PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Rapacciuolo G, Beman JM, Schiebelhut LM, Dawson MN. 2019 Microbes and macro-invertebrates show parallel β-diversity but contrasting α-diversity patterns in a marine natural experiment. *Proc. R. Soc. B* **286**: 20190999.
http://dx.doi.org/10.1098/rspb.2019.0999

Received: 30 April 2019
Accepted: 11 September 2019

Subject Category:
Ecology

Subject Areas:
ecology, microbiology

Keywords:
biogeography, macroecology, community assembly, microbes, macro-organisms

Author for correspondence:
Giovanni Rapacciuolo
e-mail: giorapac@gmail.com

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.4673129.

THE ROYAL SOCIETY PUBLISHING

Microbes and macro-invertebrates show parallel β-diversity but contrasting α-diversity patterns in a marine natural experiment

Giovanni Rapacciuolo[1,2], J. Michael Beman[2], Lauren M. Schiebelhut[2] and Michael N. Dawson[2]

[1]Institute for Biodiversity Science and Sustainability, California Academy of Sciences, San Francisco, CA, USA
[2]Life and Environmental Sciences, University of California Merced, Merced, CA, USA

GR, 0000-0003-1494-9017; LMS, 0000-0002-5417-5426; MND, 0000-0001-7927-8395

Documenting ecological patterns across spatially, temporally and taxonomically diverse ecological communities is necessary for a general understanding of the processes shaping biodiversity. A major gap in our understanding remains the comparison of diversity patterns across a broad spectrum of evolutionarily and functionally diverse organisms, particularly in the marine realm. Here, we aim to narrow this gap by comparing the diversity patterns of free-living microbes and macro-invertebrates across a natural experiment provided by the marine lakes of Palau: geographically discrete and environmentally heterogeneous bodies of seawater with comparable geological and climatic history, and a similar regional species pool. We find contrasting patterns of α-diversity but remarkably similar patterns of β-diversity between microbial and macro-invertebrate communities among lakes. Pairwise dissimilarities in community composition among lakes are positively correlated between microbes and macro-invertebrates, and influenced to a similar degree by marked gradients in oxygen concentration and salinity. Our findings indicate that a shared spatio-temporal and environmental context may result in parallel patterns of β-diversity in microbes and macro-invertebrates, in spite of key trait differences between these organisms. This raises the possibility that parallel processes also influence transitions among regional biota across the tree of life, at least in the marine realm.

1. Introduction

A key step towards understanding the general processes that underlie diversity and abundance in ecological communities is to search for patterns that are repeated across spatially, temporally and taxonomically diverse communities [1]. Recent syntheses of previously identified repeated patterns propose that the diversity and structure of most ecological communities may be driven by the same suite of processes [2,3]: deterministic differences in fitness between individuals of different species (selection), random changes in relative abundances (drift), the movement of organisms (dispersal) and the origin of new genetic variants (speciation/mutation). However, these syntheses are based predominantly on studies of 'macro'-organisms (i.e. multicellular plants and animals), which together make up a relatively small proportion of the tree of life. Comparing ecological patterns across a broad spectrum of life forms therefore remains a major gap in our understanding of the general processes shaping ecological communities and the functioning of ecosystems [4,5]. Here, we aim to narrow this gap by comparing the diversity patterns of evolutionarily and functionally disparate microbes and macro-invertebrates within a marine natural experimental system. Collectively, these groups provide a broad picture of diversity within marine ecosystems, with thousands of microbial groups

found throughout the ocean [6,7] and macro-invertebrates being among the most speciose groups of macro-organisms in marine communities [8].

Quantifying relationships between microbial and macro-organismal diversity is central to a general understanding of ecosystems [9–11], because associations between microbes and macro-organisms underlie multiple ecosystem processes and properties—including productivity [12], nutrient cycling [13] and resilience to environmental change [14]. Yet, the degree to which the processes structuring macro-organismal communities also structure microbial communities, and whether parallel processes result in parallel biodiversity patterns remains unclear [9,15,16]. Applying approaches developed for macro-organismal community ecology to microbial communities has revealed microbial patterns coherent with macro-organismal patterns and potentially driven by the same suite of general processes [17–20]. Nevertheless, given fundamental trait differences between these taxa, it is plausible that the mechanisms through which selection, drift, dispersal and speciation structure microbial and macro-organismal communities lead to contrasting patterns in some aspects of their diversity [15,16]. First, the greater metabolic diversity of microbial communities multiplies the potential abiotic and biotic pathways through which selection may act [18]. For instance, the concentration of particular compounds (e.g. methane, sulfate) may strongly influence the microbial groups that directly use them as substrates, while having little or no direct influence on macro-organisms. Second, the generally higher capacity for passive dispersal (via water or air) may lead to microbial communities that are more spatially and temporally homogeneous [21–23], and more strongly structured by environmental selection [24,25] than communities of macro-organisms. Third, the high degree of rarity and functional redundancy within microbial communities, coupled with the higher rates of passive dispersal, may make microbial communities particularly susceptible to ecological drift [19]. Finally, owing to potentially rapid growth and shorter generation times, speciation could influence microbial community dynamics over a shorter temporal scale than for macro-invertebrates [15,16].

Reliable tests of whether microbial and macro-organismal communities display distinct or parallel diversity patterns require comparisons across shared spatio-temporal domains and environmental gradients influencing diversity in both sets of organisms [9]. Previous studies comparing realized microbial and macro-organismal communities have faced a number of challenges. First, they had to grapple with the blurred boundaries and high connectedness of their study ecosystems (e.g. mountainsides: [26–28]; streams: [21,22,29,30]; forests: [31–33]), which make it difficult to select a suitable spatial scale for comparing microbes and macro-organisms. Second, they had to address whether the environmental gradients under study were relevant for both microbes and macro-organisms, and whether those effects were observed at the spatio-temporal scale most appropriate for each taxon (e.g. [5,26,31]). Third, they had to disentangle the relative influence of direct ecological interactions from the influence of shared environmental constraints between microbial and macro-organismal communities [34–38]. While *in situ* manipulative experiments can partially resolve these challenges in terrestrial ecosystems, experiments involving a high diversity of taxa are much more challenging in open and dynamic marine systems [39]. As a result, to our knowledge, no previous study has directly compared the diversity of communities of free-living microbes and macro-organisms in marine ecosystems.

The marine lakes of Palau provide an unprecedented opportunity to compare the diversity patterns of microbial and macro-invertebrate communities in the marine realm (electronic supplementary material, figure S1). Marine lakes are inland basins flooded by rising seas at the end of the Last Glacial Maximum. They represent natural ecological–evolutionary experiments that are geographically discrete and exist at intermediate spatial (hundreds of metres) and temporal (thousands of years) scales where island-like patterns are apparent [40,41]. Lakes can be broadly categorized as either mixed—vertically unstratified bodies of oxygenated and high-salinity water; or stratified—bodies of water where a shallower oxygenated brackish layer is separated from a deeper anoxic layer by a chemocline [42,43]. However, both within and among these broad categories, differences in shape, size, depth and connectivity to the surrounding sea result in finer differences in the physical composition of lakes—including marked lake-level gradients in dissolved oxygen concentration, salinity, light availability and productivity among lakes. In this way, marine lakes are microcosms for examining effects of ecologically meaningful environmental variation found across marine ecosystems worldwide, such as changing dissolved oxygen concentration, salinity and solar radiation. Despite their physical differences, all marine lakes share a similar regional history of geological and climatic change and probably have been exposed to similar rates of propagule pressure from the surrounding lagoon over the last 6000–12 000 years. As a result, marine lakes provide an ideal opportunity to ask if microbial and macro-invertebrate communities display parallel local and regional diversity patterns (figure 1) within a simplified system with shared bounded spatial and temporal scales—a fundamental attribute of study design in comparative biogeography (e.g. [44]).

