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ABSTRACT

Microorganisms form the basis of ocean ecosystems yet the effects of perturbations such as decreasing pH on microbial community structure, interactions and functionality remain compared to multicellular organisms. Using an experimental manipulation of Southern Ocean seawater, we subjected bacterioplankton and mycoplankton to artificial pH decreases, which are predicted to occur in the future. We show that acidification led to substantial increases of bacterioplankton diversity, while in contrast it had no effect on mycoplankton diversity. Our analyses revealed a loss of putative keystone taxa and a decrease in predicted community interactions as a response to lower pH levels. Bacterioplankton shifted from generalist to specialist community members, suggesting a specific stress response to unfavourable conditions. In addition, enzyme activities involved in nitrogen acquisition were lower at reduced pH levels, suggesting altered organic matter cycling in a more acidic ocean. Our findings suggest that bacterioplankton and mycoplankton may respond differentially to future ocean acidification, with potentially negative impacts on community structure and biogeochemical cycling in the Southern Ocean.

IMPORTANCE

Oceans absorb the majority of anthropogenically produced CO_2, the consequence of which is ocean acidification, a phenomenon already negatively impacting key marine organisms. Marine microbial communities form the basis of ocean food webs by generating nutrients for higher trophic levels, yet the response of these key microbial drivers to acidification remains unclear. This knowledge deficit is particularly true for understudied marine ecosystems such as the Southern Ocean. Using a mesocosm approach, we found that acidification severely impacts microbial community stability, by altering bacterioplankton community structure, reducing network complexity, and augmenting enzyme activities

associated with nitrogen acquisition. This study adds to our understanding of the effects of ocean acidification on microbial communities, particularly within an environment expected to be largely effected by future anthropogenically driven climate change.

INTRODUCTION

The ocean absorbs roughly 30% of anthropogenic carbon dioxide emissions, which leads to changes in oceanic carbonate chemistry, resulting in both carbonation and acidification (Orr et al., 2005; Zeebe et al., 2008; Doney et al., 2009; Adler et al., 2011; Kottmeier et al., 2016). Globally, ocean pH is predicted to decrease by as much as 0.4 and 0.8 units within the next 100 and 300 years, respectively (Caldeira and Wickett, 2003; Orr et al., 2005), a change which is nearly eight times higher than what has been observed within the last 25 million years (Pearson and Palmer, 2000). Ocean acidification (OA) can negatively impact marine macro-organisms, such as calcifiers, adversely affecting their growth and physiology (Doney et al., 2009; Gazeau et al., 2013; Brandenburg et al., 2019). However, the extent to which decreasing pH affects diverse bacterioplankton and mycoplankton communities remains largely unclear.

A null hypothesis proposed by Joint and colleagues (2011b) states that due to the ubiquitous abundance, fast generation time and genetic plasticity of microorganisms, microorganisms will rapidly adapt to future pH perturbations. However, despite the large genetic flexibility microorganisms possess, a physiological tipping point, where individual organisms are no longer able to adapt to perturbations, may be reached (Allison and Martiny, 2008b; Hutchins and Fu, 2017). There is considerable uncertainty and disagreement regarding the impact of acidification on bacterioplankton and mycoplankton communities, with reports demonstrating both resistance and susceptibility. Evidence of microbial

72 community resistance to acidification include: unchanged bacterial cell abundance (Grossart
73 et al., 2006; Allgaier et al., 2008; Krause et al., 2012; Newbold et al., 2012), consistent
74 composition (Lindh et al., 2013; Roy et al., 2013; Oliver et al., 2014), unaffected or increased
75 heterotrophic production (Grossart et al., 2006; Arnosti et al., 2011) and increased nitrogen
76 fixation (Joint et al., 2011a; Rees et al., 2016). Susceptibility has been seen through,
77 alterations in bacterial and fungal cell numbers (Krause et al., 2013; Endres et al., 2014;
78 Engel et al., 2014), compositional shifts (Allgaier et al., 2008; Krause et al., 2012; Zhang et
79 al., 2013) with changes towards pathogenic members (Vega-Thurber et al., 2009; Reich et al.,
80 2017) and decreased nitrification rates (Beman et al., 2011). Moreover, the increased
81 expression of proton pump genes identified in a metatranscriptomic study (Bunse et al., 2016)
82 suggests that acidification may be energetically costly for microbial cells, with possible
83 negative implications for microbial functionality and biogeochemical cycling. These results
84 emphasise the need for further investigations into the effects of acidification on complex and
85 diverse bacterioplankton and mycoplankton assemblages (Hutchins and Fu, 2017).

86 Although the Southern Ocean (SO) covers less than 20% of the global ocean it is a
87 highly productive ecosystem, facilitating the inter-ocean exchange of nutrients which drives
88 over 70% of ocean productivity (Mayewski et al., 2009). While the SO has naturally low pH,
89 it remains vulnerable to anthropogenic induced acidification due to its' low temperature, the
90 upwelling of CO_2 enriched deep waters, and the fact that approximately 40% of the global
91 oceanic uptake of CO_2 occurs in the SO (Riebesell and Gattuso, 2015). Several studies have
92 shown that acidification results in increased resilience in diatoms (Valenzuela et al., 2018)
93 and functional biodiversity loss (Teixidó et al., 2018). In contrast to other environments
94 (Newbold et al., 2012; Chen et al., 2013; Huggett et al., 2018; James et al., 2019), the precise
95 responses of bacterioplankton and mycoplankton remain largely speculative and often

96 unexamined in pristine marine environments. This knowledge is crucial for understanding the
97 effects of climate change in regions most sensitive to future environmental change.

98 To address this deficit, we assessed the effect of future predicted pH levels (based on
99 IPCC projections for the years 2100 and 2300, respectively) on both SO bacterioplankton and
100 mycoplankton diversity, composition, predicted interactions, and functionality using
101 mesocosm experiments. We predicted that; (1) ocean acidification will decrease both
102 bacterioplankton and mycoplankton diversity and will significantly alter community
103 structure; (2) microbial functionality will be both positively and negatively affected by ocean
104 acidification in terms of carbon and nitrogen/phosphorus decomposition, respectively, and (3)
105 the total number of covariations and subsequently network stability will decrease with lower
106 pH levels.

107 **RESULTS**

108 **Microbial diversity and community structure in control and acidified mesocosms**

109 Alpha diversity for bacteria and archaea was significantly higher in the low and
110 medium pH groups in comparison to the control (ANOVA, p-value < 0.001), and both
111 Shannon diversity (Fig.1a) and OTU richness (Fig.1b) significantly correlated with pH. On
112 the contrary, fungal alpha diversity was not significantly different between the three
113 treatment groups (ANOVA, p-value > 0.05), and did not correlate significantly with pH (Fig.
114 1ab). Bacterial and archaeal community structure was significantly affected by pH treatment
115 (PERMANOVA, p-value < 0.05, betadisper p-value > 0.05), and clustered according to
116 treatment type (Fig. 2a). In contrast to bacteria and archaea, fungal community structure was
117 not significantly influenced by pH treatment (PERMANOVA, p-value < 0.05, betadisper p-
118 value < 0.05), as no distinct clusters were seen according to treatment group (Fig. 2b). Thus,
119 bacterioplankton and mycoplankton community composition clearly display differential

120 responses to acidification. Whereas several bacterioplankton genera either increased or
121 decreased in abundance under acidified conditions, mycoplankton composition remained
122 stable, with the exception of Agariomyctes. Specific genera and species differences are
123 further described in Supporting Information (Fig. S3).

