences are reported. [†]Excluded for uncertain molecular identification. [‡]excluded for the low amount of associated Miseq reads. [§]excluded due to a similarity of the COI sequence lower than 90% if compared to the other samples of the *Platynereis* dataset.

of ASVs), Chao1 richness estimate, and abundance-based coverage estimators (ACE) (mathematically correcting the abundance for the number of rare ASVs). For equitability, which gives the relative abundance of different species of a community in terms of their evenness of distribution, we used Shannon's diversity, Simpson diversity, inverted Simpson diversity, and Fisher indexes.

For each index, the normality of distribution and homogeneity of variance were evaluated using the Shapiro–Wilk normality test and the Levene test, respectively. Unpaired *t*-test and ANOVA were applied to parametric distributions and Wilcoxon and Kruskal tests to nonparametric ones (*stats* and *car* packages). Welch's *t*-test was applied to parametric distributions, which did not show homogeneity of variance among groups.

For the analysis of beta diversity, the Bray–Curtis dissimilarity matrix was generated and visualized using principal coordinate analysis (PCoA). The environmental variables (site or pH zone) covarying and potentially changing the microbial community structure were overlayed onto the Bray–Curtis-based nonmetric multidimensional scaling-ordination using the envfit function (*phyloseq* and *vegan* packages) (Dixon 2003).

Type II permutation MANOVA using distance matrices (adonisII function) and pairwise permutation MANOVAs (PERMANOVAs) were used to test the significance of the separation between groups of samples and for multiple comparisons between sites and pH zones (*RVAideMemoire* and *lefff/mctoolsr* packages). To separate the turnover (beta.SIM) and nestedness (beta.SNE) components of beta diversity, the Sorensen dissimilarity index (beta.SOR) was calculated using the *betapart* package (Baselga 2010). Finally, ASVs differentially expressed across sites and pH zones were individuated for each dataset (alpha = 0.01, *DESeq2* package) (Love *et al.* 2014). Data were visualized using the *ggplot2* package (Villanueva & Chen 2019).

RESULTS

Molecular identification

Morphological identification of samples carried out by experts during collection at the Castello Aragonese vents was confirmed by molecular analyses (Table 1). Genetic distance between clades associated with the *Platynereis* spp. complexes was previously observed to be around 20% (Wäge *et al.* 2017). Most of the samples morphologically identified as belonging to *Platynereis* spp. were assigned to the *P. massiliensis* complex (hereafter *P. massiliensis* complex); particularly our samples are similar to the *P. massiliensis* clade 2 described by Wäge *et al.* (2017). One sample, most likely belonging to the *P. dumerilii* species complex, showed low similarity (from 81.4% to 82.6%) to all the other samples and was excluded from subsequent analysis. Additionally, one sample was eliminated because we were not able to amplify its COI sequence, probably due to low DNA concentration. All the remaining sequences showed high similarity to each other (from 100% to 92.94%). The COI markers of the individuals morphologically identified as *S. prolifera* were also analyzed and showed a similarity from 100% to 93% to each other and from 96.79% to 99.14% to *S. prolifera* sequences in Genbank (PP494883.1 and PP494887.1), except for one sample that was excluded from the following analysis due to uncertain sequence identification. Sequences were submitted to GenBank (OQ673176- OQ673212) (Table 1).

Sequencing overview

Illumina MiSeq sequencing of bacterial 16S ribosomal RNA gene amplicons produced a total of 2 739 684 sequences from 40 samples (average = 68492.1 ± 26490.12). After quality filtering, chimera removal,

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Table 2 Samples retrieved in the phyloseq analysis

	Platynereis massiliensis complex	*Syllis prolifera*
Site 1 (S1)	6	8
Site 2 (S2)	7	8
Site 3 (S3)	0	7
Total	13	23

taxonomy assignment, and removal of mock sequences, 1 365 621 sequences were maintained (average = 34140.525 ± 12167.5). After the exclusion of one sample having less than 30 000 reads and of the individuals of uncertain classification, the composition of the microbial community of 36 samples was analyzed, 13 *P. massiliensis* complex individuals and 23 *S. prolifera* individuals (Table 2).

In the whole dataset, a total of 3883 taxa were identified before filtering for prevalence; 401 764 sequences were associated with *P. massiliensis* complex (from a maximum of 47 950 to a minimum of 19 194, mean: 30 904 ± 8050.722) and 537 264 sequences with *S. prolifera* (from a maximum of 46 830 to a minimum of 4177, mean: 23359.3 ± 12062.28). After filtering for prevalence, 135 taxa (260 428 sequences) were retrieved for *P. massiliensis* complex and 236 taxa (273 304 sequences) for *S. prolifera* (Figs S1,S2, Supporting Information).

After rarefication, 135 taxa (126 230 sequences) were recovered for *P. massiliensis* complex and 236 taxa (53 728 sequences) for *S. prolifera*, indicating that rarefaction did not lead to a loss of taxa in the dataset (Supporting Information S2,S3).

Relative abundance of microbiome phyla in the two polychaete species

In the *P. massiliensis* complex dataset, 97 of the 135 taxa were Proteobacteria (82 Gammaproteobacteria, 15 Alphaproteobacteria), 14 Firmicutes, 11 Actinobacteriota, 6 Bacteroidota, 2 Campylobacterota and Cyanobacteria, and 1 Fusobacteriota, Spirochetota, and Verrucomicrobiota (Fig. 2a). The most prevalent sequence in the zone at normal pH (S1) was $ASV8_{PL}$, while in the low pH zone (S2) was $ASV1_{PL}$, accounting for 11% and 12% of the total sequences of each site, respectively. $ASV8_{PL}$ and $ASV1_{PL}$ sequences were 96.75% and 96.94% identical to KF786746.1 (Uncultured Alphaproteobacterium) and MK048701.1 (Uncultured *Colwellia* sp., Proteobacteria), respectively.

