Microbial communities network structure across strong environmental gradients: How do they compare to macroorganisms?

Arboleda-Baena[a,b], C.M; Freilich[c,d], M. A; Pareja, C.B[b]; Logares, R[e]; De la Iglesia, R[b,f]; Navarrete, S.A [a,f]

[a] Estación Costera de Investigaciones Marinas, Center for Applied Ecology and Sustainability (CAPES), Millenium Nucleus NUTME, Pontificia Universidad Católica de Chile, Chile

[b] Laboratorio de Microbiología Marina, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

[c] Department of Earth, Atmospheric and Planetary Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

[d] Department of Physical Oceanography, Woods Hole Oceanographic Institution,Woods Hole, Massachusetts 02543 USA

[e] Instituto de Ciencias del Mar – CSIC, Paseo Marítimo de la Barceloneta, 37 – 49, 080003 Barcelona, Spain

[f] Marine Energy Research & Innovation Center (MERIC), Avda. Apoquindo 2827, Santiago, Chile.

Corresponding author e-mail: claraarboledab@gmail.com

1. Some data are already published, with those publications properly cited in this submission: Broitman et al. 2001 and Freilich et al. 2018.

2. Sequence data will be deposited at the European Nucleotide Archive (ENA) public database under accession number PRJEB45042 and released after the journal acceptance.

3. We don´t have novel code. But we will provide a copy in Github of the script used (https://github.com/ClarArboledaBaena) after the journal acceptance with the Zenodo DOI.

Abstract

The way strong environmental gradients shape multispecific assemblages has allowed us to examine a suite of ecological and evolutionary hypotheses about structure, regulation, and community responses to fluctuating environments. But whether the highly diverse co-occurring, free-living microorganisms are shaped in similar ways as macroscopic organisms, across the same gradients, has yet to be addressed in most ecosystems. The 'everything is everywhere' hypothesis suggests they are not, at least not to the same extent. Here we characterize the structure of intertidal microbial biofilm communities and compare the intensity of zonation at the 'species' level, changes in taxonomic diversity and composition at the community level, and network attributes, with those observed in co-occurring macroalgae and invertebrates. At the level of species and OTUs, for dominant macro and microorganisms respectively, microbes showed less variability across the tidal gradient than macroorganisms. At the community-level, however, microbes and macro-organisms showed similarly strong patterns of tidal zonation, with major changes in composition and relative abundances across tides. Moreover, the proportion of 'environmental specialists' in different tidal zones was remarkably similar in micro and macroscopic communities, and taxonomic richness and diversity followed similar trends, with lower values in the high intertidal zone. Network analyses showed similar connectivity and transitivity, despite the large differences in absolute richness between the groups. A high proportion of positive co-occurrences within all tidal zones and mostly negative links between the high and low tidal zones were observed among habitat specialist taxa of micro-and macro-organisms. Thus, our results provide partial support to the idea that microbes are less affected by environmental variability than macroscopic counterparts. At the species-level, the most common microbe species exhibit less variation across tides than most common macroscopic organisms,

47 suggesting the former perceive a more homogeneous environment and/or are more resistant to
48 the associated stress. At the community-level, most indicators of community and network
49 structure across the gradient are similar between microbes and macro-organisms, suggesting that
50 despite orders of magnitude differences in richness and size, these two systems respond to stress
51 gradients, giving rise to zonation patterns.

52 **Key words:** Zonation, microorganisms, co-occurrence networks, stress gradients, individual-
53 and community-responses, network attributes.

54

55 **Introduction**

56 Understanding the mechanisms and processes responsible for patterns of abundance and
57 distribution of species across environmental gradients is one of the main goals of community
58 ecology (Mittelbach and McGill 2019), and one of the most striking patterns is the zonation of
59 dominant organisms that occupy different parts of the environmental gradient. These patterns
60 have served as the basis to test core hypotheses about the organization of communities (e.g.
61 Whittaker 1959) and the ecological processes shaping fundamental and realized species' niches,
62 such as physiological tolerances and species interactions (Connell 1961a, 1961b, Menge and
63 Sutherland 1987). However, this deep understanding of communities of macroscopic organisms
64 contrasts sharply with the still scarce knowledge about communities of free-living
65 microorganisms (Fontaneto 2011, Hanson et al. 2012). The 'everything is everywhere, but the
66 environment selects' hypothesis, set forth by Gerhard Baas Becking in the early 1930s, has
67 inspired much of the microbial biogeographical and ecological research of the past decades (de
68 Wit and Bouvier 2006, O'Malley 2007) and suggests that microbial communities are primarily
69 determined by moderate rates of dispersal and secondarily by environmental variation

(selection). It is clear that microbial biodiversity is shaped by environmental gradients, especially by those factors that alter soil or water pH (Rousk et al. 2010, Krause et al. 2012), temperature (Logares et al. 2020), and resource availability (Follows et al. 2007). The local environment ´selects´ which microorganisms are present and, like in macroscopic organisms, patterns of abundance and composition across environmental gradients result from the constraints set by abiotic conditions and the interactions among co-occurring microbes (Fontaneto 2011, Hanson et al. 2012, Mandakovic et al. 2018). Yet, the question remains, are communities of microbial organisms less structured across the same environmental gradients than their co-occurring macroscopic counterparts? If both vary across sharp environmental gradients, are the patterns of community structure towards the stressful and benign ends of a gradient similar to those observed in macroscopic organisms? Addressing these and other similar questions allows us to challenge our ecological models and get valuable insight into how different is the organization between the micro and macroscopic worlds.

Probably the most studied zonation pattern is the one occurring between the highest and lowest tides along most marine rocky shores of the world (Barnes and Hughes 1999, Raffaelli and Hawkins 2012). In this habitat, environmental conditions range from entirely aquatic to completely terrestrial in a few meters (Thompson et al. 2002, Harley and Helmuth 2003); and, within their absolute tolerance limits, species become consistently more abundant at some tidal levels than others (Stephenson and Stephenson 1949, Connell 1961a, 1961b). Also, co-occurrence network analyses show modules (cluster of species highly connected) related to a specific zonation band due mainly driven by shared environmental preferences (Freilich et al. 2018). Beyond the tidal regime that sets the stage for the stress gradient (Denny and Paine 1998, Raffaelli and Hawkins 2012), intertidal zonation of macroscopic organisms results from multiple

93 interactive physical factors, including wave exposure and the associated mechanical stresses,
94 desiccation (Evans 1947), temperature (Wethey 1983), and solar radiation (Santelices 1990), to
95 name a few. Additionally, species interactions, including interspecific competition for space
96 (Connell 1961b), food acquisition (Underwood 1972), facilitation (Bertness and Leonard 1997),
97 and predation (Connell 1961a, Paine 1966), profoundly modify intertidal zonation. As a result,
98 zonation of organisms results from a combination of environmental stressors affecting
99 differentially some species, and the propagation of these effects throughout the community via
100 species interactions. To what extent the intertidal gradient imposes similar patterns of
101 community structure on macro and microscopic communities has not been addressed.
102 Intertidal microbial communities on rocky shores can be found as individual cells or forming
103 well-defined biofilms that can grow on virtually all surfaces (Callow and Callow 2011). Biofilms
104 are composed of taxa belonging to the three domains of life, associated with extracellular
105 polymeric substances (EPS), acquiring important emergent properties such as hydrophobicity,
106 viscoelasticity, drug resistance among others (Davey and O'toole 2000, Schuster et al. 2019).
107 Studies have shown that variability in abundance and the broad composition of intertidal epilithic
108 biofilms are influenced by temperature, desiccation, UV radiation, waves (Thompson et al. 2004,
109 2005), and the concentration of ions and nutrients (Decho 2000, Dang and Lovell 2016).
110 Competition and predation between microorganisms (Dang and Lovell 2016), competition with
111 macroscopic sessile species (Callow and Callow 2011), and top-down control by grazing
112 macroinvertebrates (Lubchenco 1978, Underwood 1984) all influence abundance and tidal
113 distribution of major groups of microbial biofilms. However, most ecological studies have
114 treated biofilms as homogeneous taxonomic units composed of few entities, precluding a
115 community- or ecological-network approach comparable to macroscopic organisms. Since 2006,