Here, we compare the diversity patterns of microbial and macro-invertebrate communities across 12 of Palau's marine lakes to infer potential responses and feedbacks between the abiotic and biotic components of these ecosystems. For simplicity, we begin by testing the assumptions that organisms across the tree of life are subject to similar regional and local constraints on diversity ([4,15]; figure 1*d*). As a result, we hypothesize (i) positive correlations in the relative richness and evenness (α-diversity) of microbial operational taxonomic units (OTUs) and macro-invertebrate species across lakes, and (ii) a positive correlation in the compositional dissimilarity of microbial and macro-invertebrate communities among lakes (β-diversity). However, given the expected lower degree of dispersal limitation in microbes than macro-invertebrates [16,45], we also hypothesize that (iii) microbes display a lower overall β-diversity, and (iv) microbes display a stronger association with environmental factors than do macro-invertebrates.

2. Methods

(a) Study design

We sampled 12 marine lakes in the Republic of Palau (see the electronic supplementary material, figure S1) to obtain comprehensive lake-level estimates of (i) the relative abundance and taxonomic composition of free-living microbes, (ii) the relative abundance and taxonomic composition of benthic macro-invertebrates, and (iii) environmental variation. We used these data to estimate and

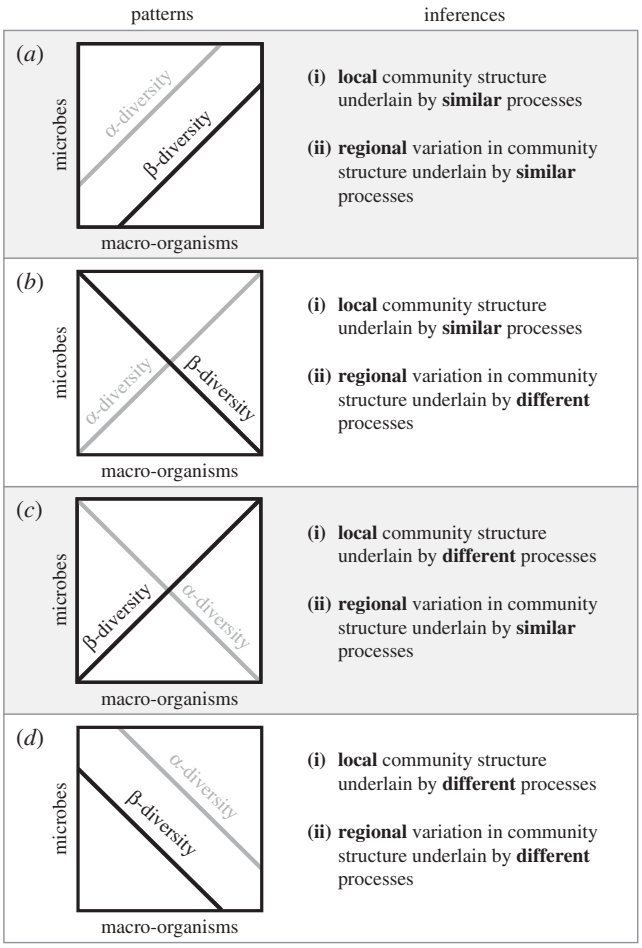

Figure 1. Potential relationships in α-diversity and β-diversity between microbes and macro-organisms and corresponding potential inferences on underlying processes. Curves are for illustrative purposes and slopes may vary substantially from the strong positive or negative linear relationships shown. Inferences highlighted provide one potential set of inferences that could be derived from each set of patterns. However, conclusive evidence will require more detailed tests than correlative tests. For instance, it is also possible that parallel patterns may be driven by different processes, while non-parallel patterns may be driven by similar processes [9].

compare patterns and environmental correlates of α- and β-diversity in microbes and macro-invertebrates (figure 1).

Our objective was, to our knowledge, the first to generate reliable and consistent estimates of relative differences in diversity across lakes using the most appropriate methodology for either microbes or macro-invertebrates; we subsequently compared differences among lakes between microbes and macro-invertebrates. While differences in microbial versus macro-invertebrate sampling protocols could potentially affect diversity estimates (particularly absolute estimates of α-diversity within each lake), we took several steps to mitigate disparities as best possible; these included using DNA sequence-based identification of OTUs for both microbes and macro-invertebrates, and using α- and β-diversity measures insensitive to under-sampling of ecological communities. We reflect on the potential influence of these methodological choices in the Discussion.

(b) Microbial data collection and analysis

Microbial diversity was assessed by sequencing and analysis of 16S rRNA genes in DNA samples extracted from marine lake water samples. To examine microbial diversity at the level of each lake, 250 ml water samples were collected at seven to eight regularly spaced depth intervals from the centre of each lake; the vertical spacing of samples scaled with total lake depth (electronic supplementary material, table S1). Following DNA extraction (see the electronic supplementary material, Methods), 16S rRNA genes were amplified using universal archaeal/bacterial primers [46] and sequenced on an Illumina MiSeq according to Earth Microbiome Project protocols (electronic supplementary material, Methods). Sequence processing and analysis was conducted in mothur (http://www.mothur.org/), following the approach of [47], modified from the mothur MiSeq SOP [48]. Following quality control, 85 000 16S rRNA sequences were randomly and equally subsampled from each lake to maintain even sampling across lakes (electronic supplementary material, Methods). Analysis of lake-level diversity patterns using differently sized sequence libraries (including resampling using 10 000, 20 000, 40 000 and 160 000 sequences per lake) all produced highly consistent results (electronic supplementary material, Methods; tables S2 and S3). All sequences were clustered into OTUs based on the commonly used 97% identity threshold using the furthest-neighbour algorithm in mothur, and we generated an OTU-sample matrix for subsequent ecological analyses as described below. Analysis of microbial communities via 16S rRNA amplicons is potentially subject to biases introduced through primer design and polymerase chain reaction amplification that favour particular segments of the microbial community, and so, we analysed these data using both abundance-based and presence–absence-based metrics described below. Sequence data are available in the Sequence Read Archive under accession number PRJNA555354 at https://www.ncbi.nlm.nih.gov/sra/PRJNA555354.

(c) Macro-invertebrate data collection and analysis

Macro-invertebrate community composition was estimated using point intercept transects placed randomly at 13–14 sites in each lake. At each site, divers using SCUBA sampled three parallel transect lines from a depth just above the chemocline of stratified lakes or the basin bottom of mixed lakes. Along each transect, four to eight equally spaced depths were selected, depending on lake size, at which four points were sampled at 15 cm intervals orthogonal to the transect. The total number of points surveyed in each lake was between 504 (lake code: SLM) and 1344 (lake codes: CLM, NLK). At each point, the first macro-invertebrate encountered was photographed, attributed a field identification and its tissue biopsied. If any additional organisms were observed directly under the original sample, they were also sampled and recorded. After all depths on a transect were surveyed, back at the surface, samples were transferred to 95% ethanol, then returned to the field station and stored at −20°C within 6 h.

