Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions

Running title: Metacommunities under shifting environment

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Abstract

Temporal variations in microbial metacommunity structure and assembly processes in response to shifts in environmental conditions are poorly understood. Hence, we conducted a temporal field study by sampling rock pools in four-day intervals during a 5-week period that included strong changes in environmental conditions due to intensive rain. We characterized bacterial and microeukaryote communities by 16S and 18S rRNA gene sequencing, respectively. Using a suite of null-model approaches to assess dynamics in community assembly, we found that strong changes in environmental conditions induced small but significant temporal changes in assembly processes and triggered different responses in bacterial and microeukaryotic metacommunities, promoting distinct selection processes. Incidence-based approaches showed that the assemblies of both communities were mainly governed by stochastic processes. In contrast, abundance-based methods indicated the dominance of historical contingency and unmeasured factors in case of bacteria and microeukaryotes, respectively, which we distinguished from dispersal-related processes using additional tests. Taken together, our study highlights that community assembly processes are not static, and the relative importance of different assembly processes can vary under different conditions and between different microbial groups.

Introduction

Different assembly processes such as environmental selection, dispersal and/or stochastic processes can simultaneously influence community composition [1]. The relative importance of the different processes is highly context–dependent and dynamic and may therefore vary in importance over time [2–5] as a consequence of processes such as ecological succession [4, 6], seasonality [3, 5, 7] or changes in connectivity between sites [8, 9]. Despite the increased recognition that community assembly processes are not static, the majority of studies is based on snapshot sampling which cannot adequately capture their dynamics [7].

Besides contemporary changes in environmental conditions and dispersal processes [10], past environmental conditions and dispersal events (i.e., historical contingency) may also influence the temporal dynamics of assembly processes [11–13]. For instance, changes in the variation in environmental heterogeneity could affect the relative importance of species-sorting or selection processes [8], while changes in dispersal rates could affect the possibilities for mass effects [14] or the extent of dispersal limitation [15]. Further, the importance of historical contingency may also depend on the environmental context. For example, priority effects – the impact of particular species on community development due to prior arrival at a site – may be affected by environmental disturbances that initiate colonization events that intensify the importance of the phenomenon [11]. Several studies detected a trajectory from stochastic to deterministic assembly processes in time following a disturbance [16, 17], which might reflect effects of initially strong, but transient priority effects that diminish over time as more species arrive and establish in the post-disturbance community. Finally, the probability of priority effects may also increase when productivity is high [18], because the growth of early colonizers is promoted [11].

Only a few studies have directly compared assembly mechanisms between different groups of microorganisms such as bacteria and microeukaryotes. Based on the differences in e.g., cell sizes, generation times and life history traits, differences in assembly processes are expected between these two groups [19–21]. For instance, it has been suggested that marine protist communities are governed by species-sorting to a greater extent than are marine bacterial communities [22, 23], while on the contrary, other studies indicated the opposite [21]. Microeukaryotes have been suggested to be mainly shaped by stochastic mechanisms (i.e. drift) [21, 24] and to be more subject to the effect of dispersal than bacteria [25]. Hence, there are to date conflicting results on how assembly processes differ and persist through time in bacterial and microeukaryotic communities.

The statistical 'toolbox' that is currently used to gain insights into the importance of different community assembly processes consists of several complementary approaches that all have their own strengths and limitations. Recently, null model approaches that quantitatively compare assembly processes have been increasingly used [26, 27]. For example, the elements of metacommunity structure (EMS) method allows to distinguish randomly assembled communities from those assembled by species-sorting processes [28, 29]. The incidence-based (Raup-Crick) beta-diversity (β_{RC}) [30] has been used to differentiate between deterministic and stochastic assembly processes [18, 31]. In addition, based on the assumption that phylogenetic relatedness is indicative of shared environmental response traits [32], null model approaches have been extended to integrate phylogenetic information [33]. Specifically, Stegen et al. [15, 27] have combined null model approaches based on phylogenetic and abundance-based (Raup-Crick) beta-diversity (β_{RCbray}) measures to quantitatively estimate the relative importance of processes such as selection, drift, dispersal limitation and mass effects. Furthermore, this quantitative process estimate (QPE) method

also differentiates between heterogenous/variable (i.e., beta-diversity enhancing) and homogeneous (i.e., beta-diversity diminishing) selection processes.

We carried out an extensive field study of bacterial and microeukaryotic communities in rock pools, which are particularly variable habitats both in space and time. The above-mentioned statistical approaches were applied to assess the temporal changes in community assembly processes. We hypothesized that temporal changes in the importance of different assembly processes should occur in dependence on changes in environmental conditions and, further, that these changes differ between bacterial and microeukaryotic communities.

Material and methods

Sampling procedure

Samples were taken from 20 neighboring rock pools – referred to as a 'metacommunity' – located along the Baltic Sea coast on the island of Gräsö, Sweden (60°29'54.0" N, 18°25'48.9" E) (Supplemental Fig. S1). The rock pools were sampled ten times, starting on 14 August 2015 and ending on 19 September 2015 in four-day intervals (Fig. S2). Four of the pools dried out at certain occasions during the sampling period. Intensive rain (starting August 31) occured in the middle of the study period and separated a cooler wet period (air temperature (°C): 13.98±1.35, precipitation (mm): 3.97±6.94, wind speed (m/s): 6.7±2.91) from an extended dry period (air temperature (°C): 17.71±0.98, precipitation (mm): 0.09±0.36, wind speed (m/s): 5.6±1.61) prior to the rain (Fig. S2).

Numerous abiotic and biotic variables were measured at each sampling occasion. Specifically, conductivity and temperature were measured using a WTW Conductometer (Cond 3210 SET 2 incl. TetraCon 325-3 measuring cell, Germany). Morphological parameters such as maximum length, width and depth were recorded for each pool.

Zooplankton samples were collected by filtering 2 l of water through a net (250 μm) and fixed with 70% ethanol for subsequent analysis. Five litres of water were collected in sterile plastic bottles and transported back to the laboratory where the samples were further processed. For quantification of bacterial abundance, samples were preserved with sterile-filtered formaldehyde at a final concentration of 2% and stored at 4 °C, while for bacterial and eukaryotic community analyses, pre-filtered (250 μm) water samples (100–500 ml) were collected by vacuum filtration onto 0.2 μm 47 mm membrane filters (Supor-200, Pall Corporation, Port Washington, NY, USA), and then, stored at –80 °C until further processing (see below).