124 **Functional community responses to ocean acidification**

125 Extracellular enzymatic activities were detected in all samples, where the activities of
126 carbon and phosphorus acquiring enzymes did not vary significantly with pH treatment (Fig.
127 3). In contrast, nitrogen acquiring enzymes NAG and LAP significantly decreased at lower
128 pH. Enzymes involved in carbon acquisition, namely BG and BX, showed no significant
129 increase in response to pH treatment (ANOVA, p-value > 0.05). AP, involved in phosphorus
130 acquisition, showed no significant increase or decrease in activity in response to the pH
131 treatment (ANOVA, p-value > 0.05). NAG activity decreased in the low group across
132 sampling days, although this was not significant from the control group (ANOVA, p-value >
133 0.05). Notably, LAP was shown to be significantly lower in the medium and low pH
134 treatment groups relative to the control at day 21 of the mesocosm experiment (ANOVA, p-
135 value < 0.05). Activities were correlated to pH levels using both linear regression (Fig. 3) and
136 Spearman's rho. Both results indicated that only NAG was significantly positively associated
137 with pH (r = 0.42, p-value < 0.05; Fig. 3a), whereby NAG activity decreased at lower pH
138 levels. LAP activity, although significantly different between treatment and control groups,
139 could not be predicted by pH, likely due to the lower activity in the medium pH group than
140 the low pH group.

141 **Community structures and system level changes associated to ocean acidification**

142 Table S2 shows topological features of bacterial and archaeal community networks.
143 All networks exhibited scale-free characteristics, typical of biological association networks,

were small world, modular, and significantly different from generated random networks (see Supporting information for details).

Network structure, complexity and community composition differed markedly between the three treatment groups (Table S2, Fig. 4). The medium network was the largest followed by the low and control networks, showing that acidification resulted in larger networks. Interestingly, although the medium network displayed the highest diversity and connectivity, the low network was the least complex (Table S2). The medium network contained the greatest number of edges, the largest average degree (i.e. average number of edges per node in the network) and the shortest harmonic geodesic distance (i.e. distance between all nodes in the network).

The number of modules in the medium and low networks were greater than the control network (Table S2). Modules were largest and most connected in the medium network, followed by the control and the low network, reflecting patterns observed from overall network structure. Intriguingly, the percentage of positive associations increased in response to acidification, where the low network had 77.82% positive associations, the medium network contained 77.25% and the control network only 74.70%, highlighting that reduced pH levels resulted in a slight increase in positive associations as opposed to co-exclusions (Fig. 4, Table S2). The overall taxonomic composition of the largest modules at both phylum and class level did not differ markedly between the three treatment groups, although some modules exhibited a dominance of different taxonomic groups (Fig. 4). Alphaproteobacteria, Bacteroidetes and Gammaproteobacteria were the dominant taxa in almost all modules and were also the dominant taxa in the overall bacterioplankton community. Actinobacteria occurred more frequently in the medium and low networks, and Euryarchaeota was found exclusively in the control network (Fig. S2 and Table S1). Unassigned sequences constituted a relatively high proportion in several modules, and were

highest in the low network, suggesting that cryptic unknown species may increase in abundance in response to acidification in the SO. Furthermore, no modules consisted of a single class or phylum, showing a low degree of phylogenetic assortativity within the association networks, which suggest a potential high capacity for biotic interactions in SO microbial communities.

Network topological metrics can be computed for each node/OTU by assessing the within-module connectivity (*Zi*) as well as the between-module connectivity (*Pi*), which can indicate the potential ecological role of each OTU. These parameters can then be used to classify each OTU into four network-based categories; peripherals, connectors, module hubs and network hubs (Fig. 5a). Ecologically, peripherals may be considered as specialists, while connectors and module hubs may represent generalists and network hubs could be classified as super generalists. Additionally, the last three can be regarded as potential keystone taxa, on the sole basis of their high connectivity, since the overall network structure would be significantly altered if they were removed.

The majority of OTUs in all three networks were classified as peripherals, with most edges occurring within their own modules (Fig. 5a). Several peripheral OTUs had a *Pi* value of 0, indicating that these nodes were connected solely to other OTUs within the same module (74% of OTUs in the control network, 70% in the medium network and 84% in the low). No network hubs (super generalists) were detected in any network. Interestingly, the relative abundance of several peripheral taxa changed in response to acidification (Fig. 5b, see Supplemental Information for details).

The number and taxonomic affiliation of module hubs and connectors detected (Table S3) differed noticeably between the three networks. Five module hubs were detected in the control network, five in the medium network and only three in the low network (Fig. 4, 5a).

All control hubs consisted of phylum Bacteroidetes OTUs and unassigned sequences, with the orders *Flavobacteriales* and *Saprospirales*. Medium hubs belonged to classes Flavobacteriia, Gammaproteobacteria and Alphaproteobacteria. Low hubs consisted of Alphaproteobacteria, Flavobacteriia and unassigned bacteria. The relative abundance of module hubs ranged from 0.01% to 0.74%. The control network contained five connectors, the medium network contained three and the low network had only one. Control and medium connectors consisted of Alphaproteobacteria (order *Rhodobacterales* and *Rickettsiales*, respectively) and Flavobacteriia (order *Flavobacteriales*), whereas the low connector belonged to Bacteroidetes (order *Cytophagales*). Connector relative abundance ranged from 0.005% to 3.24%, with all but one OTU with an abundance of less than 1%, underlying the disconnection between species centrality and relative abundance. As hubs and connectors can be considered potential keystone taxa, Alphaproteobacteria and Flavobacteriia represented prominent putative keystone taxa within all networks, although differences between networks can be detected at finer taxonomic resolution (Table S3).

DISCUSSION

Diversity does not equal stability

Microbial community stability amidst perturbations is thought to be promoted by increased diversity, due to the link between diversity and ecosystem health and multifunctionality (Girvan et al., 2005; Delgado-Baquerizo et al., 2016; Shade, 2017). Similarly, loss of community diversity following environmental disturbance is often linked to community susceptibility. However, it has recently been suggested that assumptions of increased diversity leading to increased stability are false, at least for some ecosystems, as the role of diversity is dependent on the environment or ecosystem and disturbance itself (Shade, 2017). Community members are not necessarily interacting mutualistically in diverse

compositions, and taxonomic diversity does not absolutely equate to functional diversity (Louca et al., 2016). In this study, we found that bacterioplankton alpha diversity is significantly higher in acidified compared to ambient mesocosms, in contrast to what has been hypothesised. Simultaneously, bacterioplankton community structure was significantly altered in response to CO_2, with the relative abundances of several key community members significantly changing. We suggest that increased bacterioplankton diversity is an indication of community susceptibility, or "scramble" rather than stability in the Southern Ocean. The high diversity identified for bacterioplankton communities highlight that polar marine regions, and the SO specifically, contain high levels of unexplored diversity (Dickinson et al., 2016).

Mycoplankton resistance to acidification

In contrast to our initial hypothesis, we did not observe a significant effect of ocean acidification on mycoplankton diversity. Instead, fungal community diversity and structure was stable, with the exception of Agaricomycetes, which increased in relative abundances at lower pH levels (Table S3). Agaricomycetes is a class of Basidiomycota that contains several yeast species (Jones, 2011; Jones et al., 2015), suggesting an increase in abundance of adapted fungal morphologies in response to acidification. However, the increase of Agaricomycetes phylotypes did not significantly affect overall fungal diversity or community structure. We argue that mycoplankton may contain a higher genetic plasticity and degree of physiological adaptation than bacterioplankton and are therefore resistant to future acidification. Culture dependant studies have shown that both terrestrial and marine fungi can rapidly adapt to a variety of environmental conditions, such as hot and cold temperatures and anoxia (Richards et al., 2012; Tsuji et al., 2013; Rédou et al., 2015). Additionally, fungi have larger pH tolerance ranges and optima for growth than bacteria (Rousk et al., 2010). Moreover, fungi possess chitin-rich cell walls (Taylor and Cunliffe, 2016), which may

provide enhanced resistance. Source habitat significantly influences mycoplankton community structure (Panzer et al., 2015), further suggesting that SO fungal communities may be adapted to the pelagic environment and therefore less effected by pH variations. The lack of a significant mycoplankton community response in SO waters to pH levels predicted in the next 300 years, suggests the resistance of marine fungi to acidification in these systems.