We chose a subset of specimens for DNA barcoding to corroborate field identifications. DNA was purified and amplified using several primer sets and thermocycle conditions (electronic supplementary material, table S4) targeting the cytochrome *c* oxidase subunit I (COI) barcode locus. Sequences were visually checked and manually corrected for errors and aligned by major taxonomic group—ascidians, bivalves, bryozoans, cnidarians, crustaceans, echinoderms, gastropods, polychaetes and sponges. Open reading frames were confirmed and pairwise sequence distance was calculated (see the electronic supplementary material, Methods for additional details). OTUs, or clusters of sequences, similar at 97% were identified for each taxonomic group, except for sponges, which were clustered at 99% sequence similarity, given their slow sequence evolution. We estimated the taxonomic composition and relative abundance of macro-invertebrate species in each lake in the final dataset by combining field identifications with DNA-sequencing results (see the electronic supplementary material, Methods for additional details). Data and associated metadata on the abundance of all macro-invertebrates, COI barcoding sequences and alignments are available through the National Science Foundation's Biological and

Chemical Oceanography Data Management Office at https://www.bco-dmo.org/dataset/768138.

(d) α- and β-diversity measures

We summed the number of sequences attributed to each microbial OTU and the number of individuals attributed to each macro-invertebrate species across all samples in each lake to obtain lake-level estimates of the relative abundance of all taxonomic units. Additionally, we also derived presence–absence datasets for both microbes and macro-invertebrates across the 12 lakes by converting abundance data to binary presence–absence.

We used the relative abundances of OTUs/species to estimate three measures of α-diversity for microbes and macro-invertebrates in each lake: richness, evenness and dominance. We estimated richness using the observed number of OTUs/species, the abundance-based coverage estimator (ACE; [49]) and Chao1 [50]. We estimated evenness using the Simpson's equitability index [51] and Pielou's evenness metric [52]. We estimated dominance using the Berger–Parker index [53]. We also calculated the Shannon diversity, the exponential of Shannon entropy [54], as a measure of the effective number of species in each lake that accounted for both richness and evenness. We assessed the correlation between microbial and macro-invertebrate α-diversity measures using Spearman's rank correlation tests. All α-diversity analyses were run in R v. 3.5.1 [55] using the package 'SpadeR' [56]. Owing to the relatively low number of lakes in our analyses, we quantified the uncertainty around observed correlations in α-diversity using 95% confidence intervals estimated from observed data for ACE and Shannon diversity. Specifically, we sampled 9999 values of ACE and Shannon diversity for each taxon in each lake from a uniform distribution bounded by the lower and upper limits of the estimated 95% confidence intervals; we used these values to generate 9999 potential correlations.

We examined patterns of β-diversity by calculating the dissimilarity in the composition of microbial and macro-invertebrate communities between all pairs of lakes using the complement of the Morisita–Horn overlap index [57]. We calculated the complement of the Morisita–Horn index using either the relative abundance data or the binary presence–absence data. We assessed the correlation between microbial and macro-invertebrate pairwise dissimilarity matrices using the Mantel tests (Pearson's correlation); this test assesses significance by comparing the observed correlation to the distribution of correlations obtained via 9999 random permutations of the observed matrices. To summarize β-diversity for each lake from all pairwise dissimilarities, we calculated the median (± variation) dissimilarity value for each lake with respect to all others (see the electronic supplementary material, Methods for additional details). All β-diversity analyses were run in R v. 3.5.1 [55] using the package 'vegan' [58].

(e) Environmental variables

The 12 marine lakes were profiled vertically at 1 m intervals for dissolved oxygen concentration, temperature, pH, chlorophyll fluorescence, conductivity/salinity and photosynthetically active radiation once annually between June and October 2011–2015. We summarized the environment of each lake by calculating the median and standard deviation of each of these six environmental factors and removed highly inter-correlated variables (electronic supplementary material, figure S2). Additionally, we also estimated the size (surface area, m^2) and isolation (minimum distance from a lake's edge to the surrounding lagoon, m) of each lake using satellite data in a geographical information system (see the electronic supplementary material, figure S1). This resulted in a final dataset comprising eight lake-level environmental variables: oxygen median, oxygen variation, conductivity median, temperature median, productivity median, radiation variation, lake size and lake isolation.

(f) Environmental correlates of α- and β-diversity

We examined the effects of each environmental predictor on α-diversity measures using univariate linear models. Additionally, we examined the effects of environmental predictors on β-diversity using distance-based redundancy analysis (RDA), as implemented in the 'capscale' function in the R package 'vegan' [58]. To avoid overfitting given our small number of data points (12 lakes) and to generate RDA models comparable between microbes and macro-invertebrates, we identified a minimum adequate model comprising the four environmental variables best explaining the ordinations of microbes and macro-invertebrates (see the electronic supplementary material, Methods for additional details).

We assessed the similarity between microbial and macro-invertebrate RDA models using Procrustes tests, which test the non-randomness between two ordination configurations by comparing observed ordinations to ordinations obtained from 9999 random permutations of the observed dissimilarity matrices; significant results (i.e. $p < 0.05$) indicate that two configurations are non-random with respect to each other, and significantly correlated as indicated by a Pearson's correlation coefficient r.

3. Results

(a) Contrasting patterns of α-diversity in microbes and macro-invertebrates

Contrary to our hypothesis (i), microbial and macro-invertebrate communities did not display positively correlated patterns of α-diversity across lakes, irrespective of the measure used (figure 2; electronic supplementary material, table S5). Instead, rank correlation coefficients between the richness, evenness and dominance of microbial and macro-invertebrate communities were consistently negative (ACE richness: $\rho = -0.028$, $p > 0.05$; Shannon diversity: $\rho = -0.266$, $p > 0.05$; Simpson's evenness: $\rho = -0.336$, $p > 0.05$; Berger–Parker dominance index: $\rho = -0.287$, $p > 0.05$; see the electronic supplementary material, table S5 and figure S3 for more measures) and statistically non-significant at $\alpha = 0.05$. Permutation tests based on 9999 values sampled from the 95% confidence intervals estimated from observed data indicated that correlations in ACE and Shannon diversity between microbes and macro-invertebrates were never statistically significant across estimated uncertainty bounds (figure 2 insets).

Microbes and macro-invertebrates displayed different correlations between α-diversity and environmental variables (electronic supplementary material, figure S4 and table S6). Compared with stratified lakes, mixed lakes had more even macro-invertebrate communities ($F_{1,10} = 5.76$, $p < 0.05$) but less even microbial communities ($F_{1,10} = 10.72$, $p < 0.01$); lakes with more variable dissolved oxygen concentrations had less even macro-invertebrate communities ($r = -0.64$, $p < 0.05$) but more even microbial communities ($r = 0.63$, $p < 0.05$); and lakes with higher median dissolved oxygen concentrations had richer macro-invertebrate communities ($r = 0.58$, $p < 0.05$) and less even microbial communities ($r = -0.61$, $p < 0.05$). Finally, lakes more distant from the surrounding ocean had significantly less rich ($r = -0.78$, $p < 0.01$) and less even ($r = -0.58$, $p < 0.05$) macro-invertebrate communities.