In the *S. prolifera* dataset, 129 of the 236 taxa were Proteobacteria (80 Gammaproteobacteria, 49 Alphaproteobacteria); 41 Firmicutes; 24 Actinobacteriota; 15 Cyanobacteria; 10 Verrucomicrobiota; 7 Bacteroidota; 2 Campylobacterota, Myxococcota, Planctomycetota, and SAR324 clade; and 1 Chloroflexi and WPS-2 (Fig. 2b). The most prevalent sequence in site/zone at normal pH (S1) was $ASV5_{SY}$, while in the low pH zones (S2 and S3 together) was $ASV1_{SY}$, accounting for 10.5% and 12.6% of total sequences, respectively. $ASV5_{SY}$ and $ASV1_{SY}$ sequences were 100% identical to sequences of the *Escherichia-Shigella* genera (Proteobacteria) and MT573192.1 (*Exiguobacterium profundum*, Firmicutes), respectively.

In the *P. massiliensis* complex dataset, 23 ASVs (17%) were specifically found at normal pH, 22 (16%) in the low pH zone, and 90 ASVs (67%) in both environments. In the *S. prolifera* dataset, 12 ASVs (5%) were specifically found at normal pH, 52 (22%) in the low pH zone (S2 & S3), while 172 ASVs (73%) were found in both conditions (Supporting Information S4 and Fig. S3, Supporting Information).

Changes in the relative abundance at a broad taxonomic level were investigated (Fig. 2a,b). Among the major bacterial phyla, accounting for almost 90% of all bacterial ASVs (Proteobacteria, Actinobacteriota, and Firmicutes), a coherent change was noted only in the case of Actinobacteriota, whose abundance decreases in the lower pH zone in both the species. Conversely, Proteobacteria and Firmicutes showed a contrasting trend. Particularly, Proteobacteria decreased in the *S. prolifera* samples collected in the low pH zones, while increased in the same condition in *P. massiliensis* complex. Firmicutes showed an opposite trend.

Despite being less prevalent, the abundance of Verrucomicrobiota was lower at lower pH in both the species, while the abundance of Cyanobacteria and Campylobacterota changed in the two species following an opposite direction.

In *P. massiliensis* complex, Fusobacteriota were not present in the S2 site, despite being retrieved in S1. In this species, the major changes were observed for Spirochetota and Campylobacterota (11-fold and 19-fold increase at lower pH) (Fig. 2c).

In *S. prolifera*, we were able to compare the change in prevalence throughout two different sites at decreasing pH (S2 and S3). Most phyla showed the same trend of prevalence reduction or increase in the two low pH zones (S2 and S3) compared to S1, with Cyanobacteria and WPS-2 being the phyla whose prevalence mostly

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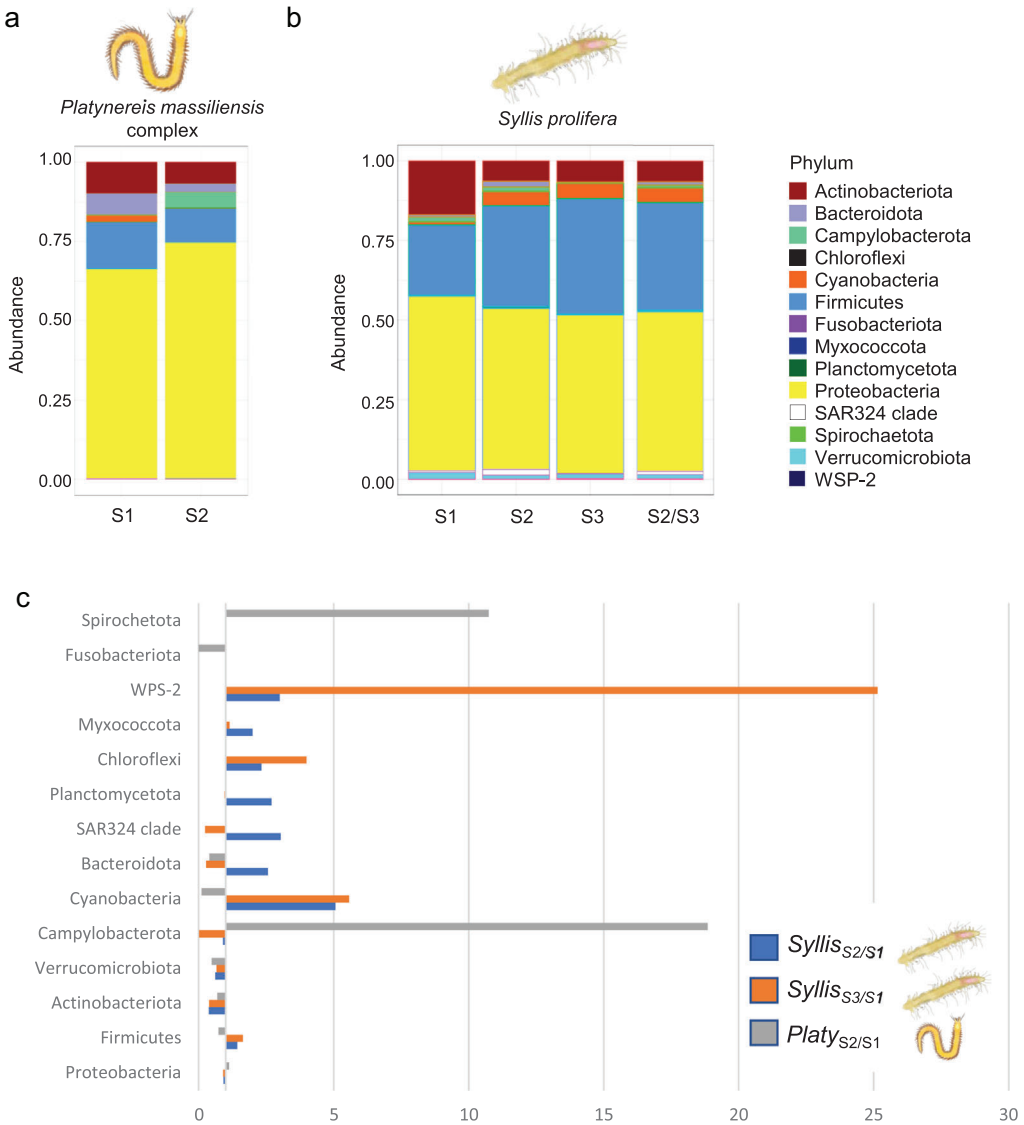

Figure 2 Prevalence of bacterial phyla in zones at different pH. The variation of prevalence of phyla within different pH conditions or sites is reported for *Platynereis massiliensis* complex (a) and *Syllis prolifera* (b) datasets. (c) The change in prevalence was measured as the abundance in sites at low pH normalized for the abundance at normal pH (site S2 divided for site S1 and site S3 divided for S1 for *S. prolifera* dataset; site S2 divided for site S1 for *P. massiliensis* complex).

increased at lower pH (5-fold and 25-fold increase, respectively) (Fig. 2c).