Altered organic matter cycling in a future acidic ocean

Microbially produced extracellular enzymes break down complex organic substrates, mediating heterotrophic carbon and nutrient recycling in marine environments (Arnosti, 2014). Enzymes measured for nutrient acquisition showed activity in all samples, indicating active bacterioplankton and/or mycoplankton heterotrophic communities within surface SO waters, since members of both assemblages are capable of producing each enzyme (Jones, 2011; Arnosti, 2014; Hoarfrost and Arnosti, 2017). The observed results of exoenzyme activities were congruent with our original hypothesis. Carbon and phosphorus degrading enzymes showed no substantial shifts in activity in response to pH treatment, suggesting constant, and not elevated carbon and phosphorus utilisation. Nitrogen acquiring enzymes, LAP and NAG, however, were negatively associated with pH, implying altered heterotrophic nitrogen recycling in a future more acidic ocean.

LAP and NAG activity responses contrasted to previous studies, which have found LAP activity to increase (Piontek et al., 2010; Endres et al., 2014) or remain unchanged (Yamada et al., 2008; Burrell et al., 2016). The decreased LAP and NAG activities suggest that nitrogen acquisition by bacterioplankton may be negatively impacted by pH, possibly due to increased physiological stress placed on microbial cells in a more acidic ocean. Bunse *et al*. (2016) found that bacterioplankton respond to a pH decrease of 0.2 units, applied over a

period of only nine days, by increasing the expression of pH homeostasis genes. The exposure to lower extracellular pH likely increased physiological stress, resulting in intensified energy expenditure on cell maintenance, at the expense of bacterial growth (Bunse et al., 2016). Therefore, it is likely that the microbial communities producing LAP and NAG were negatively impacted by acidification. Reduced pH may have caused a loss of community members responsible for producing the nitrogen acquiring enzymes (Allison and Martiny, 2008a), ultimately decreasing cellular growth and fitness. Ultimately, microbial communities are essential produces of enzymes responsible for recycling organic matter, especially within nutrient deficient regions such as the SO. Although further research is necessary, alterations in bacterioplankton communities and their functions within the oligotrophic SO suggest negative future effects on ocean nutrient cycling.

Bacterioplankton community network shifts as a response to acidification

Network topology, size and structure shifted markedly in response to reduced pH, with prominent differences between the control, medium and low bacterioplankton networks, congruent with our third hypothesis. Altered network structure and the loss of potential keystone taxa at lower pH levels suggest that marine microbial communities will be impacted by future acidification. Modules within networks may represent specific ecological niches (Olesen et al., 2007; Dupont and Olesen, 2012), and high interconnectedness between modules may indicate communication or co-operation between niches. The control group, although containing a large number of modules, did not contain a high number of connections between modules, and several between module connections were negative associations (Fig. 4). The large number of modules observed in the control group suggests that SO bacterioplankton communities form distinct niches and that both intermodular and intramodular predicted interactions may represent a balance between competition and mutualism. For example, microorganisms compete for nutrients and exhibit different growth

291 constraints (Steele et al., 2011; Eiler et al., 2012; Brum et al., 2016) and also communicate
292 via mechanisms such as quorum sensing (Hmelo, 2017). This study supports the notion that
293 SO bacterioplankton communities are highly complex with a multitude of putative biotic
294 interactions.

295 The higher network connectivity and complexity in the medium pH group suggests
296 that bacterioplankton responded to a pH decrease of 0.4 units, associated with an increase in
297 network stability (Fig. 4). Indeed, pH has been shown to alter community network structure
298 (Barberan et al., 2012), resulting in larger and more complex phylogenetic and functional
299 networks in response to elevated soil CO_2 levels (Zhou et al., 2010; Zhou et al., 2011).
300 Furthermore, network stability is thought to be promoted by increased modularity, as
301 disturbances may spread more slowly through a modular than a non-modular network
302 (Olesen et al., 2007). Module 4 contained the highest number of intra-module connections,
303 where the majority of co-occurrences were positive (Fig. 4). The high intra-module
304 connectivity is likely indicative of cooperation or mutualism between phylogenetically distant
305 species within a specific niche. In contrast, several intermodular connections were negative,
306 suggesting that increased physiological stress may result in more competitive interactions
307 between niches at lower pH levels within the SO. This competition may be due to increased
308 physiological stress on weaker community members, leading to the development of
309 specialists with more efficient mechanisms for using the available nutrients. However, more
310 research is needed to determine the cause of competition observed. Ultimately, future pH
311 decreases of 0.4 units may promote increased bacterioplankton community interactions and
312 network stability.

313 In contrast, the low network was the least complex, with reduced intermodular
314 connectivity. This may suggest that microbial niches or guilds decreased the amount of
315 interactions compared to the control and medium networks. This decreased cooperation may

be due to the increased physiological stress placed on bacterioplankton communities at pH levels expected in the next 300 years. Acidification can promote direct physiological changes of microbial communities, whereby the increased energetic cost associated with cellular maintenance may lead to reduced microbial growth and fitness (Gilbert et al., 2008; Bunse et al., 2016). Decreased cellular fitness may result in altered functionality (Bunse et al., 2016) eventually perturbing community interactions. The increased stress induced by a pH decrease of 0.8 units likely resulted in the substantial alterations of the network structure, with potentially deleterious effects on bacterioplankton community structure in a future acidified ocean scenario.

Keystone bacterioplankton in the SO and responses to acidification

Several potential keystone taxa were identified in all three networks, defined by their integral topological role in maintaining network structure and stability (Deng et al., 2012). The number of connectors decreased in the medium network, suggesting a switch between single microorganisms responsible for connecting niches to several community members linking different modules. The number of module hubs and connectors decreased dramatically in the low network. Loss of keystone members in a network decreases network stability and can lead to a collapse of network structure due to their high level of connectivity (Dunne et al., 2002; Olesen et al., 2007). The observed decrease in keystone nodes in the low network therefore correlates to the less stable network structure. Moreover, the reduced frequency of module hubs and connectors yet again suggests a lower frequency of community interactions or relationships in response to extreme levels of acidification.

The constructed networks are based on co-abundances and do not necessarily represent true biological interactions (Shi et al., 2016; Röttjers and Faust, 2019). However, co-occurrence networks have been instrumental for investigating the structure of microbial

assemblages across biomes (de Menezes et al., 2015; Murdock and Juniper, 2019), in the detection of true viral host interactions (Chow et al., 2014; Soffer et al., 2015), the association of phylotype shifts to specific environmental parameters and functions (Valverde et al., 2015; Van Goethem et al., 2017), as well as in the detection of metabolic dependencies resulting in the culture of previously uncultivable microorganisms (Duran-Pinedo et al., 2011). Furthermore, networks provide valuable insight into complex community structures and stability, particularly in the face of perturbations, and give information on community organisation not portrayed by diversity metrics alone (Zhou et al., 2010). Thus, our results provide a framework for complex community predicted interactions in response to acidification (Fig. 6) where individual species interactions may be experimentally tested in future studies.

In summary, we have shown that ocean acidification significantly alters bacterioplankton, but not mycoplankton, communities. Exoenzyme activities involved in nitrogen acquisition were substantially reduced at lower pH levels, suggesting a diminished availability of nutrients in surface waters and amended nutrient recycling. Additionally, bacterioplankton network structure and keystone taxa were significantly impacted by increased CO_2. We propose that future acidification may have adverse consequences on microbial communities and biogeochemical cycling, particularly within regions most sensitive to future climatic changes.