116 massive sequencing techniques allow us to explore and document the diversity of the microbial
117 world as never before (Hug et al. 2016), opening the doors to address ecological questions.
118 Here we compare patterns of zonation in taxonomic diversity, richness, and network structure of
119 the microbial intertidal communities on wave-exposed rocky shores of central Chile and the
120 well-documented patterns of co-occurring macroscopic organisms (Castilla 1981, Santelices
121 1990, Kéfi et al. 2015, Freilich et al. 2018). Using the previously defined zonation bands for
122 macroorganisms, we test four hypotheses: 1) that across the same environmental gradient,
123 microbial communities show proportionally less differentiation across tidal levels than their
124 macroscopic counterpart, 2) that taxonomic richness and diversity do not follow similar trends
125 across the gradient, 3) that the proportion of environmental specialist is higher in the
126 macroscopic community than in microscopic organisms, 4) that co-occurrence networks of
127 macroorganisms have modules corresponding roughly with zonation bands, while microbial
128 networks do not form zonation-related compartments.
129
130 **Methods**
131 The comparison of macro and micro-communities represents a large methodological, logistical,
132 and economic (DNA sequencing) challenge. We propose and describe here a reproducible
133 method to quantify microbial communities across tidal levels for comparison with macroscopic
134 communities.
135 ***Macroscopic community data***
136 Quantitative comparisons with macroscopic communities were based on intertidal surveys
137 conducted since 1998 across sites in central Chile from the 33º 42’ S, 71º 70’ W to 33º 59’ S, 71º
138 62’ W, including the study site for microscopic studies. Field methods have been described in

detail (Broitman et al. 2001, Freilich et al. 2018). Briefly, surveys at each site quantified the density of mobile species and the cover of sessile species of all macroscopic organisms (>1mm) in 8-10 50 x 50 cm quadrats along transects parallel to shore at three tidal levels, low, mid, and high shore. Details of surveys included in this study are presented in **Appendix S1: Table S1**.

Microbial community data

Microbial community studies were conducted in the wave-exposed rocky shore at Las Cruces, central Chile, 33º 50' S, 71º 63' W. The rock is formed by postmetamorphic granite with basaltic intrusions and high quartz content and tidal range is about 1.8 m, with semidiurnal regime. Since our goal was to compare the organization of microbial communities across different tidal levels, it was critical to set a similar successional time and reduce interference with surrounding macroscopic biota as much as possible. We therefore developed a simple method that can be replicated in future studies. First, we collected rocks from the same study site and cut them into coupons of 3 x 8 x 2 cm with a COCH Bridge saw machine that prevented overheating and potential mineral transformations. Rock coupons were washed with deionized water, dried, maintained at room temperature, and then attached individually, with a stainless-steel wire, inside stainless-steel cages 12 x 12 x 4 cm and 5 mm mesh. Cages prevented grazers and other macroscopic organisms from crawling on the rock coupons. Twelve rock coupons inside a separate stainless cage were affixed with a stainless-steel screw at each of three tidal zones haphazardly distributed along tens of meters parallel to the shore. Tidal zones followed the same levels used in macroscopic studies described above(Santelices 1990, Flores et al. 2019). Five rocks selected at random were not deployed in the field and were used as rock surface control. After 40 days of exposure, rock coupons were retrieved and, together with rock surface controls, were sonicated in sterile marine water, and the biofilm was recovered through 0.22 µm pore

filters of hydrophilic polyethersulfone (Merck). Samples were preserved in liquid nitrogen at -196°C for molecular analyses. A total of five cages with rock coupons were lost due to storms. A 40-day elapsed time has been shown to allow intertidal biofilm communities to reach a comparatively stable structure at the end of succession, and macroorganisms do not cover them yet (authors, personal observation).

DNA extraction, 16S rRNA sequencing and molecular data analyses

DNA extraction from filters was conducted with the Phenol-Chloroform method (Fuhrman et al. 1988) and DNA samples were clean by the membrane dialysis method with a Millipore 0.025 uM filter (Devaney and Marino 2001). DNA concentration was measured with the Qubit HS dsDNA Assay kit in a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and the DNA purity was quantified using a NanoDrop-1000 (NanoDrop Technologies, Wilmington, USA), according to manufacture protocols. The V4-V5 region of the 16S was amplified with the primers 515FB: GTGYCAGCMGCCGCGGTAA and 926R: CCGYCAATTYMTTTRAGTTT (Parada et al. 2016). Amplicons were sequenced in a MiSeq Illumina platform (2x300 bp). Both PCR and sequencing were done at the Dalhousie University CGEB-IMR (http://cgeb-imr.ca/). Sequence data were deposited at the European Nucleotide Archive (ENA) public database under accession number PRJEB45042.

Macro and Micro community analyses

Amplicon reads were analyzed using the DADA2 pipeline (Callahan et al. 2016, Lee 2019), generating Amplicon Sequence Variants (ASVs) that were used as Operational Taxonomic Units (OTUs). Rarefaction curves (Simberloff 1978) for bacterial diversity were generated with the R package phyloseq v1.30.0 (McMurdie and Holmes 2013) and allowed us to define a sampling effort of 16,888 reads for microbial diversity comparisons across tidal levels (**Appendix S1:**

Table S2). The number of different OTUs and macroscopic species per unit area (hereafter called 'richness'), were separately compared among treatments (three tidal zones, fixed factor) with Welch ANOVA, and one-way ANOVA for macro and micro communities, respectively, since the former displayed heteroscedasticity. The Games-Howell and a Tukey´s posteriori tests were performed after significant ANOVA results, for macro and microbial communities, respectively. Species and OTUs Shannon diversity indices were compared among tidal zones with separate Welch ANOVAs and Games-Howell a posteriori tests for macro and microorganisms, as they both exhibited heteroscedasticity. To quantify tidal variation in abundance and thus the intensity of zonation patterns, we calculated coefficients of variation (CV) across tidal levels of the most abundant macro and microorganisms at the level of species and Orders. To characterize community compositional differences and general structure among tidal zones, we conducted multivariate ordinations on presence/absence data using Jaccard distance index, and on relativized species/OTU abundances using Bray Curtis distance for Non-Metric Multidimensional Scaling ordinations. To test for statistical significance in community composition and structure among the intertidal zones, we conducted permutational analysis of variance (PERMANOVA) (Anderson and Walsh 2013), followed by pairwise a posteriori tests using FDR correction (Benjamini and Hochberg (1995), separately for micro and macroscopic organisms. We also conducted quantitative Principal Coordinate Analyses (PCoA) to represent tidal zones in the multispecies space to recover the centroid that defined the compositional groups for macro and microorganisms. This allowed us to calculate multivariate Mahalanobis distances among community centroids at the three intertidal zones. All multivariate analyses were conducted with the R package vegan v2.5-6 (Oksanen et al. 2015).

Co-occurrence Network analyses

To further compare the structure of macro- and microbial communities across tidal zones, we constructed co-occurrence networks for both groups using maximal information coefficient analysis applied to presence/absence data in the software MICtools (Albanese et al. 2018) to derive the strength and sign of correlation. Tidal zone specialists were identified with the Indicator Value (IndVal) (Dufrene and Legendre 1997) in the labdsv R package (Roberts and Roberts 2016), which identifies Species/OTUs associated to a given tidal zone based on fidelity and relative abundance. To compare network structures, we computed connectivity, the proportion of all possible edges that are realized in the network (Newman 2018), and transitivity, the ratio of the number of triangles to the paths of length two, which quantifies the clustering of the network (Wasserman and Faust 1994). Both metrics were calculated for micro and macroorganisms separately. We then identified the habitats specialists and not specialist in each network, according to the IndVal index, and represented positive and negative links within and between tidal zone compartments. To summarize patterns of positive and negative links, we calculated connectivity within and between entire compartments of habitat specialists and not specialist. The statistical significance of network connectivity and transitivity was evaluated against Erdos-Renyi random networks of the same size (Connor et al. 2017). The existence of compartments in the macro- and microbial networks was examined using the leading eigenvalue method (Newman 2006). Network metrics were computed using the R package iGraph (Csardi and Nepusz 2006).