Alpha diversity

Richness and equitability were evaluated in the *P. massiliensis* complex dataset according to the two zones where the animals were collected (S1 and S2) (Fig. S4, Supporting Information). Differences in alpha diversity were not statistically significant (Table S1, Supporting Information).

The *S. prolifera* dataset was analyzed in two different ways: (i) considering the three zones (S1, S2, and S3) separately or (ii) comparing the zone at normal pH (S1) to the two zones at low pH (S2 and S3 together). In both analyses, there was no change in the

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bacteria alpha diversity (Fig. S4 and Table S1, Supporting Information).

Beta diversity (distances)

The PCoA of the Bray–Curtis distance matrix, supported by MANOVA statistical analysis, showed that the *P. massiliensis* complex samples grouped according to their provenience (S1 or S2 zones; *F*-value = 2.5462, *P* = 0.003996; Fig. 3a). The variable that most influenced the grouping of the samples is the site of collection (R^2: 0.4333, $P = 0.009$).

In a similar analysis, the *S. prolifera* samples also grouped according to the site of collection (Bray–Curtis: MANOVA, *F*-value = 2.2825, *P* = 0.000999; Fig. 3b). Interestingly, the statistical analysis supported the separation between the S1 samples and the S2 or S3 samples, while it did not support the separation between S2 and S3 samples (pairwise comparison S1-S2 $P_{Adj} = 0.003$; S2-S3 $P_{Adj} = 0.714$, S1-S3, $P = 0.006$). When the same samples were analyzed as collected from the zone at normal pH (S1) and zones at low pH (S2 and S3 together), the samples in the PCoA analysis grouped according to their zones, and this was supported by the statistical analysis (Bray–Curtis: MANOVA, *F*-value = 3.3121, *P* = 0.000999, Fig. 3b). The variables that most influenced the grouping of the samples were the site of collection (S1, S2, and S3) and the pH of the site (normal or low) (Site, $R^2 = 0.3487$, $P = 0.003$; pH, $R^2 = 0.3328$, $P = 0.002$).

Beta diversity (indexes)

The overall beta diversity between *P. massiliensis* complex individuals, calculated using the Sorensen index, was 0.83 (value of turnover component or replacement of some species with others = 0.79; value of nestedness component or species loss = 0.03). If the animals were analyzed separately based on their collection site, the overall beta diversity was 0.70 in S1 and 0.72 in S2.

In *S. prolifera*, the overall beta diversity was 0.90 (turnover component = 0.88; nestedness component = 0.02), while the beta diversity at normal pH (S1) was 0.79 and at low pH (S2 and S3) was 0.87.

The beta diversity between groups of individuals belonging to zones at different pH (normal-S1 and low pH-S2 and S3) was also calculated. In *P. massiliensis* complex, the beta diversity between groups was 0.20 (turnover component = 0.196; nestedness component = 0.004), and in *S. prolifera* was 0.16 (turnover component = 0.07; nestedness component = 0.09). *S. prolifera* samples collected in low pH zones showed a higher degree of diversification than those harvested in S1, as suggested by the beta diversity indexes. This effect was not evident in the case of *P. massiliensis* complex, even if it must be considered that the number of individuals analyzed for this species was lower.

ASVs changing abundance according to the pH

Deseq analysis on *P. massiliensis* complex samples highlighted a statistically significant variation of 22 ASVs depending on the pH zone (Supporting Information S5). 17 ASVs belonged to Proteobacteria (77%), 3 to Bacteroidota (14%), 1 to Campylobacteria (4.5%), and 1 to Cyanobacteria (4.5%). ASVs of the Bacteroidota family were generally decreased at low pH (75%), as well as Cyanobacteria, while Campylobacterota were more abundant (Fig. 3c). Proteobacteria mainly belong to the Gammaproteobacteria class (59%) and showed both a trend of decrease (51%) and increase (49%) in S2. $ASV45_{PL}$, which was at first identified as "Chloroplast" based on the alignment against the Silva database, was similar to NC_056910.1 sequence (identity: 100%; *Poterioochromonas malhamensis* strain SZCZR2049 plastid, complete genome) using refined blast against the GenBank database.

Deseq analysis of the *S. prolifera* samples (Supporting Information S5; Fig. 3d) highlighted a statistically significant increase of the following ASVs in S2-S3: *Altererythrobacter* sp. (Proteobacteria, log2FoldChange [FC]: 25.36, $P_{Adj} = 6.06e{-}22$), *Exiguobacterium aurantiacum* (Firmicutes, FC: 8.94, $P_{Adj} = 9.93e{-}06$), and *Pseudoalteromonas phenolica* (Proteobacteria, FC: 7.85, $P_{Adj} = 7.50e{-}05$).

When the S2 and S3 *S. prolifera* samples were analyzed separately, five ASVs were differently present in samples collected in S1 and S2 (*Sphingomonas paucimobilis* FC: 8.85, $P_{Adj} = 1.0e{-}03$, increasing in S2; *Altererythrobacter* sp. FC: 23.2, $P_{Adj} = 6.3e{-}13$; *Exiguobacterium aurantiacum*, FC: 9.12, $P_{Adj} = 1.16e{-}04$; *Pseudoalteromonas phenolica* FC: 8.03, $P_{Adj} = 5.59e{-}04$; Fokiniaceae sp. FC: −19.3, $P_{Adj} = 5.77e{-}06$) and three ASVs between S2 and S3 individuals (*Sphingomonas paucimobilis* FC: −8.45, $P_{Adj} = 7.51e{-}03$, decreasing in S3; *Aquaribacter albus* FC: −24.05, $P_{Adj} = 1.20e{-}08$; Fokiniaceae sp. FC: 23.61, $P_{Adj} = 1.20e{-}08$). Finally, four ASVs changed between S1 and S3 samples (*Aquaribacter albus* sp. FC: −16.79, $P_{Adj} = 6.47e{-}04$ decreasing in S3; *Altererythrobacter* sp. FC: 24.57, $P_{Adj} = 1.43e{-}13$; *Exiguobacterium aurantiacum*, FC: 8.70, $P_{Adj} = 6.47e{-}04$; *Pseudoalteromonas phenolica* FC: 7.61, $P_{Adj} = 2.69e{-}03$).