Sample analysis

Nutrient concentrations, such as total phosphorus (TP) and total nitrogen (TN) were measured spectrophotometrically (Perkin Elmer, Lambda 40, UV/VIS Spectrometer, Massachusetts, USA) and by catalytic thermal decomposition method (Shimadzu TNM-L, Kyoto, Japan), respectively according to standard procedures. Water colour was determined by measuring the absorbance of GF/C-filtered (Whatman® Glass microfiber filter, Sigma-Aldrich, USA) water at 436 nm. Chlorophyll-a was measured [34] as an estimator of the biomass of primary producers. Bacterial abundance was determined by flow cytometry as in Székely et al. [35] with the modification of using 2.27 μM of SYTO 13 fluorescent nucleic acid stain (Invitrogen, Eugene, Oregon, USA).

For both bacterial and micro-eukaryotic community composition analyses, DNA extraction was performed from the membrane filters (PowerSoil DNA Isolation Kit, MoBio Laboratories Inc., Carlsbad, CA, USA). Bacterial 16S rRNA and eukaryotic 18S rRNA genes were amplified with bacterial (341F and 805R; [36]) and eukaryotic (574*f and 1132r; [37]) primer constructs, respectively. A full step-by-step protocol for the detailed two-step PCR

125 protocol has been deposited in the protocols.io repository
126 (dx.doi.org/10.17504/protocols.io.468gzhw). Amplicons were sequenced at the SciLifeLab
127 SNP&SEQ Technology Platform (host by Uppsala University) using Illumina MiSeq v3
128 sequencing chemistry. The raw sequencing data are available at the European Nucleotide
129 Archive under accession number PRJEB30954. A detailed report of the data processing is
130 provided in the supplementary material. The taxonomic composition of both datasets is
131 visualized in Supplemental Figure S3 and S4.
132
133 *Statistical analysis*
134 All statistical analyses (see Fig. 1 for an overview) and visualizations were conducted
135 in R version 3.3.2 [38].
136
137 Shifts in environmental conditions structuring communities
138 We excluded the four rock pools that occasionally dried out from all analyses. Then,
139 we accounted for collinearity among standardized environmental variables by omitting highly
140 collinear variables (Pearson $|r| > 0.7$) based on Dormann et al. [39]. To select the variables
141 most strongly associated with the variance of the observed communities, we applied
142 redundancy analysis (RDA) on the Hellinger-transformed sequence data with forward
143 selection (based on 999 permutations; variables retained at $p < 0.05$), separately for bacteria
144 and microeukaryotes (Fig. 1A) (see Results section for details). Differences in means and
145 variances in selected variables between the dry and wet periods were tested using Kruskal-
146 Wallis test and Levene's test, respectively. Additionally, to assess the separation between the
147 two periods, permutational multivariate analysis of variance (PERMANOVA) with 999
148 permutations was performed using the function 'adonis', further, the multivariate

149 homogeneity of group dispersions (PERMDISP) was tested using the function 'betadisper' in
150 the package 'vegan' [40].
151
152 Elements of metacommunity structure (EMS)
153 Elements of metacommunity structure (EMS) were assessed for each time point
154 following the frameworks developed by Leibold & Mikkelson [28] and Presley et al. [29]
155 (Fig. 1B). EMS enables to identify metacommunity properties that emerge in a site-by-species
156 incidence matrix that is compared with null model expectations obtained through
157 randomization [41]. Random matrices were produced by the 'r1' method (fixed-proportional
158 null model). For this, the matrices (OTU table from 16S and 18S rRNA gene sequences,
159 separately) were ordinated according to the primary axis via reciprocal averaging and then
160 hierarchically analysed using three tests (coherence, turnover and boundary clumping) (for
161 more details, see supplementary material). The package 'metacom' [41] was used to detect
162 any pattern of metacommunity structure related to an idealized scenario ('metacommunity
163 type'). Following the suggested hierarchical framework of EMS, we specifically focused on
164 the outcome of coherence tests (the number of embedded absences in the ordinated matrix and
165 comparing the empirical value to a null distribution) in the subsequent statistical analyses
166 since the majority of metacommunities were associated with checkerboard and random
167 metacommunity types. Differences in the coherence (z-values) between the two periods (wet
168 and dry) were tested using a Kruskal-Wallis test.
169
170 Incidence-based beta-diversity (β_{RC})
171 The incidence-based (Raup-Crick) dissimilarity indices (β_{RC}) were calculated to test
172 whether community were stochastically or deterministically assembled (Fig. 1C). For this, we
173 used the 'raup_crick' function provided by Chase et al. [31]. When β_{RC} is not significantly

different from 0, the community is considered to be stochastically assembled. β_{RC} values close to −1 indicate that communities are deterministically assembled and more similar to each other than expected by chance, whereas β_{RC} values close to +1 indicate that deterministic processes favor dissimilar communities. The averaged dissimilarities for each time point and for each dataset (bacteria and microeukaryotes) were calculated separately. Differences in β_{RC} between the two periods (wet and dry) were tested using a Kruskal-Wallis test.

Quantitative process estimates (QPE)

To quantify the relative importance of potential species sorting, dispersal limitation, drift and mass effects (we refer to this as 'quantiative process estimates – QPE' throughout the manuscript), we followed the two-step framework developed by Stegen et al. [27] (Fig. 1D). This approach requires that phylogenetic distances (PD) among taxa reflect differences in the ecological niches they inhabit, thus, carry a phylogenetic signal. The presence of phylogenetic signals was tested using Mantel correlograms, as described in Stegen et al. [27]. We found that niche differences caused by most of the environmental variables that structured communities according to the RDA results (see above) could induce turnover in phylogenetic community composition (Fig. S5, S6) and thereby fulfill the prerequisite of this framework.