Results

Zonation of dominant taxa

A total of 88 macroalgae (n= 40) and invertebrate (n = 48) species were found at the surveyed

231 sites of central Chile (**Appendix S1: Table S3**). Dominant species in each zone were similar to
232 those reported in previous studies (Castilla 1981, Broitman et al. 2001) (**Appendix S1: Table**
233 **S3**). We obtained 472,864 good-quality sequences from 28 samples for microbial community
234 analyses. After rarefying to 16,888 reads per sample, due to the size of the smallest dataset of
235 one low intertidal zone sample, we obtained a total of 6,252 OTUs (**Appendix S1: Table S2**).
236 The main phyla in the intertidal zone were Proteobacteria (Alphaproteobacteria and
237 Gammaproteobacteria), Bacteroidetes, Cyanobacteria, and Planctomycetes (**Appendix S1:**
238 **Figure S2, Table S3)**. Patterns of zonation, i.e., sharp changes in relative abundance, of the 14
239 most abundant macroalgae and invertebrate species across the tidal gradient were very apparent
240 (**Fig. 1**). Although most species were present at all three tidal zones, they show high abundance
241 at a single (e.g., *Ectocarpus silicosus*, *Nodolittorina peruviana*) or, at the most, two tidal zones
242 (e.g., *Ralfsia californica, Siphonaria lessoni*) (**Fig. 1a**). Zonation was also apparent in the 14
243 most common OTUs, although the changes of abundance across tidal zones were attenuated
244 compared to macroscopic organisms (**Fig. 1b**). This difference was reflected in larger
245 coefficients of variation (CV) in macro- (mean= 123%) than micro-organisms (mean= 84%)
246 (**Fig. 1c,d**). Pooling species and OTUs into the main Orders (**Appendix S1: Figure S1a,b)** still
247 revealed patterns of specialization or zonation in macroorganisms, but only weakly so in
248 microorganisms (mean CV: Macro= 125%, Micro= 62%) (**Appendix S1: Figure S1c,d).** A
249 negative slope characterized the relationship between spatial variation in orders of macro- and
250 micro-organisms and the number of species (S) within the Orders (**Fig. 2a,b**), but the
251 relationship was statistically significant only for macroorganisms. The negative, buffering effect
252 of diversity on variability across the gradient was similar in micro-and macro-organisms (similar
253 slopes, ANCOVA interaction: p= 0.459), and a single linear fit across micro and macro-

organisms was highly significant (CV= 150 – 66.8 + logS , R2= 0.43, p = 0.001, **Appendix S2: Figure S1)**, but this result should be taken with caution because of the inevitable collinearity produced by differences in richness (**Fig. 2c,d**). A detailed account of main macro-organisms and microbial orders and their variability across tidal zones is displayed in **Appendix S1: Figure S1.**

Diversity across tidal zones

Species richness of macroscopic communities was significantly different across tidal zones (**Fig. 2c**, Welch´s ANOVA, p<0.0001), with highest richness in the mid zone and lowest in the high zone. The richness of OTUs (using rarefaction of equal sampling effort) also showed significantly different values among intertidal zones (**Fig. 2d**, ANOVA, p=0.0209), with highest values in the low zone and lowest in the high zone. Microbial richness in the mid-zone reached intermediate levels and could not be statististical differentiated from values observed in the low or high zones. The Shannon diversity index for macroorganisms also showed significant differences among intertidal zones (**Fig. 2e**, Welch´s ANOVA, p<0.0001), with higher and similar values in the middle and low zones than the high zone (Games-Howell post hoc test, p=0.49). Microbial diversity also varied significantly among intertidal zones (**Fig. 2f,** Welch´s ANOVA, p=0.0143) with the highest values in the low zone and lowest in the high zone (Games-Howell tests). Intermediate diversity levels in the mid zone could not be statistically separated from other tidal zones by a posteriori tests. Richness and Shannon diversity were significantly different between microbial communities of the three intertidal zones and the rock surface control (**Appendix S2: Figure S2)**.

Community and network structure

The composition and relative abundances of the macroorganisms community were different

among the three tidal zones (**Fig. 3a,c**), with sharper separation between the low zone and the other two, and largest dispersion in the mid zone (**Fig. 3a, c**). These multispecic differences among zones were statistically significant (**Appendix S3: Table S1a,b**, PERMANOVA, p=0.0009 for Jaccard and Bray-Curtis distances), and paired comparisons showed that all intertidal zones differed from each other (FDR adjusted post-hoc tests: p=0.03 Jaccard distances and p=0.02 Bray-Curtis distances). The microbial communities tended to show similar patterns to their macroscopic counterparts, with a sharply defined low intertidal zone and increased overlap between the high and mid zones (**Fig. 3b,d**). In this case, the largest dispersion was observed in the high intertidal zone. Statistically significant differences were observed in microbial community composition and relative abundances (**Appendix S3: Table S1c,d**, PERMANOVA, p=0.0009 for Jaccard and Bray-Curtis distances), and paired comparisons also showed that all zones differed from each other (FDR adjusted post-hoc test, p=0.03 Jaccard and p=0.02 Bray-Curtis distances). Including the rock surface's control in the analyses did not alter this general pattern **(Appendix S3: Figure S1).**

The connectivity and transitivity analyses of the co-occurrence networks showed similar connectivity, 0.216 and 0.222 for macro and micro-organisms, respectively, and the same undirected transitivities of 0.56. These transitivity values were in both cases significantly larger than the transitivity of random networks ($p < 0.001$). While most species and OTUs changed in abundance across tidal zones, our IndVal index analyses allowed us to classify them into habitat specialists or not specialist and identify these types of species/OTUs in the networks (**Appendix S4: Table S1a,b**). The fraction of habitat specialists in the high, mid, and low zones was remarkably similar between micro- and macroorganisms (**Fig. 4a,b**), with the highest number found in the low shore. In micro and macroorganisms networks, positive correlations dominated

300 the links detected between taxa within all tidal zone compartments, while predominantly
301 negative links were detected between species in the high and low shore compartments (**Fig. 4a,**
302 **b**). In both networks, not specialist were positively and negatively connected with habitat
303 specialists and other not specialist. These visual patterns were well captured in the connectivity
304 summary within and between compartments. This showed high and generally positive
305 connectivity within compartments, but strong and, on average, negative connection between the
306 high and low zones (**Fig. 4c,d**). Macroorganisms restricted to the high intertidal and
307 microorganisms restricted to the low intertidal are particularly highly connected within their
308 groups of habitat specialists with connectivity of 0.73 and 0.58, respectively, and strong positive
309 correlation (**Fig. 4c,d**). In both networks, the average correlation strengths between specialists
310 and nonspecialists was very weak, although the values were higher in macro-organisms than in
311 the microbial network (**Fig. 4c,d**), a pattern that was apparent in the visual representation of
312 links. Despite these remarkable similarities, the two networks had different degree distributions,
313 i.e., how the number of links of each node are distributed, with binomial distribution in
314 macroscopic organisms and power-law degree distribution in microorganisms (**Appendix S4:**
315 **Figure S1**). Modularity analyses of the entire networks showed a weak and non-significant
316 relationship of compartments to the tidal zonation in both networks (**Appendix S4: Figure S2)**.
317

318 **Discussion**

319 Our combination of intertidal surveys and experiments allowed us to characterize microbial
320 communities and make sensible comparisons with macroscopic organisms across one of the best
321 studied environmental gradients in the world. While it is true that these comparisons are
322 constrained by methodological issues, which bound our conclusions, they still shed light on the

processes driving ecological organization in microbial organisms and how different they may be from those affecting co-occurring macroscopic components of these communities. Our results partially support the idea that microbes are less affected by environmental variability than macroscopic counterparts. First, while a similar fraction of all micro-and macro-organisms can be considered as 'habitat specialists', i.e., largely restricted to a given tidal zone, the 14 most common microbial species exhibit much less variation across tides than the most common macroscopic organisms, suggesting the former perceive a more homogeneous environment and/or are more resistant to the associated stress. At the community-level, however, most indicators of community structure and attributes of co-occurrence networks across the gradient were remarkably similar between microbes and macro-organisms, suggesting that despite orders of magnitude differences in richness and size, these two systems respond to stress gradients at a multispecific level in similar ways. We discuss these results below but start with a brief discussion of the methods used, which must be borne in mind when drawing conclusions.