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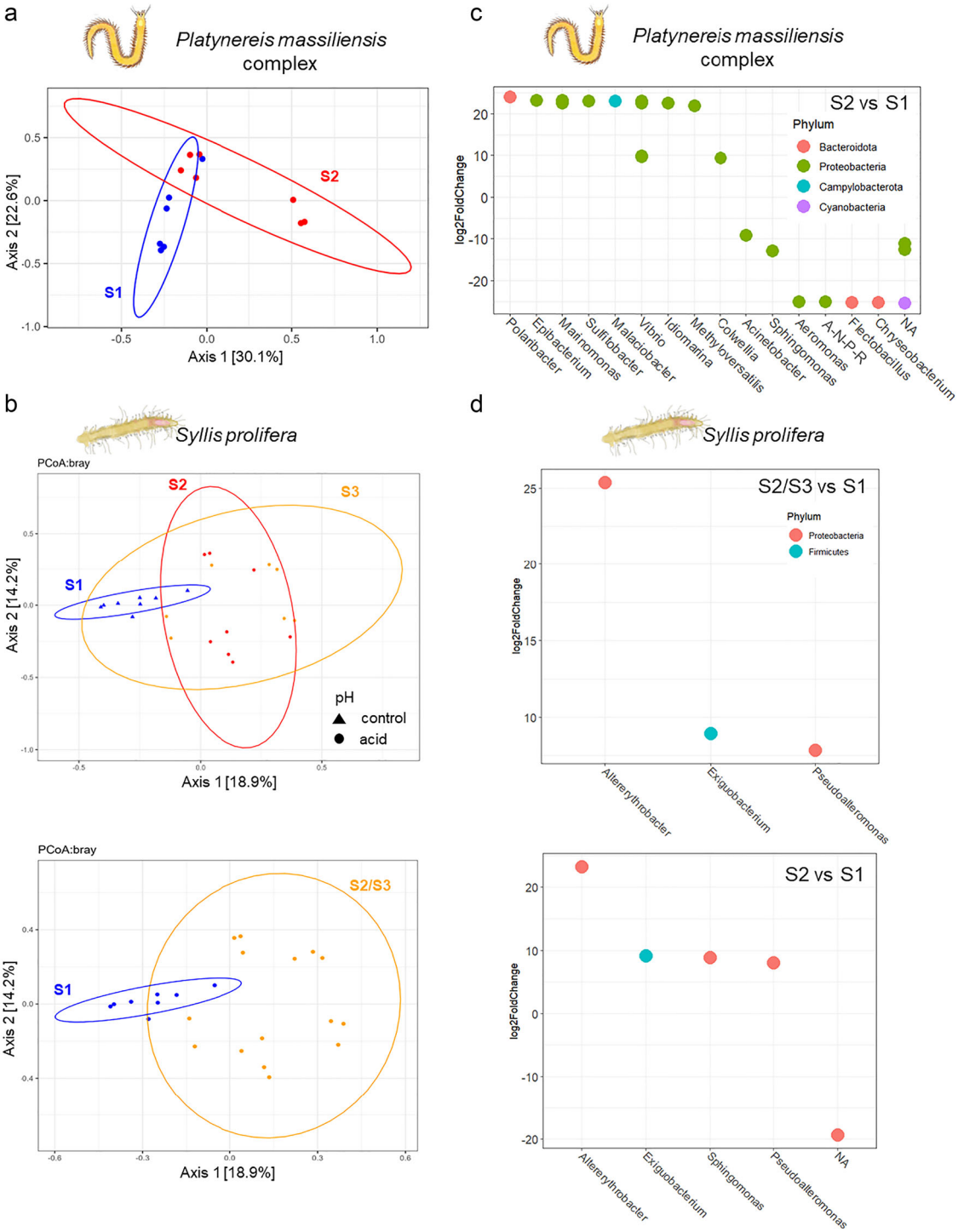

Figure 3 Beta diversity and Deseq analyses. Principal coordinate analysis (PCoA) of dissimilarity distances calculated using the Bray–Curtis method on *Platynereis massiliensis* complex samples grouped by site (a) and on *Syllis prolifera* samples (b) grouped by site (up) and pH zone (down). Deseq analysis on the *P. massiliensis* complex dataset (S2 vs S1) (c) and the *S. prolifera* dataset ((d) up: S2/S3 vs S1; (d) down: S2 vs S1). On the *y*-axis, the increase of Amplicon sequence variants (ASVs) in samples collected in S2 ((c), (d) down) or S2/S3 ((d) up) compared to S1 is represented. NA, ASVs not classified at genus level; A-N-P-R, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*.

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Summarizing the *S. prolifera* data, *Aquaribacter albus* was present in S1 and S2 but not in S3; *Sphingononas paucimobilis* was present at a higher level in S2 samples compared to both S1 and S3 individuals; Fokiniaceae sp. was present at higher levels in S1 and S3 samples compared to S2; *Altererythrobacter, Exiguobacterium aurantiacum*, and *Pseudoalteromonas phenolica* were present at higher levels in S2 and S3 compared to S1.

As it is possible to deduce by the present data, there are no shared ASVs between *P. massiliensis* complex and *S. prolifera*, which vary in the same direction and are correlated to environmental changes. Paradigmatic is the case of an ASV classified as *Sphingomonas paucimobilis*, which in *P. massiliensis* complex is more abundant in individuals collected in S1, while in *S. prolifera* is more abundant in S2 individuals.