To perform QPE based on pairwise comparisons, firstly, we determined to what extent the observed βMNTD (β-mean-nearest-taxon-distance) deviated from the mean of the null distribution and evaluated significance using the β-Nearest Taxon Index (βNTI; difference between observed βMNTD and the mean of the null distribution in units of SDs). If the observed βMNTD value is significantly greater (βNTI > 2) or less (βNTI < −2) than the null expectation, the community is assembled by variable or homogeneous selection, respectively. If there is no significant deviation from the null expectation, the observed differences in phylogenetic community composition should be the result of dispersal limitation,

199 homogenizing dispersal (mass effect) or random drift. To estimate the relative importance of
200 these processes, in the second step, the abundance-based (Raup-Crick) beta-diversity was
201 calculated using pairwise Bray-Curtis dissimilarity (β_{RCbray}) [27]. Based on the calculated
202 β_{RCbray}, we can assume that communities that were not selected in the first step, thus not
203 assembled by selection, were structured by (i) dispersal limitation coupled with drift if β_{RCbray}
204 $> +0.95$, (ii) homogenizing dispersal if $\beta_{RCbray} < -0.95$, or (iii) random processes acting alone
205 (drift) if β_{RCbray} falls in between -0.95 and $+0.95$ (Fig. 1D). The first fraction, $\beta_{RCbray} > +0.95$,
206 may either indicate 'true' effects of dispersal limitation and/or history contingency that both
207 result in more dissimilar communities than expected by chance. Hence, throughout the
208 manuscript we use the term 'dispersal limitation or historical contingency' for this fraction.
209 Differences in the QPE between the two periods (wet and dry) were tested using Kruskal-
210 Wallis test.

211 To further assess whether 'true' dispersal limitation might have occurred, Mantel
212 correlations between bacterial and microeukaryotic community dissimilarities (β_{RCbray}) and
213 geographic distances (Euclidean distances of geographical coordinates) were done using
214 Pearson correlation and 999 permutations (Fig. 1E). By this, we could confirm any potential
215 dispersal limitation and reject historical contingency if there is significant correlation between
216 β_{RCbray} and spatial distance. Further, Mantel correlation analyses between β_{RCbray} and
217 environmental dissimilarities (Euclidean distances) were done to test if community
218 dissimilarities were possibly due to selection by environmental factors that lack a
219 phylogenetic signal ('phylogenetically non-conserved selection') and was therefore not
220 detected in the first step but instead was retained in the second step of the QPE analysis. To
221 determine if significant geographic distance effects were confounded by effects of spatially
222 autocorrelated environmental variation and vice versa, partial Mantel tests were used with the

223 respective third matrix as covariate for time points where both correlations with geographic
224 and environmental distances were significant.
225
226
227 **Results**
228
229 ***Temporal variation in relevant environmental variables***
230 RDA with forward selection showed that conductivity (F = 11.37, p = 0.005), water
231 temperature (F = 2.99, p = 0.005), nutrients (TP: F = 4.71, p = 0.005 and TN: F = 2.04, p =
232 0.005), depth (F = 1.62, p = 0.03) and Daphnia abundance (F = 1.62, p = 0.015) correlated
233 significantly with the variation in bacterial community composition. For microeukaryotes, the
234 same variables and copepod abundance were significant (conductivity: F = 8.34, p = 0.005;
235 water temperature: F = 4.05, p = 0.005; TN: F = 2.84, p = 0.005; TP: F = 1.99, p = 0.005;
236 depth: F = 2.68, p = 0.005; Daphnia abundance: F = 1.87, p = 0.015; copepod abundance: F =
237 2.04, p = 0.005).
238 The temporal fluctuations of the selected variables followed similar patterns during
239 the sampling period (Fig. 2). Specifically, we found that there was a clear separation point in
240 the middle of the study period (31 August, between two sampling occasions on 30 August and
241 3 September; dashed line in Fig. 2) from when on environmental conditions became more
242 homogenous, i.e., the variance across the rock pools decreased (except in the case of depths
243 and conductivity, although the latter one was marginally insignificant) (Table S1). These
244 differences supported our separation of the study period and corresponding datasets into two
245 periods, a dry and wet period (Figs 2 and S2). Consequently, the pools had higher mean water
246 temperature, conductivity, zooplankton abundance, and nutrient concentrations, lower mean
247 pool depth and more spatially heterogeneous conditions (high variance across pools) in the

dry compared to the wet period (Fig. 2, Table S1). This separation was further supported by PERMANOVA and PERMDISP analyses, which showed that the environmental conditions ($F = 31.07$, $p = 0.001$; Fig. S7) and their variances ($F = 79.58$, $p < 0.001$) clearly differed between the two periods. There were also significant differences in the composition of the bacterial and microeukaryotic communities of the dry and wet period but no difference in their homogeneity (beta-dispersion) (Figs S8, S9). Meanwhile, at the level of individual pools significant differences in community composition (PERMANOVA) were detected in some of the pools (7 out of 16) for bacteria and for most pools (15 out of 16) in the case of microeukaryotes (Table S2) without any difference in their beta-dispersion (PERMDISP, Table S3).

Elements of metacommunity structure (EMS)

In general, the observed z-value of coherence did not show wide variation across the bacterial and microeukaryotic datasets, which were shaped by random processes at the majority of the time points in both cases. Checkerboard pattern emerged at one occasion and two occasions for the bacterial and microeukaryotic metacommunities, respectively, while a nested, clumped species loss pattern was detected once during the wet period in bacteria (Fig. 3, Table S4). Microeukaryotic metacommunities were also mainly characterized by random patterns, except for two occasions when checkerboard patterns occurred (Fig. 3). There was no significant change of coherence (z-values) over time in any of the observed datasets (bacteria: $\chi_{\text{dry vs. wet}} = 0.884$, $p = 0.347$; microeukaryotes: $\chi_{\text{dry vs. wet}} = 3.153$, $p = 0.076$), however, there were trends towards slightly higher coherence (z-values) in the wet period compared to the dry period, especially in the microeukaryote communities.

Incidence-based beta-diversity (β_{RC})

Across the 16 rock pools the average β_{RC} varied within a narrow range, not deviating strongly from the null expectations (0.066–0.227 and −0.221–0.197 in bacterial and microeukaryotic communities, respectively), which indicates stochastic assembly. For bacteria, there was no clear pattern or trend in β_{RC} associated with the shift in the environmental conditions ($\chi_{\text{dry vs. wet}} = 0.273$, $p = 0.602$) (Fig. 4). For microeukaryotes the β_{RC} values decreased at the beginning of the wet-period, but thereafter they increased rapidly (Fig. 4), although they remained within the 'stochastic' range ($-0.95 < \beta_{RC} < +0.95$) ($\chi_{\text{dry vs. wet}} = 0.535$, $p = 0.465$).