(b) Parallel patterns of β-diversity in microbes and macro-invertebrates

In agreement with hypothesis (ii), we found significant positive correlations in the β-diversity patterns of microbes and

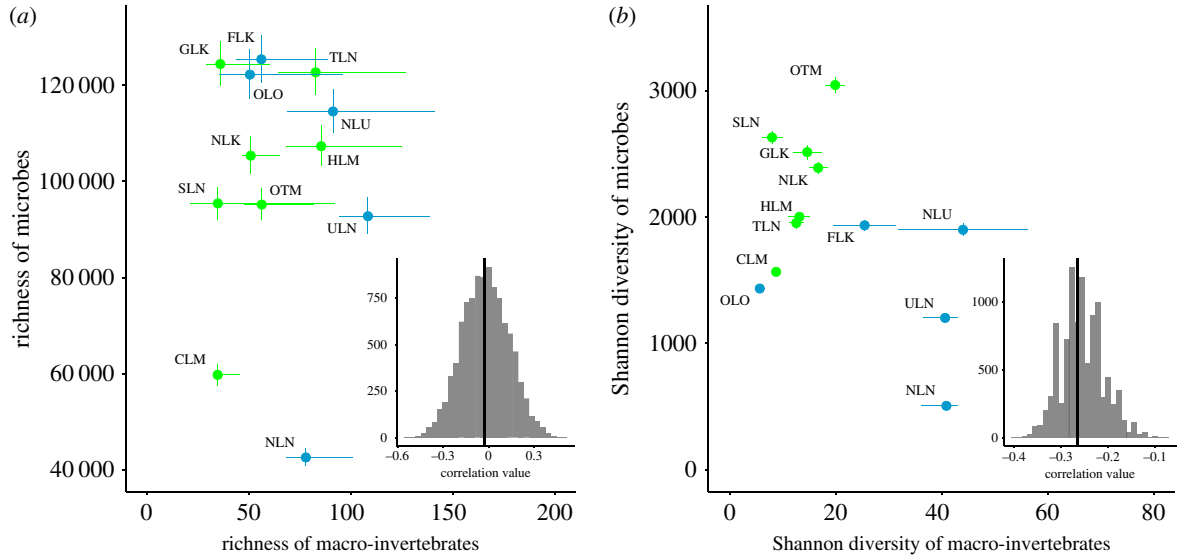

Figure 2. Correlation between the α-diversity of microbes and macro-invertebrates across 12 marine lakes. (*a*) Correlation in richness, measured using the ACE; the observed Spearman's rank correlation coefficient ρ is −0.028 ($p > 0.05$). (*b*) Correlation in Shannon diversity; the observed Spearman's rank correlation coefficient ρ is −0.266 ($p > 0.05$). Stratified and mixed lakes are in green and blue, respectively. Error bars indicate 95% confidence intervals around estimates. The insets in (*a*,*b*) show the frequency of correlation values obtained from 9999 combinations of diversity values sampled from estimated 95% confidence intervals. See the electronic supplementary material, figure S3 for correlations in additional measures of α-diversity. (Online version in colour.)

macro-invertebrates. Dissimilarity values among lakes were high on average for both microbes and macro-invertebrates and were significantly positively correlated between the two taxonomic groups (figure 3). Mantel tests indicated that, despite being based on a relatively small sample size of 12 lakes, correlations in β-diversity were more extreme than 99.8–99.99% of 9999 matrices generated randomly from our data. The pairwise dissimilarity matrices of microbes and macro-invertebrates were more strongly positively correlated when considering exclusively differences in the presence–absence of OTUs/species among lakes (Mantel test: $r = 0.578$, $p < 0.001$; figure 3*a*) than when also incorporating relative abundances ($r = 0.293$, $p = 0.020$; electronic supplementary material, figure S5A).

We found a significant positive correlation between the environmentally constrained RDA of the dissimilarity matrices of microbes and macro-invertebrates, as assessed by Procrustes tests of the symmetry between the ordination configurations (figure 4). Once again, the strength of this positive correlation was higher for presence–absence (correlation in a symmetric Procrustes rotation: $r = 0.881$, $p < 0.001$; figure 4*c*) than abundance-based dissimilarity values ($r = 0.558$, $p < 0.05$; figure 4*f*). These ordinations confirmed that the diversity of both microbes and macro-invertebrates are significantly influenced by lake type—with higher compositional differences between, rather than within, lake types—and median dissolved oxygen concentration. The gradient in median salinity among lakes was also a significant predictor in all ordinations. Environmental variables explained 41–67% and 47–59% of dissimilarity among microbial and macro-invertebrate communities, respectively (figure 4), providing no evidence towards our hypothesis (iv) of a higher influence of environmental variation on microbial than macro-invertebrate patterns.

Despite these parallel patterns, we also found noteworthy differences in β-diversity between microbes and macro-invertebrates. First, in line with hypothesis (iii), abundance-based dissimilarity values were on average higher for macro-invertebrates than microbes (mean dissimilarity: microbes = 0.69; macro-invertebrates = 0.88; $t = 5.01$, $p < 0.001$; electronic supplementary material, figure S5). However, the opposite was true for dissimilarity values based on presence–absence (mean dissimilarity: microbes = 0.91; macro-invertebrates = 0.84; $t = 6.10$, $p < 0.001$). Second, a number of the environmental factors best explaining β-diversity patterns differed between microbes versus macro-invertebrates. Microbial β-diversity patterns were significantly correlated with lake surface area and median temperature. By contrast, macro-invertebrate β-diversity patterns were significantly correlated with variation in dissolved oxygen concentration, median temperature and/or variation in solar radiation (figure 4).

4. Discussion

Our description of marine communities along environmental and connectivity gradients in marine lake ecosystems, Palau, shows that microbes and macro-invertebrates display parallel β-diversity patterns despite seemingly non-parallel constraints on α-diversity (approximating figure 1*c*). Our study provides a rare direct comparison of the diversity patterns of free-living microbial and macro-organismal communities, narrowing a key gap in our understanding of biodiversity. Our findings raise the possibility that, more broadly, transition zones between regional biotas may be shaped by large-scale processes acting in parallel on diverse taxa across the tree of life, at least in the marine realm.

A strong, direct, but largely opposite response to oxygen gradients is likely to partly underlie the combination of parallel β-diversity and non-parallel α-diversity patterns we observe in microbes and macro-invertebrates across marine lakes. Marked gradients in dissolved oxygen concentration are strong selective forces for both macro-organisms [59] and microbial communities [60,61]. Indeed, our measure of the degree of oxygenation in each lake (i.e. median dissolved oxygen concentration) was the most important predictor of community dissimilarity in both microbes and macro-invertebrates (figure 4). However, the direction of this