DISCUSSION

OA has been previously observed to drive changes in the composition of invertebrate benthic communities. Specifically considering polychaetes, some species are more prevalent in marine areas characterized by a decrease in the water pH (Gambi *et al.* 2016). This phenomenon seems to be related to specific biological traits of these polychaete species, such as brooding reproductive behavior and filter feeding (Lucey *et al.* 2015; Gambi *et al.* 2016). Despite the microbiome being considered a key plastic entity, adapting its composition and functionality in response to environmental modifications and potentially promoting holobiont acclimatization (Biagi *et al.* 2020; Palladino *et al.* 2022), the effect of OA on the composition of the microbial communities of polychaetes has never been investigated until now.

In this context, the data presented in this study are a necessary but preliminary evaluation of the effect of OA on the microbiome of polychaetes and would be a basis for future and deeper investigation of this topic, particularly referring at the functional relevance of the variation in the microbial community that we are reporting.

In both *S. prolifera* and *P. massiliensis* complex, Proteobacteria, Firmicutes, and Actinobacteriota were the most abundant bacterial phyla, an observation consistent with the microbial composition of other polychaete species (Vijayan *et al.* 2019; Hochstein *et al.* 2019; Priscilla *et al.* 2022). We investigated the eventual variation in alpha and beta diversity associated with a change in the pH or the site of collection of these polychaetes. We did not observe any change in alpha diversity of the microbial species associated with polychaete individuals collected in different pH zones, nor in the richness or evenness. Indeed, the microbiome of samples collected in sites showing a lower pH range do not differ nor for the number of observed ASVs, neither for the heterogeneity in ASV composition.

We outlined a difference in beta diversity in both polychaete species. Indeed, the animals collected in the control site shared a similar microbiome and were separated with respect to microbial composition from those collected in the acidified site. Due to the relative stability of the CO_2 vents environment and thus to the absence of stress factors other than the progressive decrease of seawater pH, this change can be associated with the variation of this key abiotic factor. The analysis of *S. prolifera* dataset, which included worms collected from three sites at decreasing pH, enabled us to appreciate that the microbial communities of the animals from the low and the very low pH sites are extremely similar. The individuals collected from S2 and S3 sites did not differ in terms of beta-diversity, despite showing few ASVs changing between them. If this phenomenon is related to the presence of an environmental microbiome homogeneous to the two zones, or a homogeneous selection of microbial species in response to low pH, is currently unknown and must be explored in the future. In this regard, additional investigation on the composition of the environmental microbiome of this area is needed, also considering that some studies have shown a variation of the microbial communities of both sediments and the water column in other CO_2 vent systems (Raulf *et al.* 2015; Arcadi *et al.* 2023 and citation therein).

More generally, it would be useful to understand the diffusion of this phenomenon in this taxon, also considering that studies in this field have been lacking until now. Indeed, in other organisms, such as corals, the change in the composition of the microbial community in response to OA was reported in some species and not in others (Biagi *et al.* 2020 and citations therein). Similarly, also in sponges, either change and no modification of the microbial community in relation to OA was reported (Morrow *et al.* 2015; Kandler *et al.* 2018; Bottè *et al.* 2019).

The species analyzed in the present study, *S. prolifera* and *P. massiliensis* complex, despite being similar with respect to their tolerance to acidified site conditions, belong to different polychaete families. This could hamper the evaluation of the role of microbial communities in favoring the permanence of polychaetes in zones at low pH, mainly because the fine analyses of ASV variation in the two species put forward a species-specific modification of the microbial community of individuals collected in acidified sites, making it hard to come up with general considerations. The analysis of beta indexes suggests a different process driving the changes in the microbial community between individuals at normal or low pH in the two species. This variation is mainly driven by species

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turnover in *P. massiliensis* complex, while both species turnover and species loss seem to characterize the modification of the microbial community of *S. prolifera*.

A broad speculation about the impact of the variation of some bacterial taxa on polychaetes living in acidified environments can be done based on previous investigations carried out in these and other organisms but needs to be corroborated by future research in this field.

For instance, as seen in *S. prolifera*, Cyanobacteria and Chloroflexi increased in the sponge *Coelocarteria singaporensis* transplanted in a CO_2 vent site (Kandler *et al.* 2018). Cyanobacteria increased also in other sponge species and foraminifera subjected to OA (Webster *et al.* 2013; Morrow *et al.* 2015). The increase of spirochaetes observed in *P. massiliensis* complex was observed also in the oyster *Saccostrea glomerate* exposed to OA (Scanes *et al.* 2021).

On the other hand, we observed the modulation of some phyla rarely described before in response to OA. For instance, Campylobacterota are chemoautotrophic bacteria that fix carbon through the reductive tricarboxylic acid cycle (Assié *et al.* 2020). These microorganisms are abundant in deep-sea hydrothermal vents and cold seeps also in association with some invertebrates (Assié *et al.* 2020; Lin *et al.* 2023) but have never been described in CO_2 vents. The observed strong increase of this phylum in *P. massiliensis* complex in S2 suggests that this species can take advantage of the CO_2 fixation by these bacteria similarly to other organisms inhabiting extreme environments.

A remarkable increase of WPS-2 was observed in *S. prolifera* from the acidified sites with respect to the ambient site. The WPS-2 phylum has unique metabolic potential, which allows it to survive in harsh environments, including acidic and polluted ones (Ji *et al.* 2021). To date, however, there are no indications regarding the modulation of these bacteria under OA conditions.

Therefore, our results suggest a potential involvement of these bacterial phyla in promoting the tolerance of polychaetes to OA. Further studies are warranted to confirm the modulation of these phyla also in other marine invertebrates subjected to OA, as well as to clarify the functional implications for the organisms related to the increase of these bacteria.