Quantitative process estimates (QPE)

The quantitative process estimates showed temporal variation over the study period with some differences between the two organism groups (Fig. 5). For bacteria, dispersal limitation or historical contingency was the dominant assembly processes (60.95–80.83% of all pairwise comparisons) followed by homogeneous selection processes (4.17–27.62%), random processes (drift, 4.76–15.38%), variable selection (0–12.5%) and homogenizing dispersal (0–1.67%). The relative proportion of homogeneous selection increased in the wet period ($\chi_{\text{dry vs. wet}} = 5.34$, $p = 0.021$), while that of variable selection decreased compared to the dry period, although this decline was not significant ($\chi_{\text{dry vs. wet}} = 1.32$, $p = 0.251$). There were no significant changes detected between the two periods in the case of dispersal limitation or historical contingency ($\chi_{\text{dry vs. wet}} = 1.10$, $p = 0.293$), drift ($\chi_{\text{dry vs. wet}} = 0.01$, $p = 0.916$) and homogenizing dispersal ($\chi_{\text{dry vs. wet}} = 0.05$, $p = 0.828$). For microeukaryotic metacommunities, dispersal limitation or historical contingency was also the dominating assembly process at all time points (56.19–85.83%). The second and third most dominant assembly processes were drift (5.00–22.86%) and variable selection (1.67–18.1%), respectively, whereas the proportions of homogeneous selection (0–2.86%) and

298 homogenizing dispersal (0–2.56%) were negligible. The proportion of dispersal limitation or
299 historical contingency decreased ($\chi_{\text{dry vs. wet}} = 5.77$, $p = 0.016$) while variable selection
300 increased during the wet period after the first rainfall ($\chi_{\text{dry vs. wet}} = 4.81$, $p = 0.028$), whereas
301 the slight increase of drift during the wet-period was not significant ($\chi_{\text{dry vs. wet}} = 2.81$, $p =$
302 0.094). The importance of homogenizing dispersal differed between the two periods ($\chi_{\text{dry vs. wet}}$
303 $= 4.51$, $p = 0.034$), while opposite to the bacterial metacommunities, homogeneous selection
304 did not change significantly ($\chi_{\text{dry vs. wet}} = 0.41$, $p = 0.522$) (Fig. 5).
305
306 Mantel correlations between community distance matrices (β_{RCbray}, the fraction
307 retrived for the second step of QPE) and geographical/environmental distance matrices were
308 generally weak, showed no consistent pattern, and were only significant for a few time points
309 (Fig. 6). Microeukaryotic communities showed significant correlations for geographic
310 distance in one case and for both environmental and geographic distances in another. In the
311 latter case the correlations were even significant when controlled for effects of covariation by
312 environmental distance in cases of geographic distance (partial $r_M = 0.23$, $p = 0.003$) or
313 geographic distance in case of environmental distance (partial $r_M = 0.19$, $p = 0.032$),
314 respectively. Meanwhile, bacterial community compositions were significantly correlated to
315 environmental distance only once.
316
317
318 **Discussion**
319
320 ***Environmental dependency of assembly mechanisms***
321 Intensive rain from the middle of our rock pool sampling campaign separated the
322 study period into a distinct dry and wet period, allowing us to specifically investigate the

environmental dependency of assembly mechanisms of microbial communities over time. According to the incidence-based beta-diversity (β_{RC}) patterns, both bacterial and microeukaryotic communities were primarily stochastically assembled throughout the study period ($\beta_{RC} \approx 0$) despite the observed environmental changes during the transition from the dry to the wet period. Testing the elements of metacommunity structure (EMS) provided further evidence for stochastic assembly. These results resonate the idea that microbial community assembly is unpredictable because stochastic occurrence patterns are due to rapid population dynamics [42]. However, in contrast to the results from the β_{RC} and EMS analyses, the quantitative process estimates (QPE) framework showed that both bacterial and microeukaryotic community assemblies were dominated by dispersal limitation or historical contingency at all time points. The relative importance of dispersal limitation or historical contingency in microeukaryotes was significantly higher in the dry period compared to the wet period, while in bacterial communities it increased towards the end of dry period, but then decreased slightly during the wet-period (Fig. 5). This suggests that a lack of connectivity among pools during the dry period lead to a temporary enforcement of dispersal limitation or history contingency (see discussion below for our interpretation). Further, the environmental shift between the dry and wet period slightly promoted the influence of homogeneous selection and variable selection for bacteria and microeukaryotes, respectively, even though none of the changes in environmental conditions that occurred throughout the study period induced strong selection processes.

While our study gives overall support for the dominance of stochastic and dispersal limitation or historical processes in the assembly of microbial communities, previous studies of bacterial communities in rock pools have shown that either both selection processes (i.e. species sorting) alone [43] or both environmental and spatial effects shape the communities [44]. However, it has also been shown that the importance of environmental vs. spatial effects over

348 time varies in response to changes in environmental conditions [5]. Here, we provide a more
349 refined picture of temporal changes in assembly processes that occur at much shorter
350 temporal scales compared to the previous studies. Since community assembly is dynamic our
351 temporal study provides a more comprehensive understanding of how microbial communities
352 respond to environmental changes on short-time scales compared to previous snapshot studies
353 [43, 44] or a study where changes were analysed over longer time periods as well as longer
354 sampling intervals [5]. The present study also differs from the previous ones in that a broader
355 suit of statistical methods was applied, allowing the analysis of further assembly mechanisms
356 than in the studies where primarily variation partitioning was used [5, 45].
357
358 ***Comparison of null model approaches***
359 Our results show that different null model approaches led to different conclusions
360 about the dominant community assembly processes. Generally, the key differences among the
361 applied three null model approaches are that EMS and β_{RC} are developed for detecting
362 patterns in binary presence-absence matrices based on taxonomic beta-diversity estimates
363 only, while the QPE framework is based on quantitative, abundance-based matrices
364 integrating phylogenetic information. One possible explanation why selection processes were
365 only detected by the QPE but not the non-quantitative methods (EMS and β_{RC}) could be that
366 species sorting is to a great extent related to changes in the relative abundances of species but
367 not the replacement of species. This highlights that abundance-based metrics might be more
368 suited to describe microbial beta-diversity and the underlying assembly mechanisms at spatial
369 scales similar to those studied here [46, 47].
370
371 ***Differences between bacterial and microeukaryotic communities***