Methodological approximation to intertidal biofilm studies has followed two general approaches. Firsts, removal of sections of natural, existing rocks from the environment with different exposure time (De la Iglesia et al. 2012, Taylor et al. 2014, Tan et al. 2015, Kerfahi et al. 2020), or the installation of artificial surfaces such as plexiglass, polystyrene, or glass slides (Zhang et al. 2013, Sanli et al. 2015). The first approach is useful for examining the composition of microbial communities but makes it difficult to make sensible comparisons across gradients because the successional time is unknown. The second approach allows for comparative studies, but the specificity of microbial communities for different surfaces limits interpretation to natural systems. Our study attempts to solve these issues using the same natural rock surface, with the same area, physical attributes, chemical composition, and the same exposure time for all

346 treatments, allowing us to compare among treatments and extrapolate to natural rock surfaces.
347 Still, some variation in micro-flows concerning a flat rocky platform is inevitable. Moreover, we
348 opted to excluded macro-organisms and, in this manner, reduce interactions with macroscopic
349 organisms, especially molluscan grazers. This was a difficult decision because it introduced the
350 potential artifact of having a stainless-steel mesh reducing the light incidence and probably
351 ameliorating stress on experimental rocks. We could devise no appropriate control for this
352 artifact without a prohibitively large number of treatments. Future studies should therefore
353 consider these potential cage effects and experimentally assess the effect of macroscopic
354 organisms on reported patterns of microbial zonation. Finally, we decided to use large numbers
355 of surveys for macroscopic organisms at the biofilm study site and nearby sites (see **Appendix**
356 **S1: Table S1**), to have a larger sample for co-occurrence network analyses. This can be easily
357 changed by considering only one or more sites and data are made available. Our broad
358 conclusion is not altered by this consideration.
359 Our results indicated that dominant microbial organisms across the same environmental gradient
360 showed comparatively less differentiation across tidal levels than their macroscopic counterpart.
361 i.e., the tidal gradient strongly shaped the most abundant species and OTUs. The common
362 explanation for this might be that the environmental conditions in the intertidal rocky shore
363 perceived at the microscopic level are less stringent than those perceived by macroscopic
364 organisms. While this is entirely possible, it is also possible that these organisms are more
365 resistant to similar gradients in stress, which can only be assessed by experimentally
366 manipulating stress levels. To what extent the cage used to exclude macroscopic organisms
367 reduced the amplitude of the stress gradient must also be further investigated. Regardless of the
368 mechanism accounting for differences in stress-related responses at the 'species' level, we

observed a strong buffering effect of species richness within higher taxa, such as Orders, on the response to the stress gradient when considering micro and macro-organisms together. (**Appendix S1: Figure S1**), and that the effect was apparently homogeneous between these groups (non-significantly different slopes). Thus, there is an indication that in both groups, increased species redundancy confers resistance to environmental stress as a taxon. This is an interesting result that deserves further studies as it suggests similarities between micro and macroscopic worlds. However, it must still be taken with caution because the linear fit relationship was marginally non-significant for the microscopic organism when analyzed separately. These results also call for caution when analyzing microbial communities across space without resolving to OTUs level because aggregation at higher taxa is bound to support the everything is everywhere paradigm equivocally.

At the community-level, stress gradient responses of micro and macroscopic communities become more similar. The time exposure to radiation, high temperature, ultraviolet light, and desiccation are factors that could explain the similar trends across the gradient between these two communities because, in both group lowest values of abundance, richness and diversity were observed in the high intertidal zone, increasing to the middle and low tidal levels. Other studies supported this finding and described a higher abundance of epilithic biofilms in the lower intertidal zone than the upper zone due to desiccation and UV light (Aleem 1950, Castenholz 1963, Underwood 1984, Thompson et al. 2004). Accordingly, the gradients of abiotic factors in the intertidal may promote adaptation to these stringent conditions and, as a consequence, increase the habitat specialists (Logares et al. 2013). Thus, restricted distribution of taxa in the intertidal zone may be more common than generally accepted and differences between micro- and microorganisms may not be substantial. This also suggests an imprint of marine over

392 'terrestrial' origin of intertidal microorganisms, as it occurs in rocky shore macro-organisms.
393 Co-occurrence networks of macro and microorganisms showed similar levels of connectivity and
394 transitivity between the clusters despite the large differences in absolute richness, indicative of
395 non-random clustering within the networks (Röttjers and Faust 2018); consequently, these
396 networks had modules (cluster of Species/OTUs highly connected) corresponding roughly with
397 tidal levels. Those clusters of species were generally more connected than the networks as a
398 whole. Freilich et al. (2018) observe that co-occurrence networks might represent niche
399 preferences of component species more than they reflect specific biotic interactions. Therefore,
400 networks constructed from known interactions (i.e., consumption) are not directly comparable to
401 co-occurrence networks. Also, Co-occurrence networks of microorganisms are structured by
402 environmental heterogeneity (e.g., pH, aridity, net primary productivity in soil (Delgado-
403 Baquerizo et al. 2018), and depth in a marine system (Cram et al. 2015)). With this caveat in
404 mind, the fact remains that networks of micro-and macro-organisms show several remarkably
405 similar attributes and some important differences across the tidal gradient.
406 Highly positive associations were observed for species restricted to the high intertidal zone
407 (Freilich et al. 2018), whereas highly positive correlations were observed for OTUs limited to the
408 low intertidal zone. The low intertidal zone is generally expected to be the benign end of the
409 environmental stress gradient for organisms of marine origin, which include both micro and
410 macroorganisms. Still, the resulting co-occurrence patterns of positive associations differ
411 between both networks. This supports that communities of macro-and microorganism respond in
412 different ways to the same environmental forcing due possibly to differences in their resiliency
413 to Ambiental stress, different mediating effects of biotic interactions, and dormancy in microbial
414 communities (Mestre and Höfer 2021). But, despite orders of magnitude differences in richness

and size, the macro and micro-communities respond to stress gradients, giving rise to specific zonation patterns in the intertidal rocky shore.

Acknowledgements

We thank Sergio Celis at ATIKA Ltda, André Comeau at CGEB-IMR. We are also indebted to many students and research assistants at ECIM who collaborated with us in the field and laboratory. Funding for these studies and for international collaboration was provided by CONICYT (ANID) – National PhD scholarship Program 2016 to CMAB, and by Fondecyt grants No. 1160289 and 1200636 to SAN, and No. 1171259 to RDI. Complementary funding was provided by ANID PIA/BASAL FB0002 to SAN.

References

Albanese, D., S. Riccadonna, C. Donati, and P. Franceschi. 2018. A practical tool for maximal information coefficient analysis. GigaScience 7.

Aleem, A. A. 1950. Distribution and ecology of British marine littoral diatoms. Journal of ecology 38:75–106.