MATERIALS AND METHODS

Sampling and mesocosm experiment

Seawater was collected during the Marion Island Relief Cruise aboard the RV SA Agulhas II from April 2016 to May 2016. Approximately 500 L of seawater was collected from surface waters (5 m) using the underway system at a single site (46°00.065’ S,

38°00.37' E). The water was distributed into nine mesocosms (50 L volume) with the sterile drums filled to near capacity to keep the headspace small (volume 5 - 10ml) in order to minimize the exchange of CO_2. The mesocosms consisted of three biological replicates for each pH treatment group, where treatments are defined as follows: a control (i.e. pH 8.0) which is the natural pH of the environment, a medium pH, which mimics predicted pH decreases within the next 100 years (0.4 units, i.e. pH 7.6), as well as a low pH treatment which exceeds the predicted decrease in pH within the next millennium (i.e. pH 7.2). All values were allowed to deviate by ± 0.05 pH units in order to account for natural variability within the SO environment (Kapsenberg et al., 2015). The mesocosms were exposed to light on a 12 hr day/night cycle, where light was allowed to penetrate the top of the mesocosms in order to mimic the natural light conditions at 5 m. Mixing of mesocosms was performed via consistent O_2 bubbling (HAILEA Air Compressor, Guangdong, China), as well as manual stirring throughout the experiment. Each treated mesocosm contained a pH probe (MODEL PH-301C, SAGA, Tainan City, Taiwan, pH accuracy ± 0.1% + 2 digits) which consistently recorded the seawater pH. Physico-chemical variables within each mesocosm such as temperature, salinity, conductivity and pH, were also manually recorded twice a day throughout the duration of the experiment using a HANNA probe (MODEL HI 98195, Padova, Italy, pH accuracy ± 0.02 units, temperature accuracy ± 0.15°C, conductivity and salinity accuracy ± 1% of reading). Changes in pH were induced via CO_2 bubbling and the experiment was run for a total of 21 days, during which pH changes were induced on the first day (day 0) of the experiment. Both O_2 and CO_2 bubbling were introduced into mesocosms via sterile plastic tubing, where the amount of gas was controlled using a regulator. Samples were collected every 7 days at noon, including at the start (day 0) of the experiment. Physico-chemical variables such as temperature, salinity, and nutrients were recorded throughout the experiment (see Fig S1).

Enzymatic activities

Seawater collected from each sampling point in the mesocosm experiment was assayed for the activity of 5 enzymes, including β-glucosidase (BG), β-xylosidase (BX), β-N-acetyl-glucosaminidase (NAG), alkaline phosphatase (AP), and leucine aminopeptidase (LAP). The potential activities of these enzymes were tested using fluorescent assays, and enzyme activities were performed and calculated as described previously (Sinsabaugh, 1994; Papanikolaou et al., 2010; Hoarfrost and Arnosti, 2017). Briefly, 200 µL of seawater was added to 96-well black flat-bottom microplates (Greiner Bio One, Frickenhausen, Germany), where four replicate wells were used per sample. A concentration of between 1 mM to 1.5 mM of each enzyme substrate was then added to the 96 well plates. Plates were incubated for 2 hours at 4°C in the dark. Fluorescence was measured from the black 96 well plates using a Spectramax® Paradigm Multi-Mode Microplate Reader (Molecular Devices, USA) with an emission wavelength of 450 nm and an excitation wavelength of 360 nm.

Molecular analysis

Seawater (2 L) collected from each sampling point in the mesocosm experiment was filtered through 0.2 µM pore cellulose acetate membrane filter (Sartorius Stedim Biotech, Göttingen, Germany). Nucleic acid was subsequently extracted from each of the filtered samples using the PowerWater DNA Isolation Kit (MoBio, Carlsbad, USA), according to the manufacturer's instructions. DNA was sent to MR DNA (www.mrdnalab.com, Shallowater, USA) for paired-end sequencing (2 x 300bp) of the 16S rRNA gene and the internal transcribed spacer (ITS) 1 rDNA variable region on the Illumina MiSeq platform (Illumina, San Diego, CA, USA), as detailed previously (Phoma et al., 2018; Vargas-Gastélum et al., 2019). The marine specific primer pair 515F-Y and 926R (Walters et al., 2016) was used to PCR amplify hypervariable regions V4 and V5 of the bacterial and archaeal 16S rRNA gene.

413 ITS region 1 was targeted and PCR amplified using the primer pair ITS1F (Gardes and
414 Bruns, 1993) and ITS2 (White et al., 1990). All samples were barcoded on the forward
415 primer to allow for multiplex sequencing.

416 Sequences were analysed using QIIME v 1.8.0 (Caporaso et al., 2010). Sequences
417 were demultiplexed according to barcode identity and were quality filtered according to
418 QIIME default parameters, with exceptions for the ITS dataset. For the ITS dataset, the p
419 score applied was altered to 0.45 to ensure that all reads >200bp were included in the dataset,
420 provided that they met the other default quality parameters (i.e. q 19, r 3, etc.). Chimeras
421 were detected both *de novo* and against the Greengenes reference library (DeSantis et al.,
422 2006) for 16S rRNA genes and the UNITE reference database for the ITS region (Kõljalg et
423 al., 2013) using usearch61 (Edgar et al., 2011). Operational taxonomic units (OTUs) were
424 clustered at a similarity threshold of 97% using the uclust method and the Greengenes and
425 UNITE reference database for 16S rRNA gene and ITS region datasets, respectively.

426 16S rRNA gene OTUs were assigned a taxonomy using the Greengenes (DeSantis et
427 al., 2006) reference database. Taxonomy was assigned to ITS region OTUs using a
428 combination of the UNITE database in QIIME and the Warcup Fungal ITS training set in the
429 RDP classifier (Wang et al., 2007; Kõljalg et al., 2013; Deshpande et al., 2016). For the RDP
430 classifier, taxonomic classifications were kept at the default confidence threshold of equal to
431 or greater than 80% for classification to the root only, after which the default confidence
432 threshold was decreased to 50% for all subsequent assignments. Following taxonomy
433 assignment, singletons were removed from the datasets and both datasets were rarefied to the
434 lowest number of sequences per sample (53,267 for 16S rRNA gene and 10,370 for ITS
435 region), using a single rarefaction. All sequences are available on the SRA database under the
436 following accession number: SRP126760

Network analysis

Ecological association networks were constructed using Random Matrix Theory (RMT) through the Molecular Ecological Network Analysis (MENA) pipeline (http://ieg2.ou.edu/MENA/) (Deng et al., 2012). Individual networks were constructed for the three treatment groups (i.e. one low, medium, control) where all days were combined, excluding day zero (i.e. nine biological replicates per network, see Supporting Information for details). All networks were visualised in Cytoscape v 3.4.0 (Shannon et al., 2003).

Statistical analysis

All statistical analyses were performed using R statistical software version 3.2.2. (R Core Team, 2013). Alpha diversity was calculated using the *vegan* package (Oksanen et al., 2011). Statistically significant differences in diversity measures between each treatment group were determined via the *aov* function (analysis of variance, ANOVA) in *vegan* and the post-hoc TukeyHSD test. Microbial community structure was visualised by non-metric multidimensional scaling (nMDS) ordination plots with the *metamds* function of *vegan* using Bray-Curtis dissimilarity and visualised using *ggplot2*. Significant differences in microbial community structure were determined via PERMANOVA (permutational analysis of variance) using the *adonis* and *betadisper* functions in *vegan*. The *aov* and *tukeyHSD* functions of *vegan* were used to determine significant differences between treatment groups for each of the enzyme activities. Correlations between taxon abundances, enzyme activities and pH were calculated using Spearman's rho, via the *rcorr* function of the *Hmisc* package. Relationships between pH and enzyme activities, bacterial, archaeal and fungal diversity and taxon abundance were modelled using linear regressions (*lm* function).