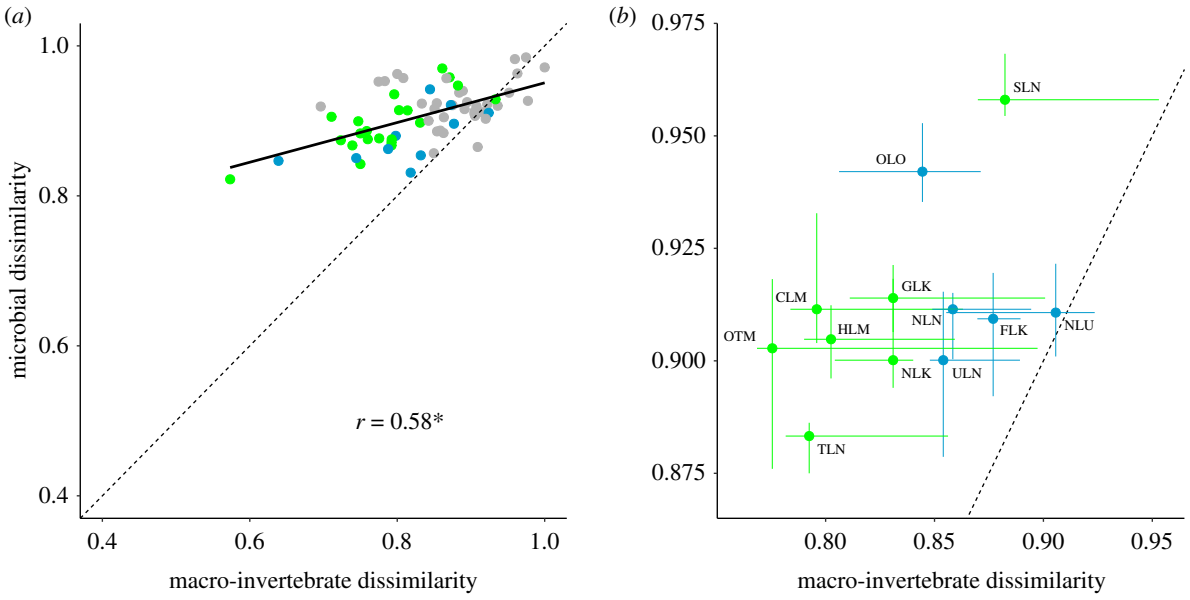

Figure 3. Correlation between the β-diversity of microbes and macro-invertebrates across 12 marine lakes. (*a*) Dissimilarity values across all pairs of lakes and (*b*) β-diversity of the 12 marine lakes based on binary presence–absence data. Dissimilarity values were quantified as 1 – Morisita–Horn overlap index. In (*a*), stratified–stratified lake comparisons are in green, mixed–mixed lake comparisons are in blue and stratified–mixed lake comparisons are in grey; also shown are the line of best fit (black line) and corresponding linear correlation coefficient *r*. In (*b*), β-diversity was quantified as the median (±50%) dissimilarity value of each lake with respect to all other lakes; stratified and mixed lakes are in green and blue, respectively. Dotted grey line shows a 1 : 1 relationship. Abundance-based β-diversity results are in the electronic supplementary material, figure S5. (Online version in colour.)

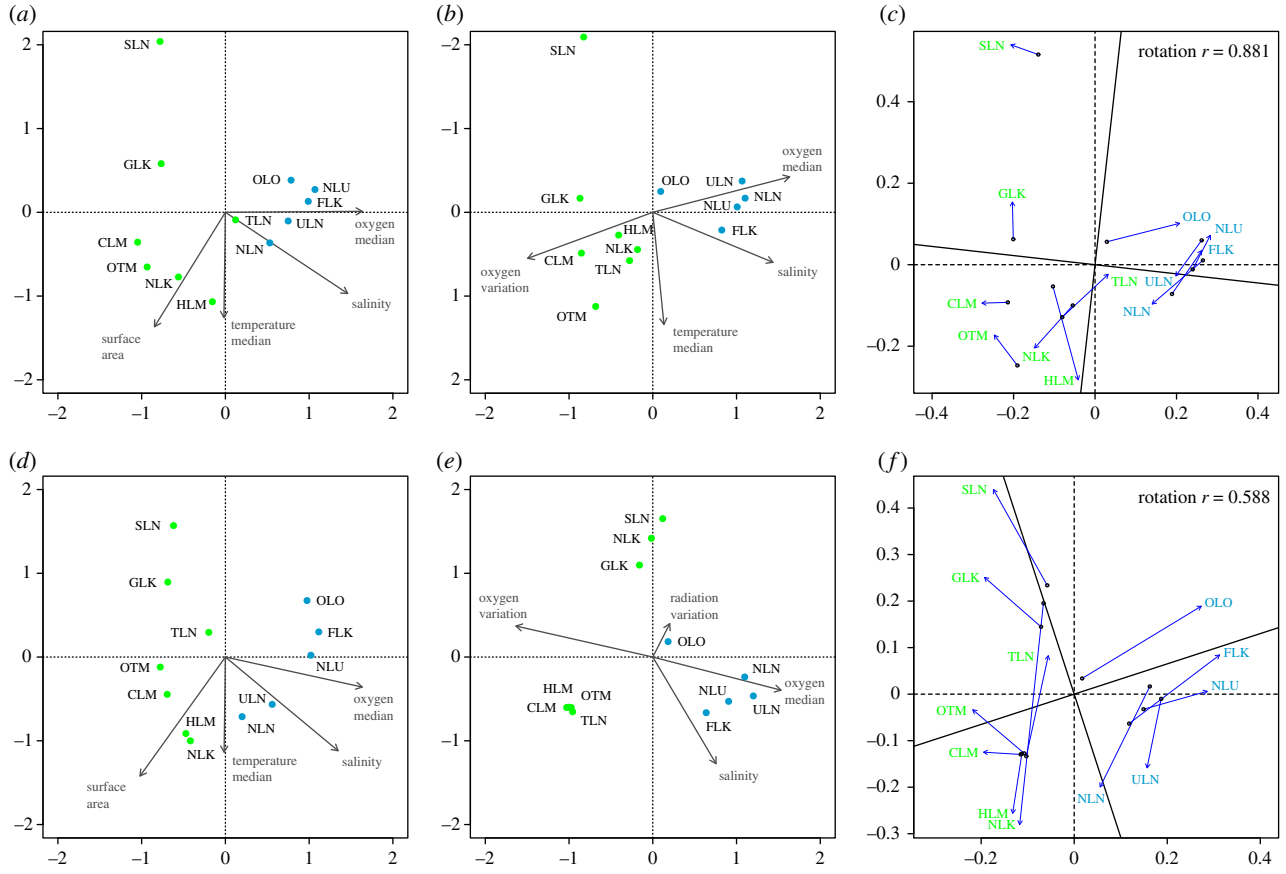

Figure 4. Environmentally constrained ordination (redundancy analysis) of the pairwise dissimilarity among microbial and macro-invertebrate communities. (*a*) Microbial ordination of dissimilarity values based on presence–absence. (*b*) Macro-invertebrate ordination of dissimilarity values based on presence–absence. (*c*) Procrustes test of the non-randomness between the configurations in (*a,b*). (*d*) Microbial ordination of dissimilarity values based on relative abundance data. (*e*) Macro-invertebrate ordination of dissimilarity values based on abundance data. (*f*) Procrustes test of the non-randomness between the configurations in (*d,e*). Dissimilarity values were quantified as 1 – Morisita–Horn overlap index. Axes in all panels are the first two axes of variation identified in redundancy analysis, respectively, as *x* and *y*. The variables shown in (*a,b,d,e*) are the four variables best explaining each ordination; we narrowed down the set of environmental variables to four from the full set of eight using backward stepwise model simplification. Procrustes tests rotate the two configurations to maximum similarity and estimate their correlation. The correlation between microbial and macro-invertebrate principal coordinates analysis configurations in a Procrustes test was 0.881 ($p < 0.001$) for presence–absence-based (*c*) and 0.558 ($p < 0.05$) for abundance-based (*f*) dissimilarity. (Online version in colour.)

response was opposite in microbes and macro-invertebrates (electronic supplementary material, figure S4). On the one hand, macro-invertebrates are unable to survive at low dissolved oxygen concentrations, with profound effects on ecosystem energetics and function [59]. In our marine lake surveys, macro-invertebrates in effect were not found below oxygen concentrations of 0.7 µg l^{-1} (electronic supplementary material, figure S6). On the other hand, some microbial taxa thrive in anoxic conditions, with gradients in dissolved oxygen both driving and being reinforced by changes in aerobic versus anaerobic microbial activity [59,61]. In marine lakes, the diversity of a subset of microbial groups—including sulfate-reducing bacteria and SAR324, for example—increases markedly in anoxic conditions [62].