As already specified, during the preparation of the samples, we did not differentiate between gut-residing microbiome and epibiotic bacteria. Epibiotic bacteria may represent up to 50% of the animal weight in some animals (like sponges) (Hentschel *et al.* 2006). This community is formed by bacteria coming from the surrounding water and colonizing the animal. Epibiotic microbes can benefit the host by providing defense against predators, antibiotics, and anti-pathogenic compounds (Thomas *et al.* 2010; Sayem *et al.* 2011; Padmavathi & Pandian 2014). Two bacteria found in the epibiotic community of *P. cf dumerilii* and *Syllis* sp. collected from the Southeast coast of India, *Exiguobacterium* sp. and *Actinobacterium* sp., have been demonstrated to have an anti-microbial activity and to inhibit adhesion of pathogenic bacteria to surfaces and biofilm formation (Shankar *et al.* 2015). Interestingly, an ASV classified as a species of *Exiguobacterium* is present at a higher level in *S. prolifera* under acidified pH conditions, suggesting a possible link between changes in the microbial community and important biological traits of the animals that might favor their survival under acidified conditions.

CONCLUSIONS

The results presented in this study underline for the first time a variation in the composition of microbial communities of polychaetes living under natural acidified conditions. The data suggest that the variation of the polychaete microbiome community is modulated by species-specific features. In this regard, the relationship between microbial variation and the higher tolerance for low pH environmental conditions of some polychaete species must be further investigated.

Our results highlighted that microbial changes were noticeable when comparing individuals collected from a site characterized by a normal pH range and individuals collected from acidified sites, but not comparing individuals collected from acidified sites with different levels of acidification. This may indicate that the microbial community associated with these animals is extremely sensitive to even small pH changes (such as in S2) with respect to control conditions, being relatively constant when facing stronger acidified conditions (as in S3). Some of the changes we reported are associated with bacteria that might be involved in biofilm formation in the sea environment, which might be linked to the ability of the animals to settle and breed in acidified zones. This demands attention to the possible consequences of the worrying projections about ocean acidification in the next few decades. Providing a first insight into the microbiome of *S. prolifera* and *P. massiliensis* complex, and of its OA-driven modification, the present study highlights the importance of studying microbial communities to understand how the change of microbiome impacts the adaptation of these animals to ocean acidification. In the future, massive efforts must be carried out to investigate the interaction between polychaetes and the bacteria associated with their microbiome to fully understand what would be the impact of OA-induced modification on this highly relevant group of benthonic organisms.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403–10.

Arcadi E, Rizzo C, Calogero R *et al.* (2023). Microbial communities inhabiting shallow hydrothermal vents as sentinels of acidification processes. *Frontiers in Microbiology* **14**, 1233893.

Arnoldi I, Negri A, Soresinetti L *et al.* (2022). Assessing the distribution of invasive Asian mosquitoes in Northern Italy and modelling the potential spread of *Aedes koreicus* in Europe. *Acta Tropica* **232**, 106536.

Assié A, Leisch N, Meier DV *et al.* (2020). Horizontal acquisition of a patchwork Calvin cycle by symbiotic and free-living Campylobacterota (formerly Epsilonproteobacteria). *The ISME Journal* **14**, 104–22.

Barreto MM, Ziegler M, Venn A *et al.* (2021). Effects of ocean acidification on resident and active microbial communities of *Stylophora pistillata*. *Frontiers in Microbiology* **12**, 707674.

Baselga A (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography* **19**, 134–43.

Biagi E, Caroselli E, Barone M *et al.* (2020). Patterns in microbiome composition differ with ocean acidification in anatomic compartments of the Mediterranean coral *Astroides calycularis* living at CO_2 vents. *Science of The Total Environment* **724**, 138048.

Botté ES, Nielsen S, Abdul Wahab MA *et al.* (2019). Changes in the metabolic potential of the sponge microbiome under ocean acidification. *Nature Communications* **10**, 4134.

Bressan M, Chinellato A, Munari M *et al.* (2014). Does seawater acidification affect survival, growth and shell integrity in bivalve juveniles? *Marine Environmental Research* **99**, 136–48.

Caldeira K, Wickett ME (2003). Anthropogenic carbon and ocean pH. *Nature* **425**, 6956.

Caldeira K, Wickett ME (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research* **110**, C09S04.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**, 581–83.

Calosi P, Rastrick S, Lombardi C, De Guzman HJ, Davidson L, Jahnke M (2013). Adaptation and acclimatization to ocean acidification in marine ectotherms: An *in situ* transplant experiment with polychaetes at a shallow CO_2 vent system. *Philosophical Transactions of the Royal Society B* **368**, 20120444.

Del Olmo I, Roma-Cavagliani J, Martín-Hervás MDR *et al.* (2024). Integrative taxonomy in *Syllis prolifera* (Annelida, Syllidae): From a unique cosmopolitan species to a complex of pseudocryptic species. *Invertebrate Systematics* **38**, IS24004.

Del Pasqua M, Gambi MC, Caricato R, Lionetto MG, Giangrande A (2019). Effects of short-term and long-term exposure to ocean acidification on carbonic anhydrase activity and morphometric characteristics in the invasive polychaete *Branchiomma boholense* (Annelida: Sabellidae): A case-study from a CO_2 vent system. *Marine Environmental Research* **144**, 203–12.

Dixon P (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* **14**, 927–30.

Ferrari C (2010). *Biodiversità: Dal Genoma al Paesaggio*. Zanichelli, Modena, Italy.

Figuerola B, Hancock AM, Bax N *et al.* (2021). A review and meta-analysis of potential impacts of ocean acidification on marine calcifiers from the Southern Ocean. *Frontiers in Marine Science* **8**, 584445.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–99.

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Foo SA, Byrne M, Ricevuto E, Gambi MC (2018). The carbon dioxide vents of Ischia, Italy, a natural system to assess impacts of ocean acidification on marine ecosystems: An overview of research and comparisons with other vent systems. *Oceanographic and Marine Biology* **56**, 237–310.

Gambi M, Musco L, Giangrande A, Badalamenti F, Micheli F, Kroeker K (2016). Distribution and functional traits of polychaetes in a CO_2 vent system: Winners and losers among closely related species. *Marine Ecology Progress Series* **550**, 121–34.

Gambi MC, Manzo M, Giangrande A, Massa-Gallucci A (2017). Pilot study on population features of *Syllis prolifera* (Annelida, Syllidae) in relation to ocean acidification. *Biologia Marina Mediterranea* **24**, 114–15.