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466

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Figures legends

Fig. 1: Relationships between bacterial and fungal diversity and experimentally induced seawater pH changes. Shannon diversity or H' index (a) and richness or observed species (b) are indicated in the top and bottom panels, respectively. Solid lines represent the fitted regression model. The percentage of variance explained by the model or R^2, as well as the significance level of the model, is indicated in the bottom left corner of each sub-figure. Fungal diversity relationships with pH were non-significant (p-value > 0.5), and therefore no regression line is shown.

Fig. 2: Non-metric multi-dimensional scaling (nMDS) ordination plots of bacterioplankton and mycoplankton communities constructed using Bray-Curtis dissimilarity. The left (a) and right (b) panels represent the 16S rRNA gene and ITS 1 region datasets, respectively. Points represent samples and are coloured and shaped according to treatment group and sampling day, respectively. Stress values are indicated at the bottom of each sub-figure.

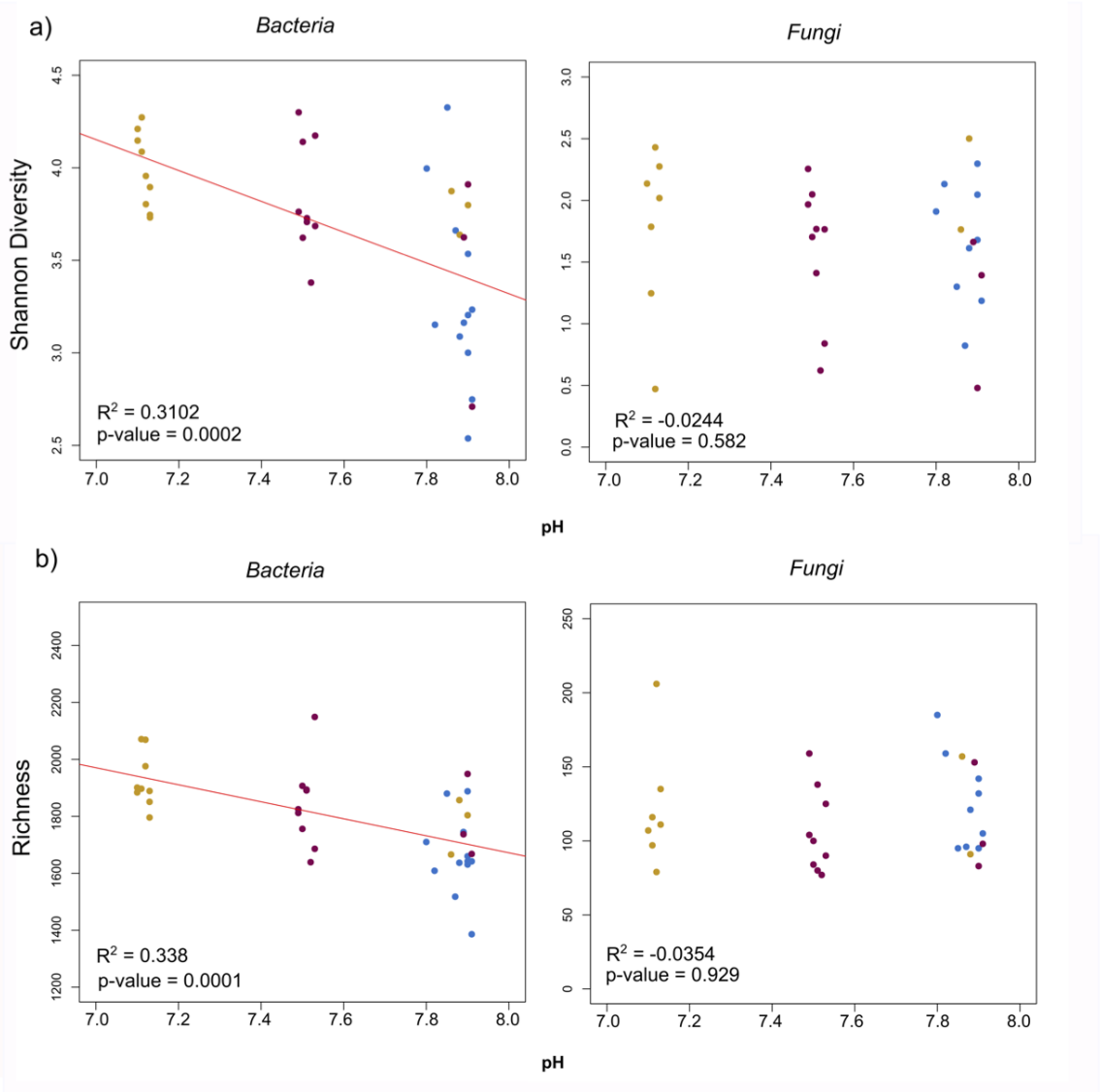
Fig. 3: Relationship between extracellular enzymatic activities and experimentally induced acidification. The solid red line represents the fitted regression model. The percentage of variance explained by the model or R^2, as well as the significance level of the model, is indicated in the left corner of each sub-figure. No fitted regression line is shown for all exoenzyme activities which were not significantly correlated with pH (p-value > 0.5). Points are coloured by the three treatment groups where orange, purple and blue indicate low, medium and control, respectively.

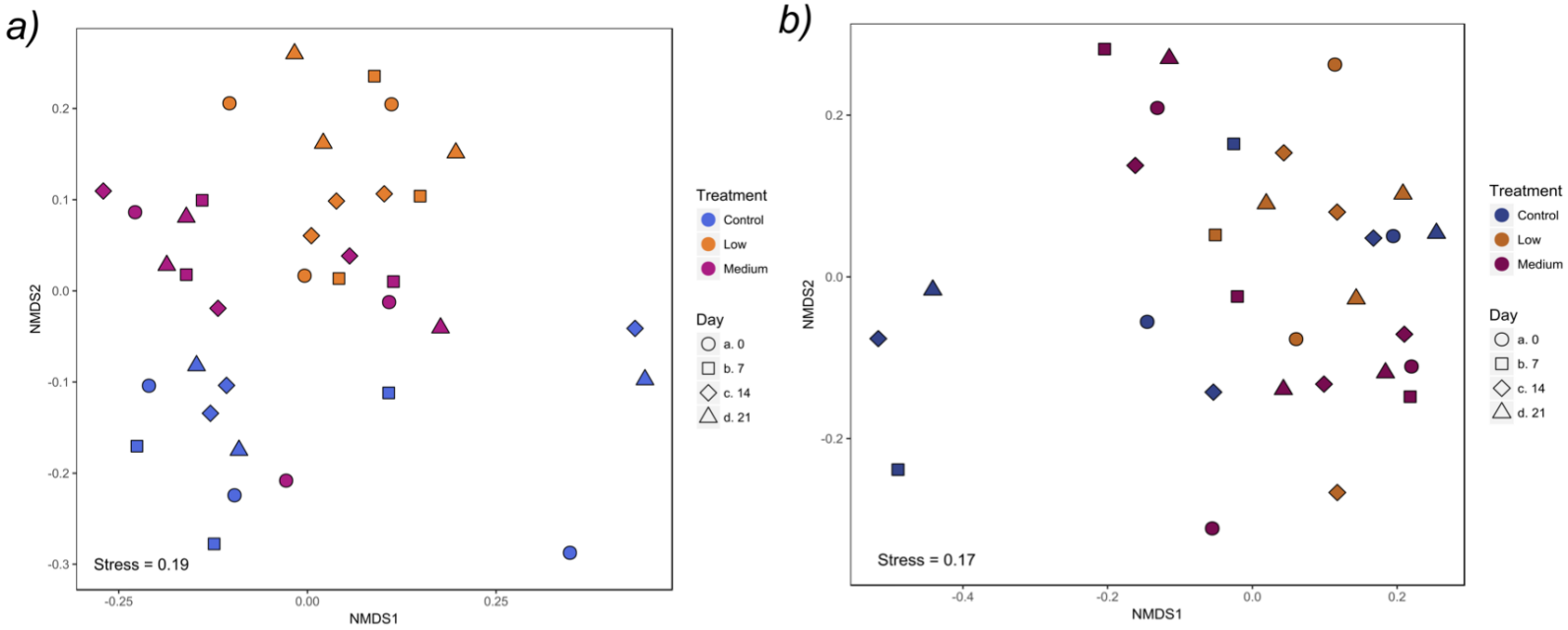
Fig. 4: Ecological bacterioplankton association networks for the control, medium and low pH treatment groups. Nodes correspond to OTUs, where node size is plotted according to node degree, or connectedness. Node colours correspond to phyla and classes. Module taxa compositions are indicated with numbered donut charts, where numbers within a specific