Anderson, M. J., and D. C. I. Walsh. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs 83:557–574.

Barnes, R. S. K., and R. N. Hughes. 1999. An introduction to marine ecology. John Wiley & Sons.

436 Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and
437 Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B
438 (Methodological) 57:289–300.
439 Bertness, M. D., and G. H. Leonard. 1997. The role of positive interactions in communities:
440 Lessons from intertidal habitats. Ecology 78:1976–1989.
441 Broitman, B., S. Navarrete, F. Smith, and S. Gaines. 2001. Geographic variation of southeastern
442 Pacific intertidal communities. Marine Ecology Progress Series 224:21–34.
443 Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes.
444 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature
445 Methods 13:581–583.
446 Callow, J. A., and M. E. Callow. 2011. Trends in the development of environmentally friendly
447 fouling-resistant marine coatings. Nature Communications 2:244.
448 Castenholz, R. W. 1963. An experimental study of vertical distribution of littoral marine
449 Diatoms. Limnology and Oceanography 8:450–462.
450 Castilla, J. C. 1981. Perspectivas de investigación en estructura y dinámica de comunidades
451 intermareales rocosas de Chile central, 2: Depredadores de alto nivel trópico. Medio
452 Ambiente (Chile) 5:190–215.
453 Connell, J. H. 1961a. Effects of Competition, Predation by Thais lapillus, and Other Factors on
454 Natural Populations of the Barnacle Balanus balanoides. Ecological Monographs 31:61–
455 104.
456 Connell, J. H. 1961b. The Influence of Interspecific Competition and Other Factors on the
457 Distribution of the Barnacle Chthamalus Stellatus. Ecology 42:710–723.

458 Connor, N., A. Barberán, and A. Clauset. 2017. Using null models to infer microbial co-
459 occurrence networks. PLOS ONE 12:e0176751.
460 Cram, J. A., L. C. Xia, D. M. Needham, R. Sachdeva, F. Sun, and J. A. Fuhrman. 2015. Cross-
461 depth analysis of marine bacterial networks suggests downward propagation of temporal
462 changes. The ISME Journal 9:2573–2586.
463 Csardi, G., and T. Nepusz. 2006. The igraph software package for complex network research.
464 InterJournal, Complex Systems, 1695.:9.
465 Dang, H., and C. R. Lovell. 2016. Microbial Surface Colonization and Biofilm Development in
466 Marine Environments. Microbiology and Molecular Biology Reviews 80:91–138.
467 Davey, M. E., and G. A. O'toole. 2000. Microbial Biofilms: from Ecology to Molecular
468 Genetics. Microbiology and Molecular Biology Reviews 64:847–867.
469 De la Iglesia, R., D. Valenzuela-Heredia, S. Andrade, J. Correa, and B. González. 2012.
470 Composition dynamics of epilithic intertidal bacterial communities exposed to high
471 copper levels. FEMS Microbiology Ecology 79:720–727.
472 Decho, A. W. 2000. Microbial biofilms in intertidal systems: an overview. Continental Shelf
473 Research:17.
474 Delgado-Baquerizo, M., A. M. Oliverio, T. E. Brewer, A. Benavent-González, D. J. Eldridge, R.
475 D. Bardgett, F. T. Maestre, B. K. Singh, and N. Fierer. 2018. A global atlas of the
476 dominant bacteria found in soil. Science 359:320–325.
477 Denny, M. W., and R. T. Paine. 1998. Celestial Mechanics, Sea-Level Changes, and Intertidal
478 Ecology. The Biological Bulletin 194:108–115.

479 Devaney, J. M., and M. A. Marino. 2001. Purification methods for preparing polymerase chain
480 reaction products for capillary electrophoresis analysis. Pages 43–49 Capillary
481 Electrophoresis of Nucleic Acids. Springer.
482 Dufrene, M., and P. Legendre. 1997. Species Assemblages and Indicator Species: The Need for a
483 Flexible Asymmetrical Approach. Ecological Monographs 67:345.
484 Evans, R. G. 1947. The Intertidal Ecology of Selected Localities in the Plymouth
485 Neighbourhood. Journal of the Marine Biological Association of the United Kingdom
486 27:173–218.
487 Follows, M. J., S. Dutkiewicz, S. Grant, and S. W. Chisholm. 2007. Emergent Biogeography of
488 Microbial Communities in a Model Ocean. Science 315:1843–1846.
489 Fontaneto, D. 2011. Biogeography of microscopic organisms: is everything small everywhere?
490 Cambridge University Press. 79:388.
491 Freilich, M. A., E. Wieters, B. R. Broitman, P. A. Marquet, and S. A. Navarrete. 2018. Species
492 co-occurrence networks: Can they reveal trophic and non-trophic interactions in
493 ecological communities? Ecology 99:690–699.
494 Fuhrman, J. A., D. E. Comeau, A. Hagstrom, and A. M. Chan. 1988. Extraction from Natural
495 Planktonic Microorganisms of DNA Suitable for Molecular Biological Studies. Applied
496 and Environmental Microbiology 54:1426–1429.
497 Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine, and J. B. H. Martiny. 2012. Beyond
498 biogeographic patterns: processes shaping the microbial landscape. Nature Reviews
499 Microbiology 10:497–506.

500 Harley, C. D. G., and B. S. T. Helmuth. 2003. Local- and regional-scale effects of wave
501 exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal
502 zonation. Limnology and Oceanography 48:1498–1508.
503 Hug, L. A., B. J. Baker, K. Anantharaman, C. T. Brown, A. J. Probst, C. J. Castelle, C. N.
504 Butterfield, A. W. Hernsdorf, Y. Amano, K. Ise, Y. Suzuki, N. Dudek, D. A. Relman, K.
505 M. Finstad, R. Amundson, B. C. Thomas, and J. F. Banfield. 2016. A new view of the
506 tree of life. Nature Microbiology 1:16048.
507 Kéfi, S., E. L. Berlow, E. A. Wieters, L. N. Joppa, S. A. Wood, U. Brose, and S. A. Navarrete.
508 2015. Network structure beyond food webs: mapping non-trophic and trophic interactions
509 on Chilean rocky shores. Ecology 96:291–303.
510 Kerfahi, D., B. P. Harvey, S. Agostini, K. Kon, R. Huang, J. M. Adams, and J. M. Hall-Spencer.
511 2020. Responses of Intertidal Bacterial Biofilm Communities to Increasing pCO2.
512 Marine Biotechnology 22:727–738.
513 Krause, E., A. Wichels, L. Giménez, M. Lunau, M. B. Schilhabel, and G. Gerdts. 2012. Small
514 Changes in pH Have Direct Effects on Marine Bacterial Community Composition: A
515 Microcosm Approach. PLoS ONE 7:e47035.
516 Lee, M. 2019. Happy Belly Bioinformatics: an open-source resource dedicated to helping
517 biologists utilize bioinformatics. Journal of Open Source Education 2:53.
518 Logares, R., I. M. Deutschmann, P. C. Junger, C. R. Giner, A. K. Krabberød, T. S. B. Schmidt,
519 L. Rubinat-Ripoll, M. Mestre, G. Salazar, C. Ruiz-González, M. Sebastián, C. de Vargas,
520 S. G. Acinas, C. M. Duarte, J. M. Gasol, and R. Massana. 2020. Disentangling the
521 mechanisms shaping the surface ocean microbiota. Microbiome 8:55.