Nevertheless, non-parallel α- and parallel β-diversity patterns between microbes and macro-invertebrates persisted even when analysing only samples collected in the oxic portion of each lake's water column (i.e. above 0.7 µg l^{-1} dissolved oxygen; electronic supplementary material, figures S7 and S8), indicating that these findings are not driven solely by steep declines in oxygen concentrations. Secondary environmental drivers may also underlie α- and β-diversity. For instance, the median salinity of each lake correlated with aspects of α- (electronic supplementary material, table S6) and β-diversity (figure 4) in both microbes and macro-invertebrates. While this could reflect direct physiological responses to salinity [63], median salinity may also reflect the degree of connectivity of lakes to the surrounding ocean and the brackish nature of stratified lakes.

Despite their extraordinariness as natural experimental systems, marine lakes encapsulate over a small geographical extent much of the ecologically meaningful environmental variation found across marine ecosystems worldwide, including marked gradients in dissolved oxygen concentration, salinity and solar radiation (though not temperature). Moreover, marine lakes also reflect diversity–environment relationships occurring at large spatial scales across the ocean, including the central role of transitions between mixed and stratified waters, as well as oxygen and salinity gradients, on α- and β-diversity in microbes [7,64] and macro-invertebrates [65,66]. Therefore, our study highlights the potential for marked marine environmental gradients across the globe to drive parallel transitions in regional biodiversity across highly taxonomically, evolutionarily and functionally disparate organisms.

Environmental constraints explained a combined 41–67% and 47–59% of dissimilarity among microbial and macro-invertebrate communities, respectively (figure 4). Additional drivers of community dissimilarity which could be responsible for parallel β-diversity patterns between microbes and macro-invertebrates include a parallel history of speciation and/or colonization [67], a comparable influence of ecological drift and/or direct interactions among subsets of the two taxa [5,37]. We assumed that microbial and macro-invertebrate taxa are unlikely to have experienced a parallel history of taxonomic diversification across marine lakes. This is because the inception of all lakes, and subsequent inoculation with both microbial and macro-invertebrate life, occurred relatively recently (putatively approx. 6000–12 000 years ago) and, while this timeline will have allowed for the creation of new genetic variants in both microbes and macro-invertebrates, it is unlikely to have allowed for speciation in macro-invertebrate taxa. Moreover, based on the putative ages of five lakes identified from lake sediments, we find no statistically significant relationship between lake age and either lake environment or α-diversity patterns (electronic supplementary material, table S7), indicating that the timing of lake inception is unlikely to have driven contemporary diversity patterns. However, dispersal limitation and ecological drift, in addition to selection, could have influenced diversity patterns in both macro-invertebrates and microbes. We found that lake isolation was significantly negatively correlated with macro-invertebrate α-diversity, potentially reflecting the signature of dispersal limitation. Furthermore, microbial community dissimilarity was higher between mixed and stratified lakes than within either lake type, suggesting that at least some microbial groups (e.g. strictly anaerobic microbes) may not easily translocate between lakes types. Microbial dissimilarity was also influenced by lake area (figure 4), with smaller lakes being on average more dissimilar than larger lakes, potentially indicating a higher influence of ecological drift in smaller lakes. Finally, rank correlations in α-diversity patterns between the most speciose individual subgroups of microbes and macro-invertebrates were occasionally positive (e.g. the positive correlation between Deltaproteobacteria and Porifera richness; electronic supplementary material, figure S9). While this may indicate direct or indirect associations between these individual subgroups, a reliable test of the influence of biotic interactions on these communities will require more in-depth sampling specifically targeting hypothesized interactions across shared substrates.

Given the diversity of life forms and life histories—and consequently of sampling protocols used—in comparisons of microbial versus macro-invertebrate communities, it is worth asking whether methodological choices could systematically bias findings. Recent studies have contended that any single method is likely to under-sample some taxa [68], so we believe the question is phrased most productively as: do chosen sampling methods misrepresent diversity in the target taxa and places studied, and what is the uncertainty around correlations in diversity estimates? We suggest four main steps can minimize the impact of methodological choices on comparative analyses generally. First, applying DNA-based protocols in independent but parallel surveys of microbial and macro-organismal diversity can generate robust estimates for each taxon and provide a 'common currency' for comparison (including with other studies, e.g. eDNA-based surveys [69]). Second, focusing on reliably estimating relative α-diversity across environmental gradients—rather than absolute site-level diversity—using multiple metrics (figure 2; electronic supplementary material, figure S3 and table S5) can facilitate comparisons; especially because estimating the total diversity of microbial communities remains mostly out of reach [70]. For this purpose, non-parametric asymptotic estimators that account for the likely incidence of undetected species and provide uncertainty estimates are an important tool [71]. Third, using a (dis)similarity metric insensitive to under-sampling, such as the Morisita–Horn index, can produce robust estimates of β-diversity even when hyper-diverse communities may have been incompletely surveyed [72]. Finally, using permutational tests—contrasting observed correlations to correlations obtained using thousands of reshuffled diversity matrices (e.g. figure 2 insets)—can help quantify the likelihood that estimated relationships among taxa occurred by chance; an important step when analysing datasets with a relatively small sample size. With these four steps, we consider our results a robust description of α- and β-diversity patterns between microbes and macro-invertebrates

across the 12 marine lakes, and a strong starting hypothesis for similar analyses in other marine systems.

While the proximate mechanisms revealed by our analyses are contingent on marine systems, our study nevertheless has important implications for a general understanding of biodiversity, as it provides novel insights into the understudied relationship between organismal scale and spatial scale. Our findings imply that similar processes may underlie the β-component of regional diversity across vastly different organisms, irrespective of their local constraints on α-diversity. At broader spatial scales (e.g. an island archipelago), taxon-specific individualistic responses to local-scale variation become less important and a common regional-scale signal—potentially resulting from shared variation in historical and contemporary climate, geology and/or dispersal—emerges across taxa. Our results are in line with previous comparisons between microbial and macro-organismal taxa in terrestrial and freshwater systems, which reported consistently positive correlations in β-diversity, irrespective of correlations in α-diversity (e.g. terrestrial: [5,31,32,36,37]; freshwater: [21,22,29]). The extent to which deviations from this general relationship may be explained by taxon-specific attributes such as physiological and/or life-history traits remains an open and interesting question that demands further scrutiny.

In conclusion, our study takes advantage of a marine natural experimental system to show that marine organisms as diverse as microbes and macro-invertebrates display parallel patterns in community dissimilarity across space, despite divergent local-scale responses potentially resulting from distinct physiology and life history. The extent to which parallel and universal processes may drive these parallel patterns in regional biodiversity across the tree of life in the marine realm is an important question that deserves increasing attention [9].

Data accessibility. All data and custom computer code used in this paper are available on the GitHub repository https://github.com/giorap/comparing-microbial-and-macro-invertebrate-diversity. Data and associated metadata on the abundance of all organisms, COI barcoding sequences and alignments for macro-invertebrates are available through the National Science Foundation's Biological and Chemical Oceanography Data Management Office at https://www.bco-dmo.org/dataset/768138. Microbial 16S rRNA sequences are available through the National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA555354 at https://www.ncbi.nlm.nih.gov/sra/PRJNA555354.

Authors' contributions. J.M.B., M.N.D. and G.R. conceived the ideas presented in this paper; J.M.B., M.N.D. and L.M.S. designed the research and collected the data; G.R. analysed the data with input from J.M.B., M.N.D., L.M.S.; G.R. wrote the bulk of the paper; and all authors wrote and edited portions of the paper.

Competing interests. We declare we have no competing interests.

Funding. Funding was provided by the National Science Foundation award no. OCE-1241255 to M.N.D. and J.M.B.