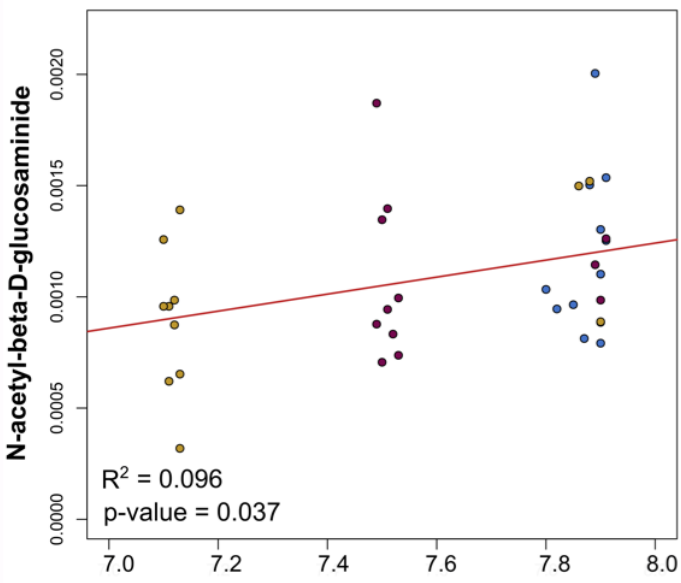
chart correspond to a particular module. Associations are defined by Spearman's' rho correlation coefficient and are deemed significant by random matrix theory (RMT). Positive co-occurrences are shown with a blue line whereas negative co-exclusions are indicated with a red line. Black boxes indicate within module keystones (module hubs) and between module keystones (connectors), respectively.

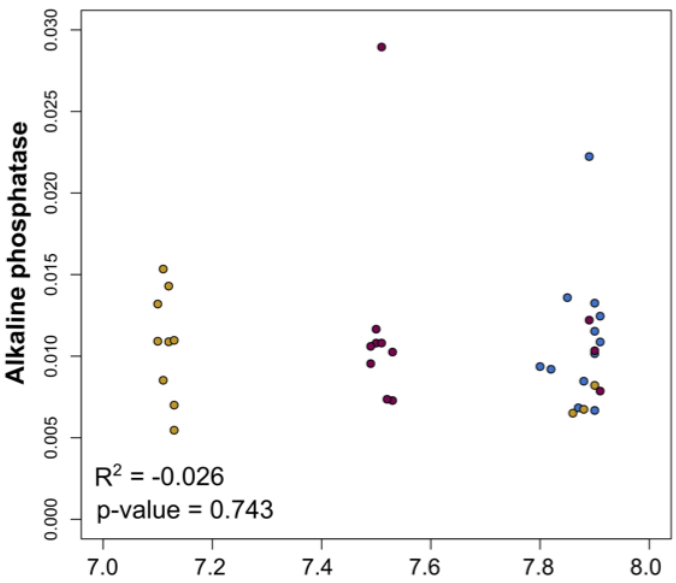
Fig. 5: Plot of the within-module connectivity (*Zi*) and between-module connectivity (*Pi*) of each node or OTU in the three ecological networks and taxon relative abundances. Topological roles of OTUs are indicated either as peripherals ($Zi \leq 2.5$, $Pi \leq 0.62$), connectors ($Zi \leq 2.5$, $Pi > 0.62$), module hubs ($Zi > 2.5$, $Pi \leq 0.62$) and network hubs ($Zi > 2.5$, $Pi > 0.62$). The abundance of peripherals, connectors and module hubs between the three treatment groups is indicated in (b), where the control group is the centremost donut ring, the medium group the middle ring and the low group the outer donut ring.

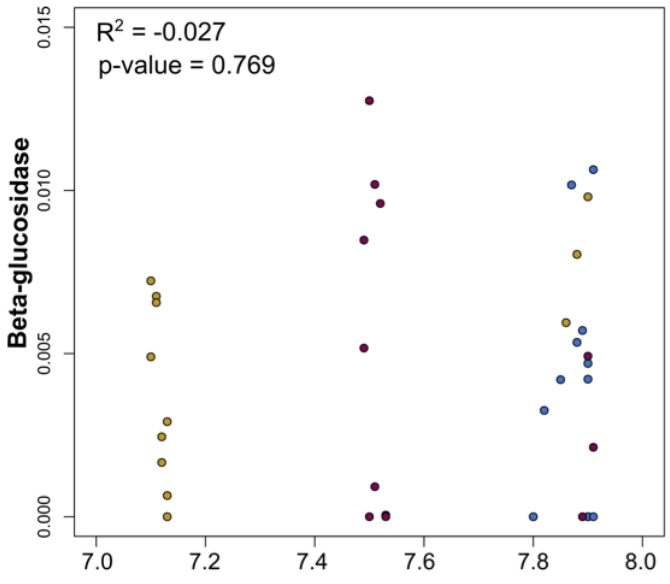
Fig. 6: Conceptual diagram depicting ocean acidification effects on SO surface microbial community structure and functionality. The left and right panel indicate current and future pH levels, respectively. Ovals represent microbial communities, where the lightest colour is indicative of bacteria, the middle colour archaea and the darkest colour fungi. Headings indicate different processes; the first panel shows the process of CO_2 exchange with the atmosphere and the associated consequences on ocean H^+ concentration, pH and a bacterial cell (bacterial cell = yellow oval; proton pump = red star); the second panel indicates current and future microbial community interactions; the last panel shows functional processes utilised to degrade high molecular weight DOM, the subsequent products, and the usage of the resultant products by microbial communities and the higher trophic system. Abbreviations are as follows: HMW = High molecular weight; LMW = Low molecular weight; C = carbon; N = nitrogen; H+ = hydrogen ions; BG = beta-glucosidase; BX = beta-xylosidase; NAG = N-acetyl-glucosaminidase; LAP = leucine aminopeptidase.

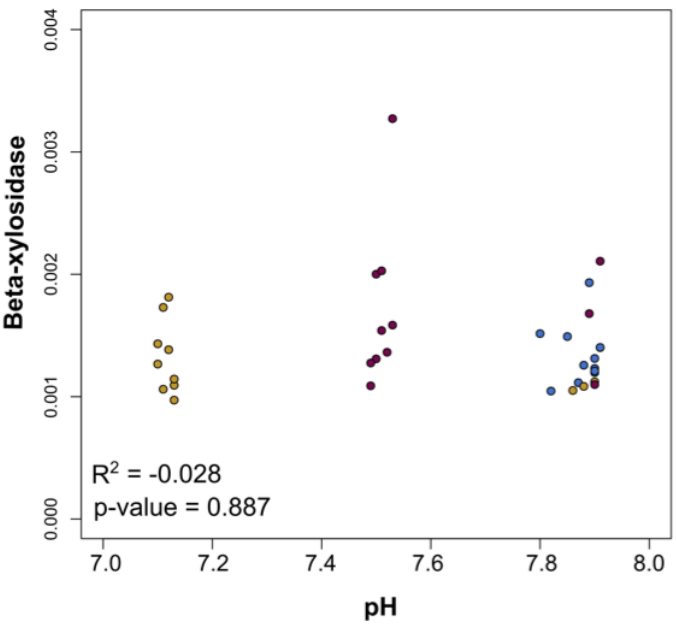

a)
Bacteria
Fungi
Shannon Diversity
R² = 0.3102
p-value = 0.0002
R² = -0.0244
p-value = 0.582
pH
b)
Bacteria
Fungi
Richness
R² = 0.338
p-value = 0.0001
R² = -0.0354
p-value = 0.929
pH