Giangrande A, Licciano M, Musco L (2005). Polychaetes as environmental indicators revisited. *Marine Pollution Bulletin* **50**, 1153–62.

González-Delgado S, Hernández JC (2018). The importance of natural acidified systems in the study of ocean acidification: What have we learned? *Advances in Marine Biology* **80**, 57–99.

Hentschel U, Usher KM, Taylor MW (2006). Marine sponges as microbial fermenters. *FEMS Microbiology Ecology* **55**, 167–77.

Hochstein R, Zhang Q, Sadowsky MJ *et al.* (2019). The deposit feeder *Capitella teleta* has a unique and relatively complex microbiome likely supporting its ability to degrade pollutants. *Science of the Total Environment* **670**, 547–54.

IPCC (2019). IPCC Special Report on the Ocean and Cryosphere in a Changing Climate (Pörtner HO, Roberts DC, Masson-Delmotte V *et al*., eds.). Cambridge University Press, Cambridge, UK and New York, NY, USA, 755 p.

Ji M, Williams TJ, Montgomery K *et al.* (2021). Candidatus Eremiobacterota, a metabolically and phylogenetically diverse terrestrial phylum with acid-tolerant adaptations. *The ISME Journal* **15**, 2692–707.

Jumars PA, Dorgan KM, Lindsay SM (2015). Diet of worms emended: An update of polychaete feeding guilds. *Annual Review of Marine Science* **7**, 497–520.

Kandler NM, Abdul Wahab MA, Noonan SHC *et al.* (2018). *In situ* responses of the sponge microbiome to ocean acidification. *FEMS Microbiology Ecology* **94**, https://doi.org/10.1093/femsec/fiy205

Kong N, Han S, Fu Q, Yu Z, Wang L, Song L (2022). Impact of ocean acidification on the intestinal microflora of the Pacific oyster *Crassostrea gigas*. *Aquaculture* **546**, 737365.

Kroeker KJ, Kordas RL, Crim R *et al.* (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology* **19**, 1884–96.

Kroeker KJ, Micheli F, Gambi MC, Martz TR (2011). Divergent ecosystem responses within a benthic marine community to ocean acidification. *PNAS* **108**, 14515–20.

Larsson A (2014). AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* **30**, 3276–78.

Lebrato M, Andersson AJ, Ries JB *et al.* (2016). Benthic marine calcifiers coexist with $CaCO_3$-undersaturated seawater worldwide. *Global Biogeochemical Cycles* **30**, 1038–53.

Lin YT, Xu T, Ip JC *et al.* (2023). Interactions among deep-sea mussels and their epibiotic and endosymbiotic chemoautotrophic bacteria: Insights from multi-omics analysis. *Zoological Research* **44**, 106–25.

Love MI, Huber W, Anders S (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**, 550.

Lucey NM, Lombardi C, DeMarchi L, Schulze A, Gambi MC, Calosi P (2015). To brood or not to brood: Are marine invertebrates that protect their offspring more resilient to ocean acidification? *Scientific Reports* **5**, 12009.

McFall-Ngai M, Hadfield MG, Bosch TCG *et al.* (2013). Animals in a bacterial world, a new imperative for the life sciences. *PNAS* **110**, 3229–36.

McMurdie PJ, Holmes S (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**, e61217.

Meron D, Atias E, Iasur Kruh L *et al*. (2011). The impact of reduced pH on the microbial community of the coral *Acropora eurystoma*. *The ISME Journal* **5**, 51–60.

Meron D, Rodolfo-Metalpa R, Cunning R, Baker AC, Fine M, Banin E (2012). Changes in coral microbial communities in response to a natural pH gradient. *The ISME Journal* **6**, 1775–85.

Mirasole A, Badalamenti F, Di Franco A, Gambi MC, Teixidó N (2021). Boosted fish abundance associated with *Posidonia oceanica* meadows in temperate shallow CO_2 vents. *Science of the Total Environment* **771**, 145438.

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Morgan M, Anders S, Lawrence M, Aboyoun P, Pagès H, Gentleman R (2009). ShortRead: A bioconductor package for input, quality assessment and exploration of high-throughput sequence data. *Bioinformatics* **25**, 2607–8.

Morrow KM, Bourne DG, Humphrey C *et al.* (2015). Natural volcanic CO_2 seeps reveal future trajectories for host-microbial associations in corals and sponges. *The ISME Journal* **9**, 894–908.

Munari M, Chiarore A, Signorini SG *et al*. (2022). Surviving in a changing ocean. Tolerance to acidification might affect the susceptibility of polychaetes to chemical contamination. *Marine Pollution Bulletin* **181**, 113857.

Orr JC, Fabry VJ, Aumont O *et al.* (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 7059.

Padmavathi AR, Pandian SK (2014). Antibiofilm activity of biosurfactant producing coral associated bacteria isolated from Gulf of Mannar. *Indian Journal of Microbiology* **54**, 376–82.

Palladino G, Caroselli E, Tavella T *et al.* (2022). Metagenomic shifts in mucus, tissue and skeleton of the coral *Balanophyllia europaea* living along a natural CO_2 gradient. *ISME Communications* **2**, 65.

Palombo C, Chiarore A, Ciscato M *et al.* (2023). Thanks mum. Maternal effects in response to ocean acidification of sea urchin larvae at different ecologically relevant temperatures. *Marine Pollution Bulletin* **188**, 114700.

Priscilla L, Rajeev M, Pandian SK *et al.* (2022). Gut associated culturable bacterial community in intertidal polychaete worms (Annelida: Polychaeta), their characterization and implications in captive shrimp aquaculture. *Regional Studies in Marine Science* **52**, 102274.

Quast C, Pruesse E, Yilmaz P *et al.* (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**, D590–96.

Range P, Teodósio Chícharo M, Ben-Hamadou R *et al.* (2014). Impacts of CO_2-induced seawater acidification on coastal Mediterranean bivalves and interactions with other climatic stressors. *Regional Environmental Change* **14**, 19–30.

Raulf FF, Fabricius K, Uthicke S *et al.* (2015). Changes in microbial communities in coastal sediments along natural CO_2 gradients at a volcanic vent in Papua New Guinea. *Environmental Microbiology* **17**, 3678–91.