522 Logares, R., E. S. Lindström, S. Langenheder, J. B. Logue, H. Paterson, J. Laybourn-Parry, K.
523 Rengefors, L. Tranvik, and S. Bertilsson. 2013. Biogeography of bacterial communities
524 exposed to progressive long-term environmental change. The ISME Journal 7:937–948.
525 Lubchenco, J. 1978. Plant Species Diversity in a Marine Intertidal Community: Importance of
526 Herbivore Food Preference and Algal Competitive Abilities. The American Naturalist
527 112:23–39.
528 Mandakovic, D., C. Rojas, J. Maldonado, M. Latorre, D. Travisany, E. Delage, A. Bihouée, G.
529 Jean, F. P. Díaz, B. Fernández-Gómez, P. Cabrera, A. Gaete, C. Latorre, R. A. Gutiérrez,
530 A. Maass, V. Cambiazo, S. A. Navarrete, D. Eveillard, and M. González. 2018. Structure
531 and co-occurrence patterns in microbial communities under acute environmental stress
532 reveal ecological factors fostering resilience. Scientific Reports 8:5875.
533 McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R Package for Reproducible Interactive
534 Analysis and Graphics of Microbiome Census Data. PLoS ONE 8:e61217.
535 Menge, B. A., and J. P. Sutherland. 1987. Community Regulation: Variation in Disturbance,
536 Competition, and Predation in Relation to Environmental Stress and Recruitment. The
537 American Naturalist 130:730–757.
538 Mestre, M., and J. Höfer. 2021. The Microbial Conveyor Belt: Connecting the Globe through
539 Dispersion and Dormancy. Trends in Microbiology 29:482–492.
540 Mittelbach, G. G., and B. J. McGill. 2019. Community ecology. Second edition. Oxford
541 University Press, Oxford, United Kingdom ; New York, NY.
542 Newman, M. 2018. Networks. Oxford university press.
543 Newman, M. E. J. 2006. Finding community structure in networks using the eigenvectors of
544 matrices. Physical Review.

545 Oksanen, J., F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, G. Simpson, P.
546 Solymos, M. Stevens, and H. Wagner. 2015. Vegan: community ecology package. R
547 package vegan, vers. 2.2-1. Worl. Agro. Cent 3:7–81.
548 O'Malley, M. A. 2007. The nineteenth century roots of "everything is everywhere." Nature
549 Reviews Microbiology 5:647–651.
550 Paine, R. T. 1966. Food Web Complexity and Species Diversity. The American Naturalist
551 100:65–75.
552 Parada, A. E., D. M. Needham, and J. A. Fuhrman. 2016. Every base matters: assessing small
553 subunit rRNA primers for marine microbiomes with mock communities, time series and
554 global field samples: Primers for marine microbiome studies. Environmental
555 Microbiology 18:1403–1414.
556 Raffaelli, D., and S. J. Hawkins. 2012. Intertidal ecology. Springer Science & Business Media.
557 Roberts, D. W., and M. D. W. Roberts. 2016. Package 'labdsv.' Ordination and Multivariate 775.
558 Röttjers, L., and K. Faust. 2018. From hairballs to hypotheses–biological insights from microbial
559 networks. FEMS Microbiology Reviews 42:761–780.
560 Rousk, J., E. Bååth, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N.
561 Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil.
562 The ISME Journal 4:1340–1351.
563 Sanli, K., J. Bengtsson-Palme, R. H. Nilsson, E. Kristiansson, M. Alm Rosenblad, H. Blanck,
564 and K. M. Eriksson. 2015. Metagenomic sequencing of marine periphyton: taxonomic
565 and functional insights into biofilm communities. Frontiers in Microbiology 6.
566 Santelices, B. 1990. Patterns of organizations of intertidal and shallow subtidal vegetation in
567 wave exposed habitats of central Chile. Hydrobiologia 192:35–57.

568 Schuster, H., M. S. Davies, S. J. Hawkins, P. S. Moschella, R. J. Murphy, R. C. Thompson, and
569 A. J. Underwood. 2019. Biofilms in Intertidal Habitats. Pages 448–473 *in* S. J. Hawkins,
570 K. Bohn, L. B. Firth, and G. A. Williams, editors. Interactions in the Marine Benthos.
571 First edition. Cambridge University Press.
572 Simberloff, D. 1978. Use of rarefaction and related methods in ecology. Page Biological data in
573 water pollution assessment: quantitative and statistical analyses. ASTM International.
574 Stephenson, T. A., and A. Stephenson. 1949. The Universal Features of Zonation Between Tide-
575 Marks on Rocky Coasts. The Journal of Ecology 37:289.
576 Tan, E. L.-Y., M. Mayer-Pinto, E. L. Johnston, and K. A. Dafforn. 2015. Differences in Intertidal
577 Microbial Assemblages on Urban Structures and Natural Rocky Reef. Frontiers in
578 Microbiology 6.
579 Taylor, J. D., R. Ellis, M. Milazzo, J. M. Hall-Spencer, and M. Cunliffe. 2014. Intertidal epilithic
580 bacteria diversity changes along a naturally occurring carbon dioxide and pH gradient.
581 FEMS Microbiology Ecology 89:670–678.
582 Thompson, R. C., T. P. Crowe, and S. J. Hawkins. 2002. Rocky intertidal communities: past
583 environmental changes, present status and predictions for the next 25 years.
584 Environmental Conservation 29:168–191.
585 Thompson, R. C., T. A. Norton, and S. J. Hawkins. 2004. Physical stress and biological control
586 regulate the producer-consumer balance in intertidal biofilms. Ecology 85:1372–1382.
587 Thompson, R., P. Moschella, S. Jenkins, T. Norton, and S. Hawkins. 2005. Differences in
588 photosynthetic marine biofilms between sheltered and moderately exposed rocky shores.
589 Marine Ecology Progress Series 296:53–63.

590 Underwood, A. 1972. Tide-model analysis of the zonation of intertidal prosobranchs. I. Four
591 species of Littorina (L.). Journal of Experimental Marine Biology and Ecology 9:239–
592 255.
593 Underwood, A. J. 1984. The vertical distribution and seasonal abundance of intertidal microalgae
594 on a rocky shore in New South Wales. Journal of Experimental Marine Biology and
595 Ecology 78:199–220.
596 Wasserman, S., and K. Faust. 1994. Social network analysis: Methods and applications.
597 Cambridge university press.
598 Wethey, D. S. 1983. Geographic limits and local zonation: The barnacles Semibalanus (Balanus)
599 and Chthamalus in New England. The Biological Bulletin 165:330–341.
600 Whittaker, R. H. 1959. On the Broad Classification of Organisms. The Quarterly Review of
601 Biology 34:210–226.
602 de Wit, R., and T. Bouvier. 2006. “Everything is everywhere, but, the environment selects”; what
603 did Baas Becking and Beijerinck really say? Environmental Microbiology 8:755–758.
604 Zhang, W., Y. Wang, O. O. Lee, R. Tian, H. Cao, Z. Gao, Y. Li, L. Yu, Y. Xu, and P.-Y. Qian.
605 2013. Adaptation of intertidal biofilm communities is driven by metal ion and oxidative
606 stresses. Scientific Reports 3:3180.
607
608
609
610
611
612

613 **Figure legend**

614 **Fig. 1.** Zonation patterns of the 14 most abundant species (in terms of relativized cover, density,
615 or reads of sessile invertebrates and macroalgal species, mobile invertebrate species, or OTUs,
616 respectively), expressed here as relative to the maximum abundance observed in a given zone for
617 **A)** Macroorganisms and **B)** Microorganisms. The coefficients of variation (CV%) across tidal
618 zones for the same species and OTUs are shown for **C)** macro- and **D)** micro-organisms. The
619 horizontal lines are mean CV for macroorganisms = 123.2% and microorganisms = 84.6%.

620

621 **Fig. 2.** Linear regression between the coefficient of variation across tidal zones and the OTUs
622 richness within Orders for macro- (**A**) and microorganisms (B). Mean (+ SE) richness (**C,D**) and
623 Shannon diversity (**E,F**) of (**C,E**) macroscopic community and (**D,F**) microbial community in
624 the high, middle, and low intertidal zones. Different letters above bars indicate significant
625 differences with a posteriori tests (**C,E, F**: Games-Howell tests, **D**: Tukey test at the experiment
626 wise error rate = 0.05).