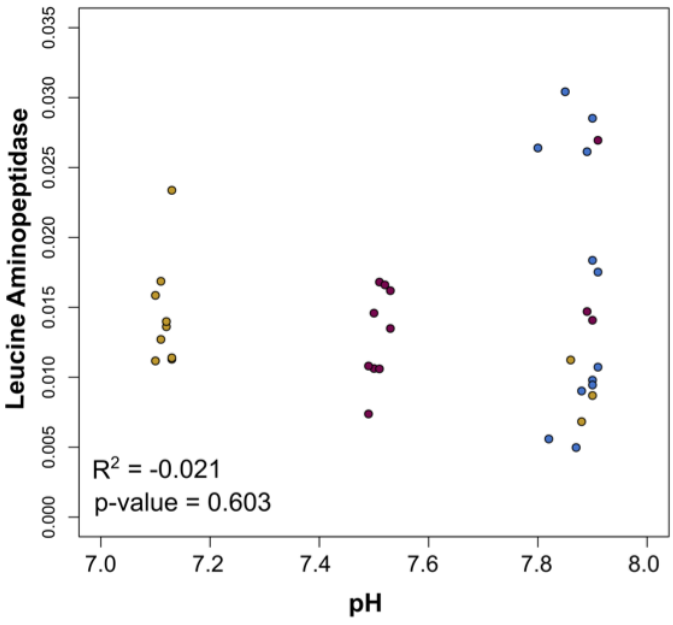

a)
b)
NMDS1
NMDS2
Stress = 0.19
Stress = 0.17
Treatment
Control
Low
Medium
Day
a. 0
b. 7
c. 14
d. 21

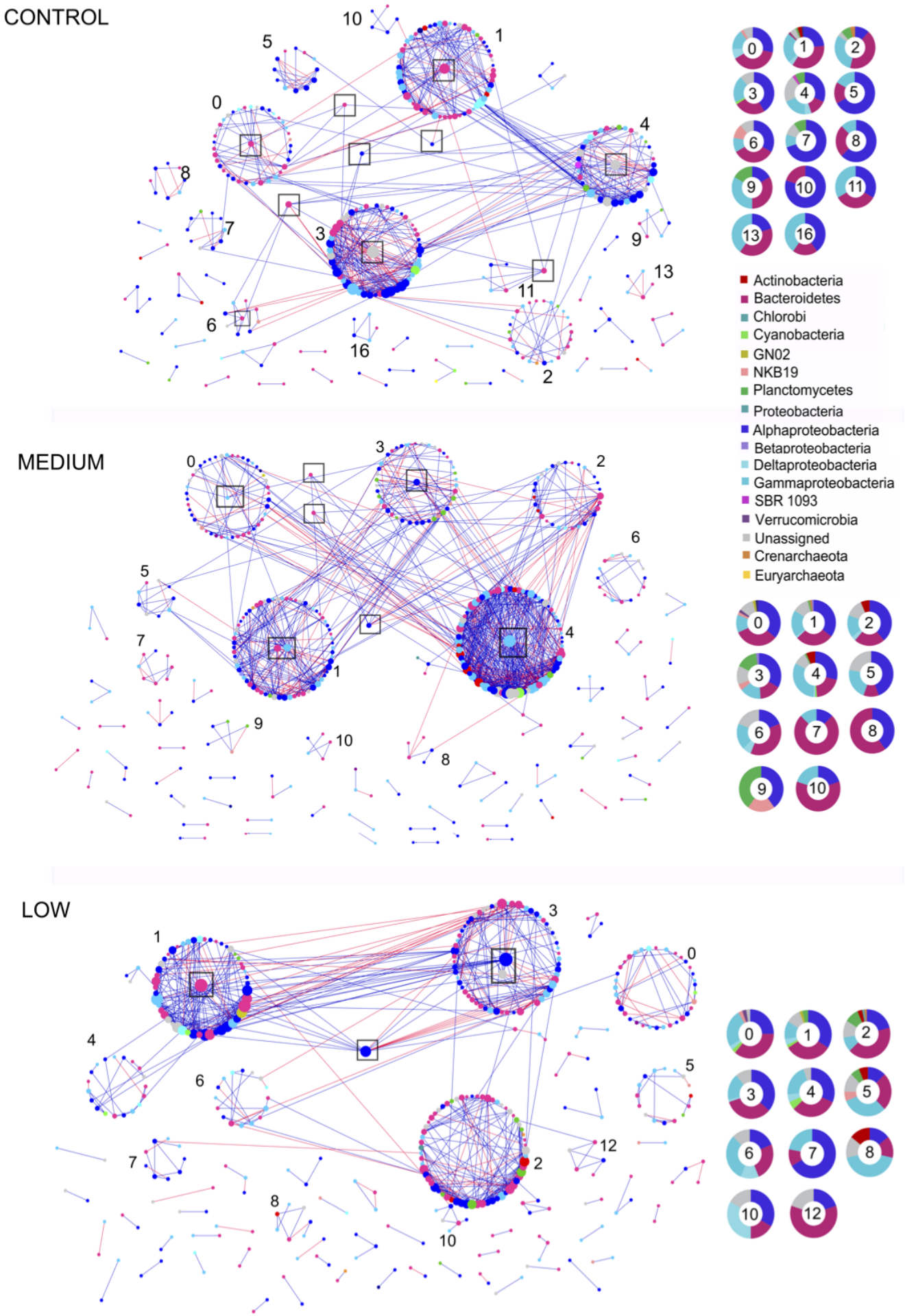

N-acetyl-beta-D-glucosaminide
R² = 0.096
p-value = 0.037
0.0000
0.0005
0.0010
0.0015
0.0020
7.0
7.2
7.4
7.6
7.8
8.0