372 Based on the results of null model approaches the hypothesis that bacteria and micro-
373 eukaryotes are assembled by different assembly processes was partly supported. More
374 specifically, the relative importance of assembly processes, and the way they changed in
375 response to changing environmental conditions differed for bacterial and microeukaryotic
376 communities. More specifically, for the bacterial metacommunity, there was a slight decrease
377 in the influence of variable selection processes in the wet period compared to the previous dry
378 period, while the relative importance of homogeneous selection processes significantly
379 increased, which is conform with the idea that homogenization in environmental conditions
380 among rock pools leads to more similarly composed bacterial communities [5]. On the
381 contrary, for microeukaryotes, homogeneous selection processes remained negligible
382 throughout the study period period, while the relative influence of variable selection
383 surprisingly increased in the wet period. One possible explanation might be that in the wet
384 period the increased water depth could have generated more gradients within each pool for
385 environmental parameters such light, which is crucial for photo- and mixotrophic
386 microeukaryotes [48, 49], thus, promoting the establishment of distinct local microeukaryotic
387 communities. In general, it is worth to mention that both the bacterial and the microeukaryotic
388 dataset consisted of several distinct groups of organisms which have very different population
389 dynamics, niche-preferences and interspecific interactions (Fig. S5, S6). This could
390 potentially mask important selection forces that act at each taxonomic level. More
391 specifically, when a metacommunity consists of sets of species that are more structured by
392 environment and others that are less so, a comprehensive perspective (pooling all groups
393 together) could result in a fuzzy, stochastic picture of assembly [50]. Hence, a separate
394 investigation of different microeukaryotic and bacterial groups (e.g. heterotrophs vs.
395 autotrophs) might reveal different influences of assembly mechanisms [3].
396

Historical contingency vs. pure dispersal limitation

As mentioned earlier the quantitative process estimate analysis showed that both bacterial and microeukaryotic communities were primarily shaped by dispersal limitation or historical contingencies. At a first glance, the dominance of dispersal limitation seems surprising, given the idea that has persisted in microbiology for a long time that microorganisms are to a great extent not dispersal limited. This idea has now been challenged as many studies have, for instance, detected spatial distance effects for microorganism [5, 44, 51, 52]. Moreover, other studies using quantitative process estimates have also shown considerable proportion of 'dispersal limitation or historical contingency' fraction [22, 53]. However, problems related to the interpretation of dispersal limitation fraction have also been raised [54], because it does not purely reflect dispersal limitation but rather a number of different processes, such as historial contingency and effects of phylogenetically non-conserved selection processes. To more explicitly test whether pure dispersal limitation was present in our study, we used Mantel correlations of abundance-based Raup-Crick beta-diversity ($\beta_{RCbray,}$ on the fraction retrived for the second step of QPE) vs. geographical distances between pools to detect spatial distance-decay relationships [55]. However, except for one case in microeukaryotes, this was not the case neither for bacteria nor for microeukaryotes (Fig 7) and we do therefore not have robust support for dispersal limitation. Likewise, there was also no indication that the dispersal limitation or historical contingency fraction masked substantial effects of phylogenetically non-conserved selection processes related to measured environmental factors as Mantel correlations between RC_{bray} and environmental distance were also not significant in most cases. Therefore, it seems most likely that the dispersal limitation or historical contingency fraction points to the importance of the outcome of historical contingency and the effect of unmeasured factors (e.g. light) that are not phylogenetically conserved. Evidence for historical contingency, such as priority

effects, would be a low temporal turnover in community composition at the level of individual rock pools despite the drastic environmental shift that occurred during the study period. In case of bacteria, most of the individual rock pools (9 out of 16) did not experience significant compositional shifts between the two periods. Hence, this suggests that these nine communities might have been influenced by priority effects, while the remaining pools might have been influenced by unmeasured environmental factors that are phylogenetically non-conserved. In contrast, microeukaryotic communities are unlikely to have experienced priority effects because most (15 out of 16) of the individual pools experienced significant compositional shift between the two periods (Table S2). Still, we could not explain these compositional shifts by spatial or measured environmental factors using Mantel tests (Fig. 6), suggesting that unmeasured environmental factors, such as light or trophic interactions are more important for microeukaryotes compared to bacteria. In summary caution needs to be taken when interpreting the results of quantitative process estimates and future refined statistical frameworks should integrate additional analyses such as those presented here to provide a more clear distinction of historical contingencies (e.g. priority effects), phylogenetically non-conserved selection and pure effects of dispersal limitation.

Conclusions

Our results show that historical contingency and selection processes can play a key role in shaping microbial communities, but that the relative contribution of selection processes varies depending on the temporal variation of environmental heterogeneity and between bacteria and microeukaryotes. Furthermore, this present study highlighted that incidence-based and abundance-based null model approaches lead to different conclusions about the dominant community assembly process in microbial communities. Further, the outcomes of the current QPE framework act merely as a guide, because the fraction expected