Acknowledgements. Support in the field and laboratory was provided by staff from the Coral Reef Research Foundation—E. Basilius, L. Bell, P. Colin, M. Mesubed, S. Patris and G. Ucharm—and C. Berg, M. Friedrich, C. Hayden, K. Henry, S. Knapp, M. Parekh, M. Rocha Souza, H. Swift and J. Wilson. Permits were issued by the Bureau of Marine Resources (RE-13-11, RE1409, RE-16-08, RE-17-15) and Koror State (13-233, 14-05, 16-15, 17-07).

References

1. Rapacciuolo G, Blois JL. 2019 Understanding ecological change across large spatial, temporal and taxonomic scales: integrating data and methods in light of theory. *Ecography* **42**, 1247–1266. (doi:10.1111/ecog.04616)
2. Vellend M. 2010 Conceptual synthesis in community ecology. *Q. Rev. Biol.* **85**, 183–206. (doi:10.1086/652373)
3. Vellend M. 2016 *The theory of ecological communities*. Princeton, NJ: Princeton University Press.
4. Locey KJ, Lennon JT. 2016 Scaling laws predict global microbial diversity. *Proc. Natl Acad. Sci. USA* **113**, 5970–5975. (doi:10.1073/pnas.1521291113)
5. Prober SM *et al.* 2015 Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol. Lett.* **18**, 85–95. (doi:10.1111/ele.12381)
6. Moran MA. 2015 The global ocean microbiome. *Science* **350**, aac8455. (doi:10.1126/science.aac8455)
7. Sunagawa S *et al.* 2015 Structure and function of the global ocean microbiome. *Science* **348**, 1261359. (doi:10.1126/science.1261359)
8. Stella JS, Pratchett MS, Hutchings P, Jones GP. 2011 Coral-associated invertebrates: diversity, ecological importance and vulnerability to disturbance. *Oceanogr. Mar. Biol.* **49**, 43–104. (doi:10.1201/b11009-3)
9. Shade A *et al.* 2018 Macroecology to unite all life, large and small. *Trends Ecol. Evol.* **33**, 731–744. (doi:10.1016/j.tree.2018.08.005)
10. Tipton L, Darcy JL, Hynson NA. 2019 A developing symbiosis: enabling cross-talk between ecologists and microbiome scientists. *Front. Microbiol.* **10**, 1–10. (doi:10.3389/fmicb.2019.00292)
11. Nemergut D, Shade A, Violle C. 2014 When, where and how does microbial community composition matter? *Front. Microbiol.* **5**, 2012–2014. (doi:10.3389/fmicb.2014.00497)
12. Van Der Heijden MGA, Bardgett RD, Van Straalen NM. 2008 The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **11**, 296–310. (doi:10.1111/j.1461-0248.2007.01139.x)
13. Georgiou K, Abramoff RZ, Harte J, Riley WJ, Torn MS. 2017 Microbial community-level regulation explains soil carbon responses to long-term litter manipulations. *Nat. Commun.* **8**, 1–10. (doi:10.1038/s41467-017-01116-z)
14. Bourne DG, Morrow KM, Webster NS. 2016 Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annu. Rev. Microbiol.* **70**, 317–340. (doi:10.1146/annurev-micro-102215-095440)
15. Martiny JBH *et al.* 2006 Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* **4**, 102–112. (doi:10.1038/nrmicro1341)
16. Barberán A, Casamayor EO, Fierer N. 2014 The microbial contribution to macroecology. *Front. Microbiol.* **5**, 1–8. (doi:10.3389/fmicb.2014.00203)
17. Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JBH. 2012 Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* **10**, 497–506. (doi:10.1038/nrmicro2795)
18. Nemergut DR *et al.* 2013 Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* **77**, 342–356. (doi:10.1128/MMBR.00051-12)
19. Zhou J, Ning D. 2017 Stochastic community assembly: does it matter in microbial ecology? *Microbiol. Mol. Biol. Rev.* **81**, 1–32. (doi:10.1128/MMBR.00002-17)
20. Stegen JC, Lin X, Fredrickson JK, Konopka AE. 2015 Estimating and mapping ecological processes influencing microbial community assembly. *Front. Microbiol.* **6**, 1–15. (doi:10.3389/fmicb.2015.00370)
21. Padial AA *et al.* 2014 Dispersal ability determines the role of environmental, spatial and temporal drivers of metacommunity structure. *PLoS ONE* **9**, 1–8. (doi:10.1371/journal.pone.0111227)
22. Astorga A, Oksanen J, Luoto M, Soininen J, Virtanen R, Muotka T. 2012 Distance decay of similarity in freshwater communities: do macro- and microorganisms follow the same rules? *Glob. Ecol. Biogeogr.* **21**, 365–375. (doi:10.1111/j.1466-8238.2011.00681.x)
23. Wang J, Meier S, Soininen J, Casamayor EO, Pan F, Tang X. 2017 Regional and global elevational patterns of microbial species richness and evenness. *Ecography* **40**, 393–402. (doi:10.1111/ecog.02216)