627

628 **Fig. 3.** Compositional similarity of the different intertidal rocky shore zones for macroorganisms
629 (N= 88 species) and microorganisms (N= 6,252 OTUs). Non-metric multidimensional scaling
630 (NMDS) ordination plots based on presence/absence data using Jaccard distances (**A**, **C**), and on
631 abundance data using Bray-Curtis distances (**B**, **D**). The symbols represent the different intertidal
632 zones, surrounded by the ellipse of 95% confidence interval. Each observation is a survey unit
633 (50 x 50 cm quadrats for macroscopic organisms, 3 x 8 cm rock surface coupons for microbes).

634

635 **Fig. 4.** Co-occurrence networks of A) macroorganisms and B) microorganisms communities.

636 The node color denotes the intertidal zone specialists at the (●/●) high, (●/●) middle and (●/●)
637 low intertidal zone. The gray nodes (●) are species/OTUs without specialisms. The edge color
638 denotes (----) positive correlation and (---) negative correlation. The magnitude of the correlation
639 is shown by the intensity of the shade, as depicted in the legend between the two networks.
640 Connectivity of subnetworks of C) macroorganisms and D) microorganisms communities. The
641 edge width is proportional to the connectivity within and between each subnetworks of tidal
642 height specialists. The edges are labeled with the connectivity. “None” indicates non-specialists.
643 The edge color is proportional to the average correlation in subnetworks within and between
644 tidal height specialists and non-specialists.
645
646
647
648

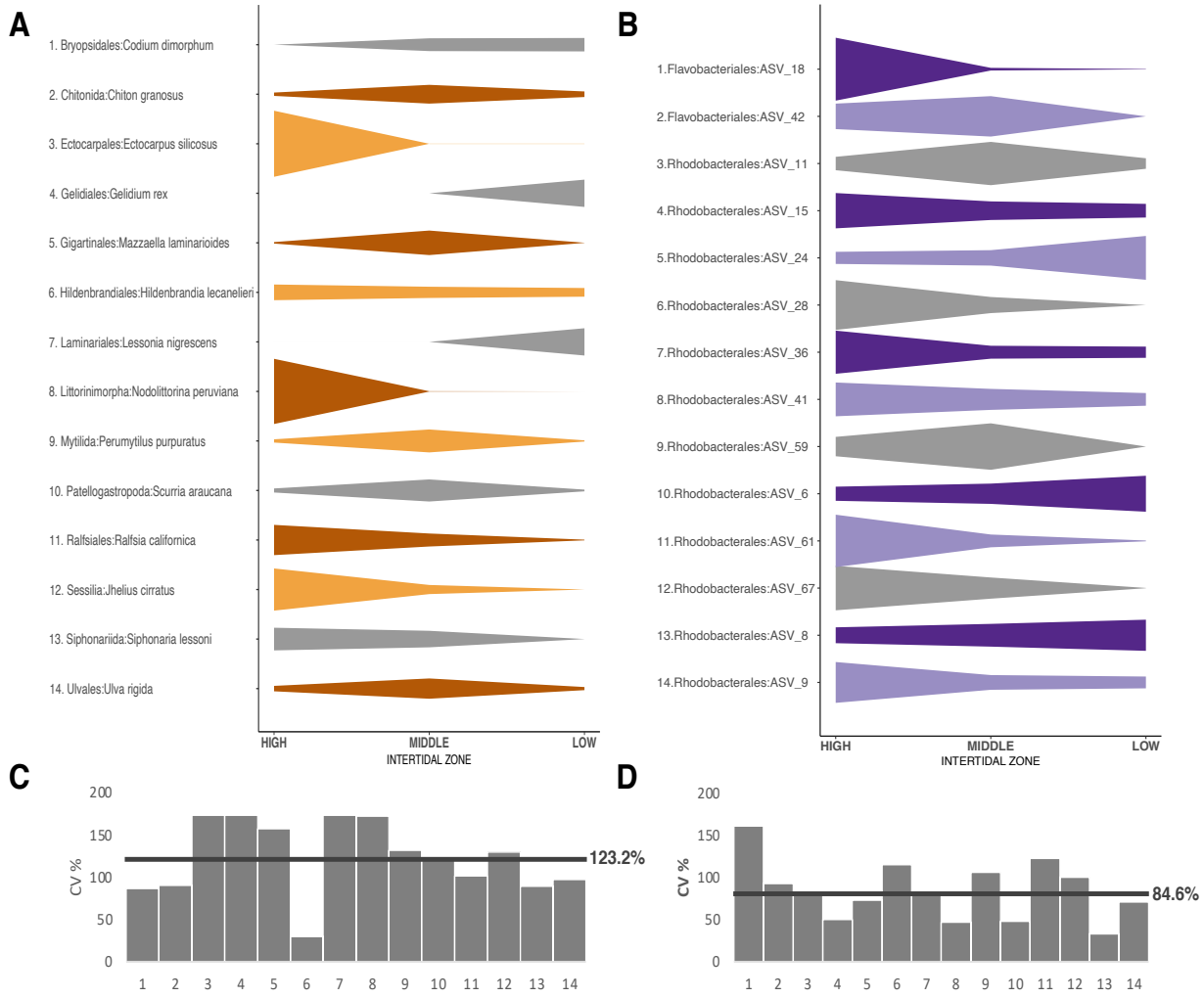
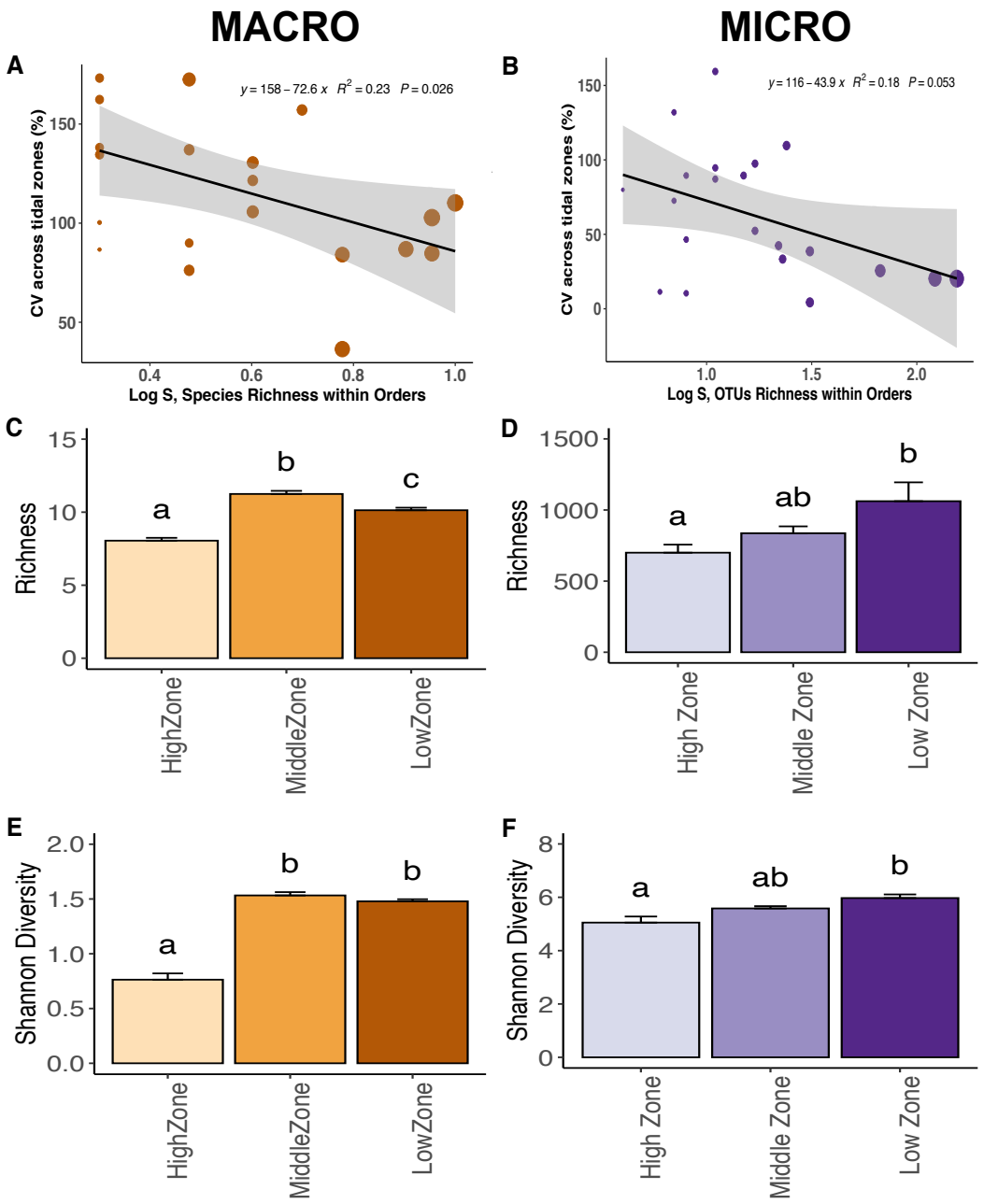
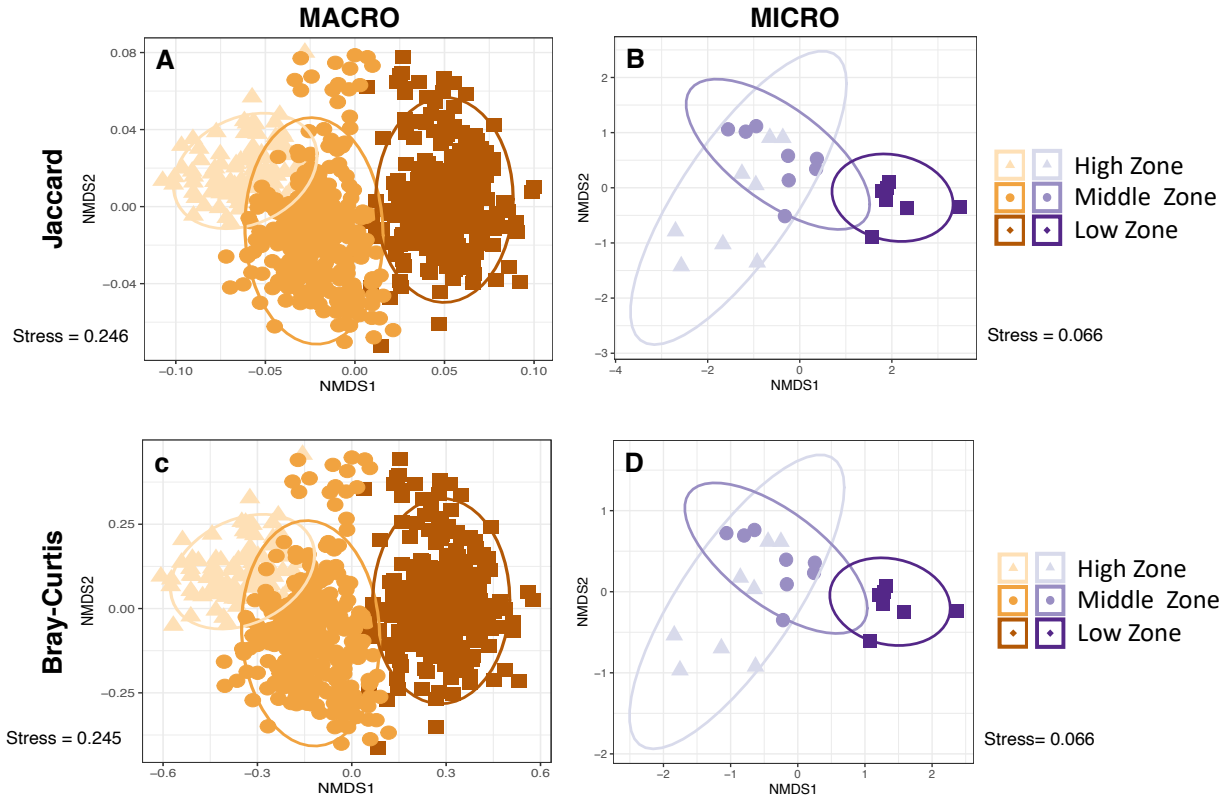