447 to indicate dispersal limitation may in reality depict other processes such as historical
448 contingency, phylogenetically non-conserved selection, or even other, unmeasured processes.
449 Our findings also show that temporal observations with high-resolution can provide more a
450 comprehensive understanding than snapshot studies. Taken together, we encourage future
451 studies to consider temporal variation of metacommunities and its environmental dependency,
452 regardless the microbial group of interest, as well as to consider historical contingency (e.g.
453 imprints of assembly history) as a potentially important assembly process.
454
455
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464
465
466 **Conflict of interest**
467 The authors declare that they have no conflict of interest.
468
469
470 **References**
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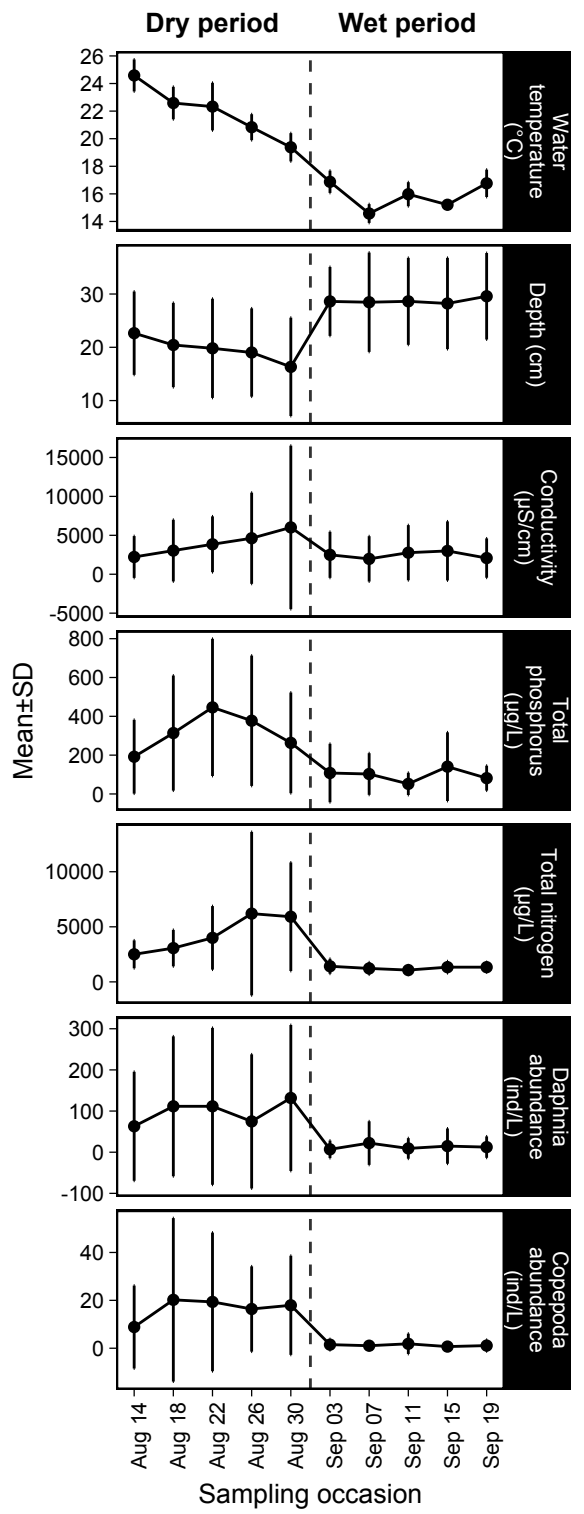
610 **Figure 1.** Flow chart of the statistical analyses performed in this study. (A) Redundancy
611 analysis with forward selection was performed to select the most important environmental
612 variables that explain variation in the community matrices. Then, we compared the variance
613 and homogeneity of environmental and community distances between the dry and wet period
614 using PERMANOVA and PERMDISP, respectively. (B-C-D) Three null-model approaches
615 were applied. (B) EMS identifies metacommunity properties emerging in site-by-OTUs
616 incidence matrix [28, 29]. (C) Incidence-based (Raup-Crick) beta-diversity (β_{RC}) tests
617 stochasticity and determinism using a metric provided by Chase et al. [31]. (D) QPE
618 quantifies assembly processes involving phylogeny and abundance-based (Raup-Crick) beta-
619 diversity (β_{RCbray}) following the framework of Stegen et al. [27]. (E) We performed (partial)
620 Mantel tests as a complement to the QPE between β_{RCbray} and geographical and environmental
621 distance matrices in order make a clear distinction of historical contingencies (e.g. priority
622 effects) and/or unmeasured factors, phylogenetically non-conserved selection and pure effects
623 of dispersal limitation. Then, we distinguished historical contingency and the effects of
624 unmeasured factors by assessing temporal change of community composition at the level of
625 individual rock pool using PERMANOVA.
626
627 **Figure 2.** Temporal dynamics of the mean values and standard deviations of environmental
628 variables that significantly affected either the composition of bacterial or the microeukaryotic
629 communities (based on RDA) during the study period. The dashed line indicates rain that
630 separated the study period into a dry and wet period.
631
632 **Figure 3.** Temporal variation of metacommunity types of the bacterial and microeukaryotic
633 datasets. Within the dotted lines ($-1.96 <$ coherence z-value < 1.96) metacommunities are
634 randomly structured. Positive significant values (coherence z-value > 1.96) indicate that