24. Lozupone CA, Knight R. 2007 Global patterns in bacterial diversity. *Proc. Natl Acad. Sci. USA* **104**, 11 436–11 440. (doi:10.1073/pnas.0611525104)
25. Auguet JC, Barberan A, Casamayor EO. 2010 Global ecological patterns in uncultured Archaea. *ISME J.* **4**, 182–190. (doi:10.1038/ismej.2009.109)
26. Fierer N, Mccain CM, Meir P, Zimmermann M, Rapp JM, Silman MR, Knight R. 2011 Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* **92**, 797–804. (doi:10.1890/10-1170.1)
27. Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL. 2008 Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proc. Natl Acad. Sci. USA* **105**, 11 505–11 511. (doi:10.1073/pnas.0801920105)
28. Yuan X, Nelman J, Gasarch E, Wang D, Nemergut D, Seastedt T. 2016 Plant community and soil chemistry responses to long-term nitrogen inputs drive changes in alpine bacterial communities. *Ecology* **97**, 1543–1554. (doi:10.1890/15-1160.1)
29. Wang J, Soininen J, Zhang Y, Wang B, Yang X, Shen J. 2012 Patterns of elevational beta diversity in micro- and macroorganisms. *Glob. Ecol. Biogeogr.* **21**, 743–750. (doi:10.1111/j.1466-8238.2011.00718.x)
30. Wang J, Soininen J, Zhang Y, Wang B. 2011 Contrasting patterns in elevational diversity between microorganisms and macroorganisms. *J. Biogeogr.* **38**, 595–603. (doi:10.1111/j.1365-2699.2010.02423.x)
31. Barberán A *et al.* 2015 Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecol. Lett.* **18**, 1397–1405. (doi:10.1111/ele.12536)
32. Li H *et al.* 2015 Aboveground–belowground biodiversity linkages differ in early and late successional temperate forests. *Sci. Rep.* **5**, 1–11. (doi:10.1038/srep12234)
33. Wang J, Zheng Y, Hu H, Li J, Zhang L, Chen B, Chen W, He J. 2016 Coupling of soil prokaryotic diversity and plant diversity across latitudinal forest ecosystems. *Sci. Rep.* **6**, 19561. (doi:10.1038/srep19561)
34. Kardol P, Wardle DA. 2010 How understanding aboveground-belowground linkages can assist restoration ecology. *Trends Ecol. Evol.* **25**, 670–679. (doi:10.1016/j.tree.2010.09.001)
35. Hiiesalu I *et al.* 2014 Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. *New Phytol.* **203**, 233–244. (doi:10.1111/nph.12765)
36. Tedersoo L *et al.* 2016 Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *ISME J.* **10**, 346–362. (doi:10.1038/ismej.2015.116)
37. Li H *et al.* 2018 Soil microbial beta-diversity is linked with compositional variation in aboveground plant biomass in a semi-arid grassland. *Plant Soil* **423**, 465–480. (doi:10.1007/s11104-017-3524-2)
38. Carney KM, Matson PA. 2006 The influence of tropical plant diversity and composition on soil microbial communities. *Microb. Ecol.* **52**, 226–238. (doi:10.1007/s00248-006-9115-z)
39. Megrey BA, Link JS, Hunt GL, Moksness E. 2009 Comparative marine ecosystem analysis: applications, opportunities, and lessons learned. *Prog. Oceanogr.* **81**, 2–9. (doi:10.1016/j.pocean.2009.04.002)
40. Dawson MN, Hamner WM. 2005 Rapid evolutionary radiation of marine zooplankton in peripheral environments. *Proc. Natl Acad. Sci. USA* **102**, 9235–9240. (doi:10.1073/pnas.0503635102)
41. Dawson MN. 2016 Island and island-like marine environments. *Glob. Ecol. Biogeogr.* **25**, 831–846. (doi:10.1111/geb.12314)
42. Hamner WM, Gilmer RW, Hamner PP. 1982 The physical, chemical, and biological characteristics of a stratified, saline, sulfide lake in Palau. *Limnol. Oceanogr.* **27**, 896–909. (doi:10.4319/lo.1982.27.5.0896)
43. Hamner PP, Hamner WM. 1998 Stratified marine lakes of Palau (Western Caroline Islands). *Phys. Geogr.* **19**, 175–220. (doi:10.1080/02723646.1998.10642647)
44. Dawson MN. 2014 Natural experiments and meta-analyses in comparative phylogeography. *J. Biogeogr.* **41**, 52–65. (doi:10.1111/jbi.12190)
45. Soininen J. 2012 Macroecology of unicellular organisms—patterns and processes. *Environ. Microbiol. Rep.* **4**, 10–22. (doi:10.1111/j.1758-2229.2011.00308.x)
46. Parada AE, Needham DM, Fuhrman JA. 2016 Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* **18**, 1403–1414. (doi:10.1111/1462-2920.13023)
47. Wilson JM, Litvin SY, Beman JM. 2018 Microbial community networks associated with variations in community respiration rates during upwelling in nearshore Monterey Bay, California. *Environ. Microbiol. Rep.* **10**, 272–282. (doi:10.1111/1758-2229.12635)
48. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013 Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* **79**, 5112–5120. (doi:10.1128/AEM.01043-13)
49. Chao A, Lee S-M. 1992 Estimating the number of classes via sample coverage. *J. Am. Stat. Assoc.* **87**, 210–217. (doi:10.1080/01621459.1992.10475194)
50. Chao A. 1984 Nonparametric estimation of the number of classes in a population. *Scand. J. Stat.* **11**, 265–270.
51. Simpson EH. 1949 Measurement of diversity. *Nature* **163**, 688. (doi:10.1038/163688a0)
52. Pielou EC. 1966 The measurement of diversity in different types of biological collections. *J. Theor. Biol.* **13**, 131–144. (doi:10.1016/0022-5193(66)90013-0)
53. Berger WH, Parker FL. 1970 Diversity of planktonic foraminifera in deep-sea sediments. *Science* **168**, 1345–1347. (doi:10.1126/science.168.3937.1345)
54. Chao A, Wang YT, Jost L. 2013 Entropy and the species accumulation curve: a novel entropy estimator via discovery rates of new species. *Methods Ecol. Evol.* **4**, 1091–1100. (doi:10.1111/2041-210X.12108)
55. R Core Team. 2018 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org/.
56. Chao A, Ma KH, Hsieh TC, Chun-Huo C. 2016 SpadeR (species-richness prediction and diversity estimation with R). R package version 0.1.1. See https://CRAN.R-project.org/package=SpadeR.
57. Morisita M. 1959 Measuring of the dispersion of individuals and analysis of the distributional patterns. *Mem. Fac. Sci. Kyushu Univ. Ser. E Biol.* **2**, 215–235.
58. Oksanen J *et al.* 2018 vegan: community ecology Package. R package version 2.4-0. See https://CRAN.R-project.org/package=vegan.
59. Gilly WF, Beman JM, Litvin SY, Robison BH. 2013 Oceanographic and biological effects of shoaling of the oxygen minimum zone. *Annu. Rev. Mar. Sci.* **5**, 393–420. (doi:10.1146/annurev-marine-120710-100849)
60. Beman JM, Carolan MT. 2013 Deoxygenation alters bacterial diversity and community composition in the ocean's largest oxygen minimum zone. *Nat. Commun.* **4**, 1–11. (doi:10.1038/ncomms3705)
61. Wright JJ, Konwar KM, Hallam SJ. 2012 Microbial ecology of expanding oxygen minimum zones. *Nat. Rev. Microbiol.* **10**, 381–394. (doi:10.1038/nrmicro2778)
62. Meyerhof MS, Wilson JM, Dawson MN, Michael Beman J. 2016 Microbial community diversity, structure and assembly across oxygen gradients in meromictic marine lakes, Palau. *Environ. Microbiol.* **18**, 4907–4919. (doi:10.1111/1462-2920.13416)
63. Rivera-Ingraham GA, Lignot J-H. 2017 Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. *J. Exp. Biol.* **220**, 1749–1760. (doi:10.1242/jeb.135624)
64. Raes EJ, Bodrossy L, van de Kamp J, Bissett A, Ostrowski M, Brown MV, Sow SLS, Sloyan B, Waite AM. 2018 Oceanographic boundaries constrain microbial diversity gradients in the South Pacific Ocean. *Proc. Natl Acad. Sci. USA* **115**, 201719335. (doi:10.1073/pnas.1719335115)
65. Bleich S, Powilleit M, Seifert T, Graf G. 2011 β-diversity as a measure of species turnover along the salinity gradient in the Baltic Sea, and its consistency with the Venice System. *Mar. Ecol. Prog. Ser.* **436**, 101–118. (doi:10.3354/meps09219)
66. Medeiros CR, Hepp LU, Patrício J, Molozzi J. 2016 Tropical estuarine macrobenthic communities are structured by turnover rather than nestedness. *PLoS ONE* **11**, 1–14. (doi:10.1371/journal.pone.0161082)

67. Castro-Insua A, Gómez-Rodríguez C, Baselga A. 2016 Break the pattern: breakpoints in beta diversity of vertebrates are general across clades and suggest common historical causes. *Glob. Ecol. Biogeogr.* **25**, 1279–1283. (doi:10.1111/geb.12507)
68. Kelly RP, Closek CJ, O'Donnell JL, Kralj JE, Shelton AO, Samhouri JF. 2017 Genetic and manual survey methods yield different and complementary views of an ecosystem. *Front. Mar. Sci.* **3**, 1–11. (doi:10.3389/fmars.2016.00283)
69. Deiner K *et al.* 2017 Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Mol. Ecol.* **26**, 5872–5895. (doi:10.1111/mec.14350)
70. Vitorino L, Bessa L. 2018 Microbial diversity: the gap between the estimated and the known. *Diversity* **10**, 46. (doi:10.3390/d10020046)
71. Gotelli NJ, Chao A. 2013 Measuring and estimating species richness, species diversity, and biotic similarity from sampling data. In *Encyclopedia of biodiversity*, 2nd edn (ed. SA Levin), pp. 195–211. Waltham, MA: Academic Press.
72. Beck J, Holloway JD, Schwanghart W. 2013 Undersampling and the measurement of beta diversity. *Methods Ecol. Evol.* **4**, 370–382. (doi:10.1111/2041-210x.12023)