Sayem SMA, Manzo E, Ciavatta L *et al.* (2011). Anti-biofilm activity of an exopolysaccharide from a sponge-associated strain of *Bacillus licheniformis*. *Microbial Cell Factories* **10**, 74.

Scanes E, Parker LM, Seymour JR *et al.* (2021). Microbiome response differs among selected lines of Sydney rock oysters to ocean warming and acidification. *Fems Microbiology Ecology* **97**, fiab099.

Shankar C, Sathianeson S, Viju N, Punitha S (2015). Antibacterial and biofilm inhibitory activities of bacteria associated with polychaetes. *Journal of Coastal Life Medicine* **3**, 495–502.

Signorini S, Munari M, Cannavacciuolo A *et al.* (2023). Investigation of the molecular mechanisms which contribute to the survival of the Polychaete *Platynereis* spp. under ocean acidification conditions in the CO_2 vent system of Ischia Island (Italy). *Frontiers in Marine Science* **9**, 1067900.

Simonetti S, Zupo V, Gambi MC, Luckenbach T, Corsi I (2022). Unravelling cellular and molecular mechanisms of acid stress tolerance and resistance in marine species: New frontiers in the study of adaptation to ocean acidification. *Marine Pollution Bulletin* **185**, 114365.

Sunday JM, Fabricius KE, Kroeker KJ *et al.* (2017). Ocean acidification can mediate biodiversity shifts by changing biogenic habitat. *Nature Climate Change* **7**, 81–85.

Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M (2014). Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS ONE* **9**, e105592.

Tangherlini M, Corinaldesi C, Ape F *et al.* (2021). Ocean acidification induces changes in virus-host relationships in Mediterranean Benthic Ecosystems. *Microorganisms* **9**, 769.

Teixidó N, Carlot J, Alliouane S *et al.* (2024). Functional changes across marine habitats due to ocean acidification. *Global Change Biology* **30**, e17105.

Teixidó N, Gambi MC, Parravacini V *et al.* (2018). Functional biodiversity loss along natural CO_2 gradients. *Nature Communications* **9**, 5149.

Thomas TRA, Kavlekar DP, LokaBharathi PA (2010). Marine drugs from sponge-microbe association—A review. *Marine Drugs* **8**, 1417–68.

Thukral A (2017). A review on measurement of Alpha diversity in biology. *Agricultural Research Journal* **54**, 1–10.

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Valvassori G, Benedetti M, Regoli F, Gambi MC (2019). Antioxidant efficiency of *Platynereis* spp. (Annelida, Nereididae) under different pH conditions at a CO_2 vent's system. *Journal of Marine Biology* **2019**, 8415916.

Vijayan N, Lema KA, Nedved BT *et al.* (2019). Microbiomes of the polychaete *Hydroides elegans* (Polychaeta: Serpulidae) across its life-history stages. *Marine Biology* **166**, 19.

Villanueva RA, Chen Z (2019). ggplot2: Elegant graphics for data analysis (2nd ed.). *Measurement: Interdisciplinary Research and Perspectives* **17**, 160–67.

Webster NS, Negri AP, Botté ES (2016). Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. *Scientific Reports* **6**, 19324.

Webster NS, Negri AP, Flores F *et al.* (2013). Near-future ocean acidification causes differences in microbial associations within diverse coral reef taxa. *Environmental Microbiology Reports* **5**, 243–51.

Wäge J, Valvassori G, Hardege JD, Schulze A, Gambi MC (2017). The sibling polychaetes *Platynereis dumerilii* and *Platynereis massiliensis* in the Mediterranean Sea: Are phylogeographic patterns related to exposure to ocean acidification? *Marine Biology* **164**, 199.

SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Information S1 ShortRead Quality Assessment.

Supplementary Information S2 Tax tables of the analyses of *Syllis* and *Platynereis* datasets.

Supplementary Information S3 Amplicon Sequence Variants recovered for each sample within *Syllis* and *Platynereis* datasets.

Supplementary Information S4 Amplicon Sequence Variants shared between samples collected in acid or normal pH conditions within *Syllis* and *Platynereis* datasets.

Supplementary Information S5 Results of the Deseq analysis.

Figure S1 Prevalence of ASVs in *Platynereis massiliensis* complex dataset before (A) and after (B) filtering and rarefaction. The prevalence of each ASV on the total number of samples within the dataset (Frac.Samples) is plotted against the number of reads associated with the ASV itself (total abundance). The dashed line indicates the prevalence threshold established for filtering. ASVs are grouped by phylum.

Figure S2 Prevalence of ASVs in *Syllis prolifera* dataset before (A) and after (B) filtering and rarefaction. The prevalence of each ASV on the total number of samples within the dataset (Frac.Samples) is plotted against the number of reads associated with the ASV itself (total abundance). The dashed line indicates the prevalence threshold established for filtering. ASVs are grouped by phylum.

Figure S3 Venn diagram representation of the amount of ASVs shared between samples collected in different pH zones. ASVs were grouped by pH zone for *Platynereis massiliensis* complex (A) and *Syllis prolifera* (B). Orange oval includes ASVs restricted to the acid pH zone (S2 for *Platynereis massiliensis* complex and S2/S3 for *S. prolifera*), while blue oval those restricted to control pH zone. Purple oval contains AVSs shared between different pH zones.

Figure S4 Alpha diversity indexes. Multiple indexes of alpha diversity were evaluated (Observed richness, Chao1, ACE richness, Shannon diversity, Simpson diversity, Inverted Simpson and Fisher diversity). The analysis was done on *Platynereis massiliensis* complex grouping samples by site (1° column), on *Syllis prolifera* dataset grouping samples by site (2° column) or pH zone (3° column).

Table S1 Report of the statystical analysis on alpha diversity indexes. For *Platynereis massiliensis* complex and *Syllis prolifera* (S1 vs S2/S3) datasets Unpaired Student *t*-test was applied, unless otherwise specified (*t* and *P* values and degree of freedom (df) are reported). For *Syllis prolifera* (S1 vs S2 vs S3) dataset, One-way ANOVA was applied, unless otherwise specified (*F* and *P* values and df are reported)

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