Figure 1.



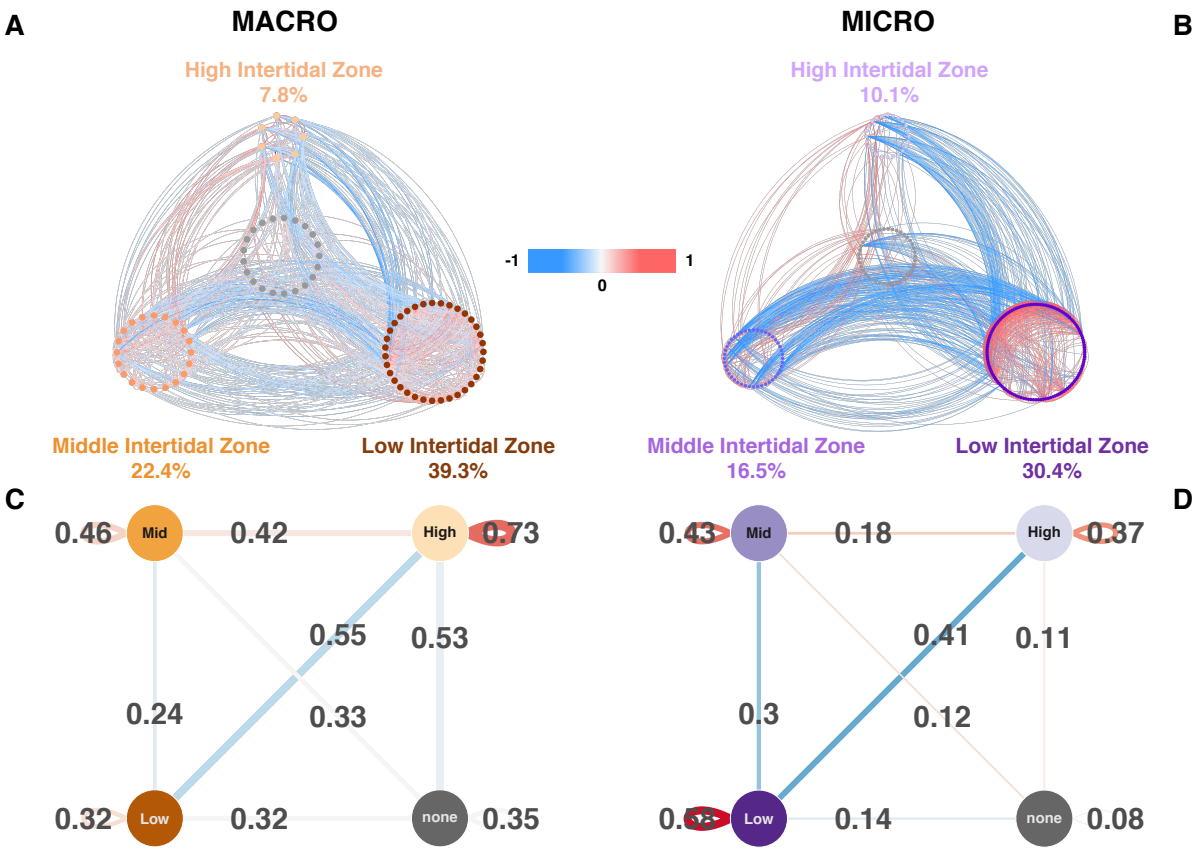
651

652 **Figure 2.**



653

654 **Figure 3.**



655

656 **Figure 4.**