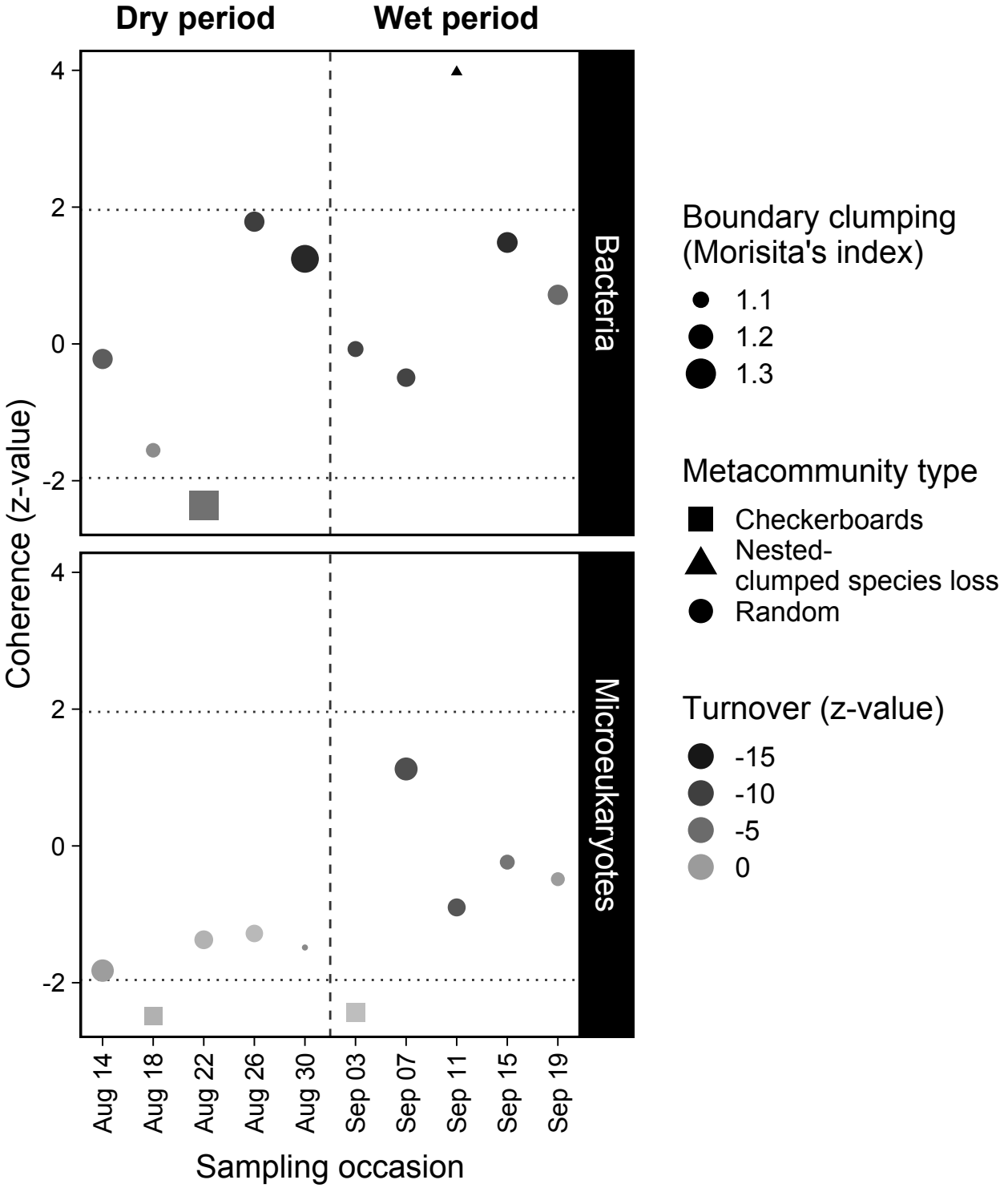
635 species' distribution occur in response to environmental variation. Significantly negative
636 coherence (coherence z-value < −1.96) indicates checkerboard distribution. The greyscale
637 represents species turnover (z-value; number of observed replacements compared to a null
638 distribution) where positive values indicate species replacements in response to environmental
639 variation and negative values nested species distributions caused by species losses. The size
640 of the symbols denotes the Morisita's index (boundary clumping) which shows the degree of
641 spatial distribution of species in a metacommunity where lower numbers indicate over-
642 dispersed boundaries and higher numbers clumped boundaries. Vertical dashed lines refer to
643 the division between the dry and wet period.
644
645 **Figure 4.** Variation of incidence-based (Raup-Crick) beta-diversity (β_{RC}) for bacteria and
646 microeukaryotic communities during the study period. Dashed line refers to the division
647 between dry and wet period.
648
649 **Figure 5.** Overall (A, B) and temporal (C, D) dynamics of the relative importance of different
650 community assembly processes expressed as the proportion of community pairs assembled
651 either by species-sorting (variable or homogeneous selection), dispersal limitation or
652 historical contingency, homogenizing dispersal or drift for bacteria (A, C) and
653 microeukaryotic (B, D) communities. Note that the scales are not equal on the C and D facet
654 plot. The dashed lines refer to the division between the dry and wet period, and red asterisks
655 indicate significant differences between them (Kruskal-Wallis test, significance at $p < 0.05$
656 level).
657
658 **Figure 6.** Mantel (Pearson) correlations between bacterial and microeukaryotic community
659 dissimilarities (abundance-based Raup-Crick beta-diversity – β_{RCbray}) and (A) environmental

660 and (B) geographic distances (Euclidean distances) for each sampling occasion. The dashed

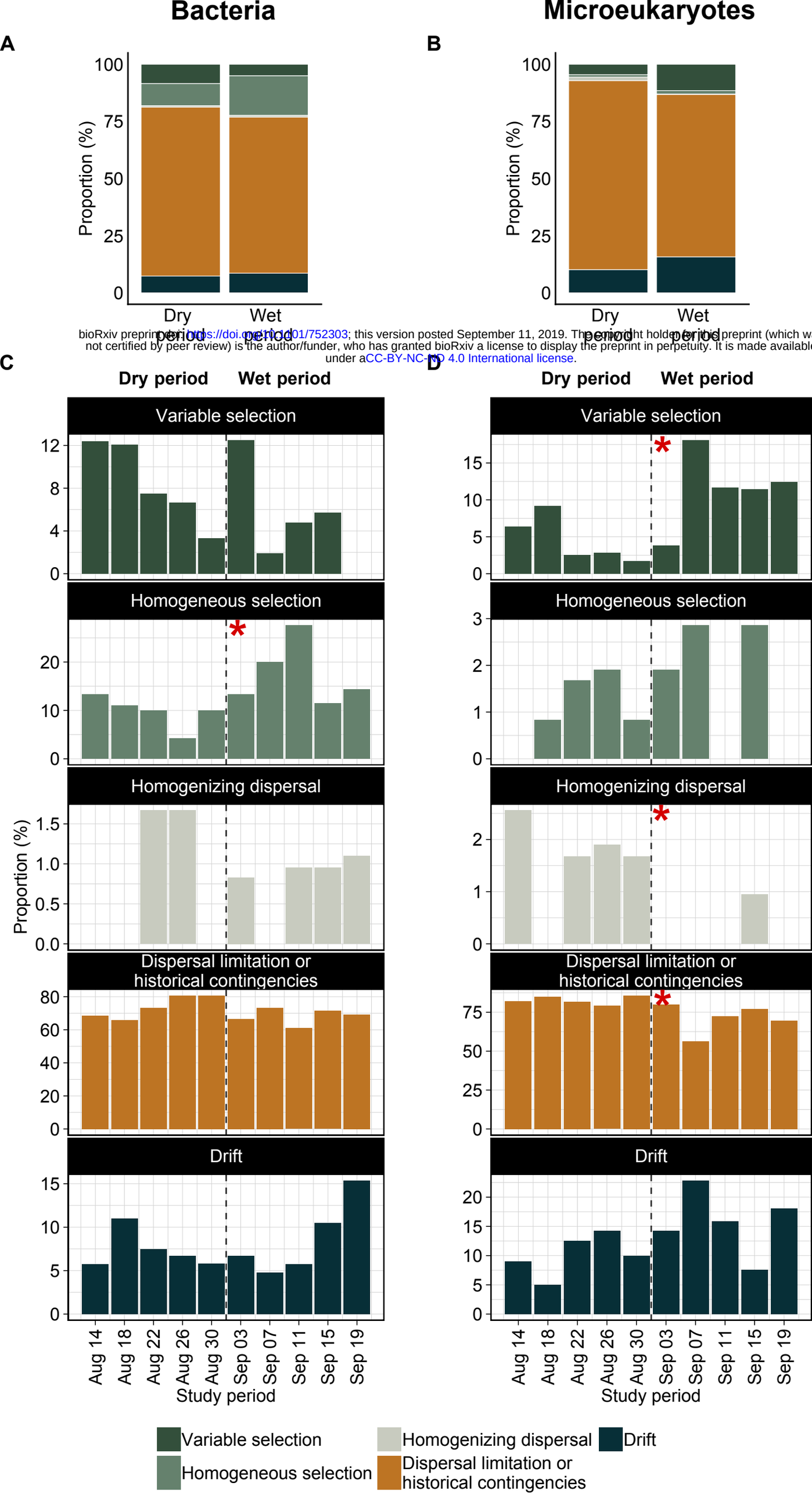
661 lines refer to the division between dry and wet period.