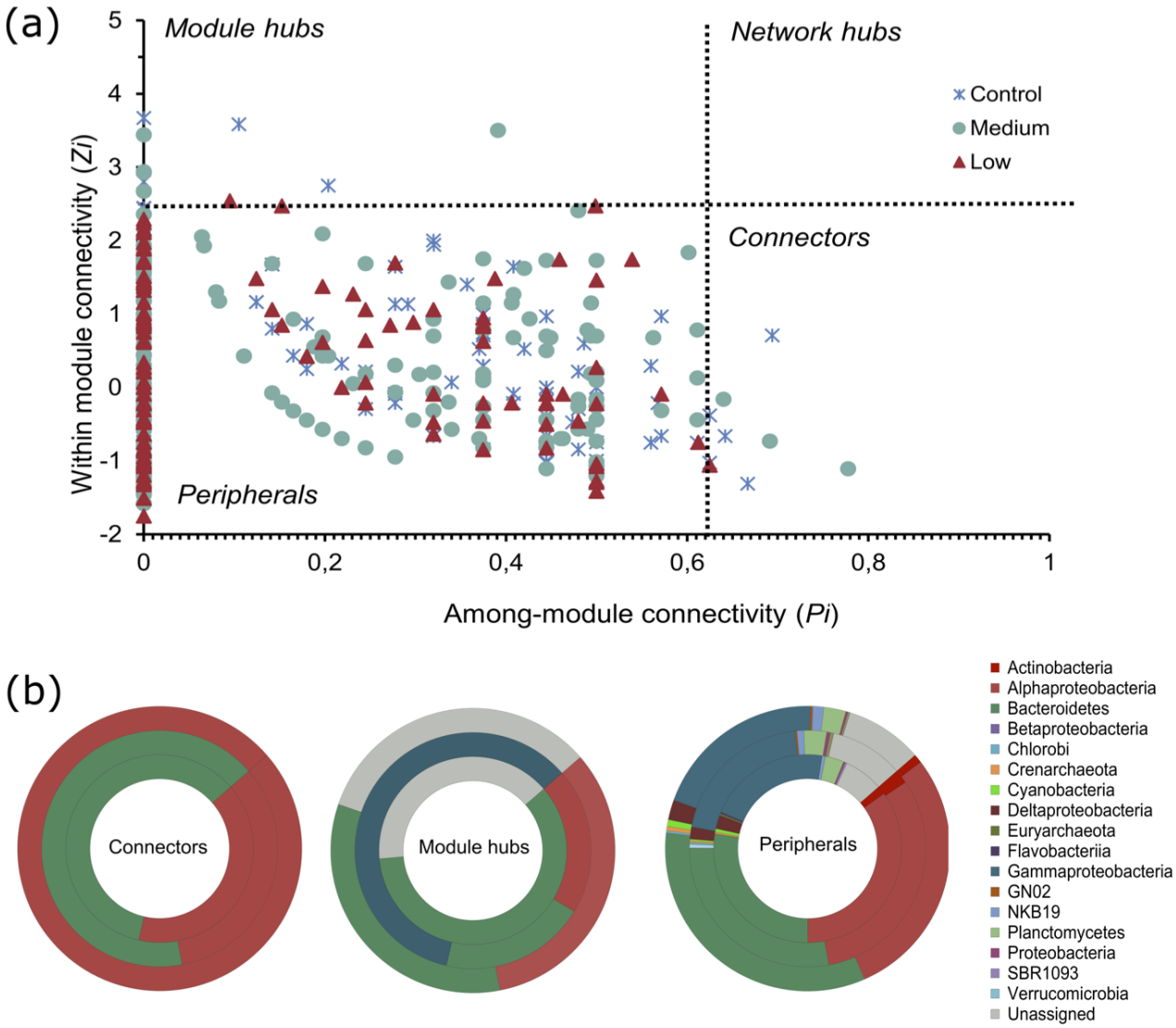

Alkaline phosphatase
R² = -0.026
p-value = 0.743
0.000
0.005
0.010
0.015
0.020
0.025
0.030
7.0
7.2
7.4
7.6
7.8
8.0

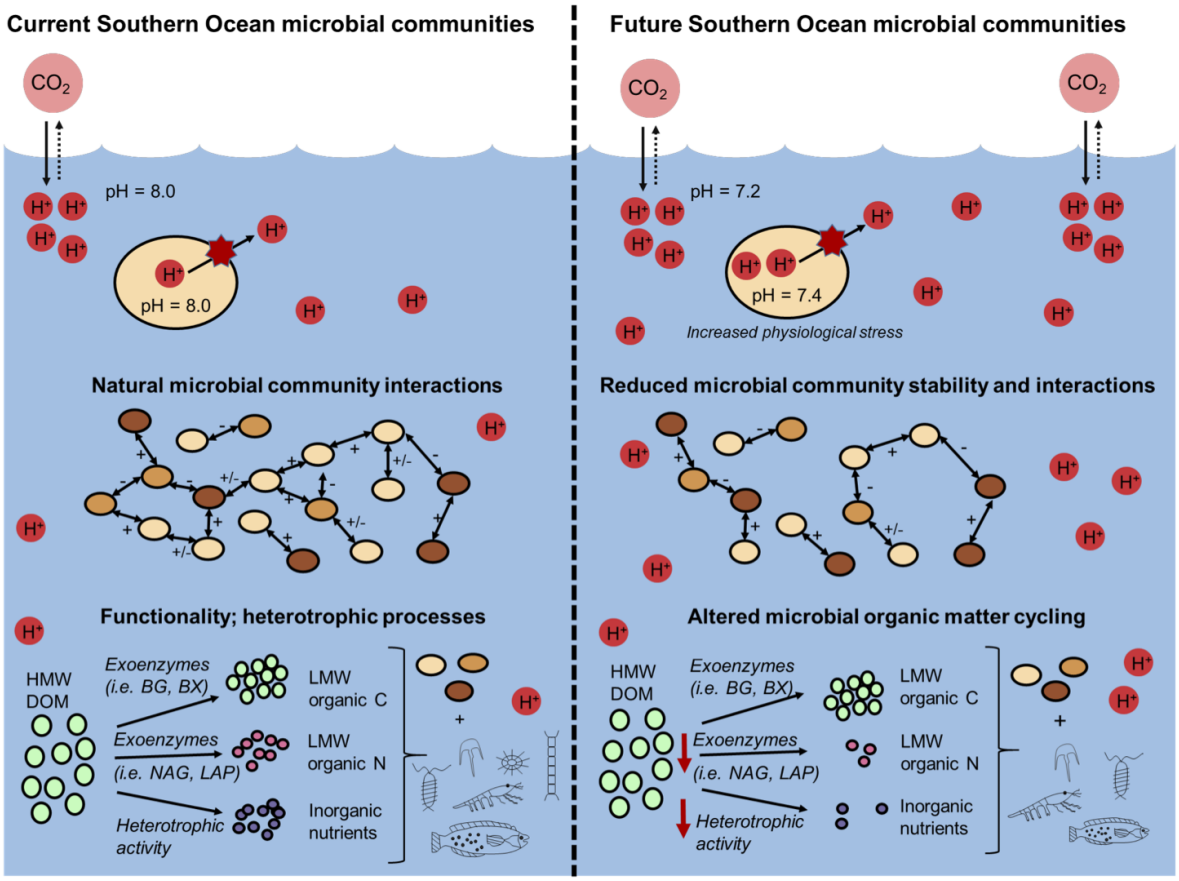

Beta-glucosidase
R² = -0.027
p-value = 0.769
0.000
0.005
0.010
0.015
7.0
7.2
7.4
7.6
7.8
8.0


Beta-xylosidase
R² = -0.028
p-value = 0.887
0.000
0.001
0.002
0.003
0.004
7.0
7.2
7.4
7.6
7.8
8.0
pH


Leucine Aminopeptidase
R² = -0.021
p-value = 0.603
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.035
7.0
7.2
7.4
7.6
7.8
8.0
pH


CONTROL
MEDIUM
LOW
Actinobacteria
Bacteroidetes
Chlorobi
Cyanobacteria
GN02
NKB19
Planctomycetes
Proteobacteria
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
SBR 1093
Verrucomicrobia
Unassigned
Crenarchaeota
Euryarchaeota


(a)
Module hubs
Network hubs
Connectors
Peripherals
Control
Medium
Low
Within module connectivity (Zi)
Among-module connectivity (Pi)
5
4
3
2
1
0
-1
-2
0
0,2
0,4
0,6
0,8
1
(b)
Connectors
Module hubs
Peripherals
Actinobacteria
Alphaproteobacteria
Bacteroidetes
Betaproteobacteria
Chlorobi
Crenarchaeota
Cyanobacteria
Deltaproteobacteria
Euryarchaeota
Flavobacteriia
Gammaproteobacteria
GN02
NKB19
Planctomycetes
Proteobacteria
SBR1093
Verrucomicrobia
Unassigned


Current Southern Ocean microbial communities
Future Southern Ocean microbial communities
CO2
CO2
CO2
pH = 8.0
pH = 8.0
pH = 7.2
pH = 7.4
Increased physiological stress
Natural microbial community interactions
Reduced microbial community stability and interactions
Functionality; heterotrophic processes
Altered microbial organic matter cycling
HMW DOM
Exoenzymes (i.e. BG, BX)
Exoenzymes (i.e. NAG, LAP)
Heterotrophic activity
LMW organic C
LMW organic N
Inorganic nutrients
H+