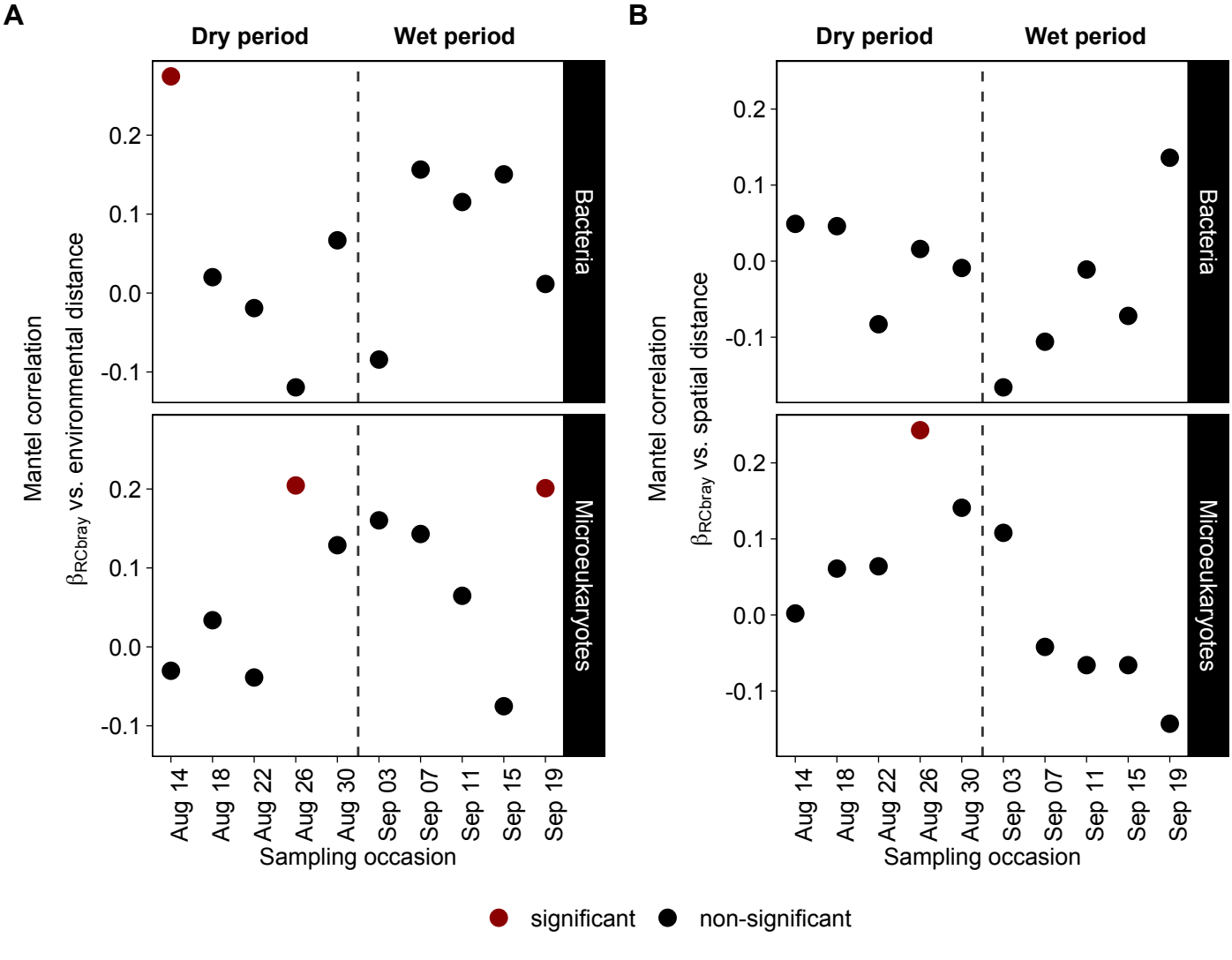
662


Dry period
Wet period
Water temperature (°C)
Depth (cm)
Conductivity (µS/cm)
Total phosphorus (µg/L)
Total nitrogen (µg/L)
Daphnia abundance (ind/L)
Copepoda abundance (ind/L)
Mean±SD
Aug 14
Aug 18
Aug 22
Aug 26
Aug 30
Sep 03
Sep 07
Sep 11
Sep 15
Sep 19
Sampling occasion


Dry period
Wet period
Bacteria
Microeukaryotes
Coherence (z-value)
4
2
0
-2
Aug 14
Aug 18
Aug 22
Aug 26
Aug 30
Sep 03
Sep 07
Sep 11
Sep 15
Sep 19
Sampling occasion
Boundary clumping (Morisita's index)
1.1
1.2
1.3
Metacommunity type
Checkerboards
Nested-clumped species loss
Random
Turnover (z-value)
-15
-10
-5
0

Dry period
Wet period
Bacteria
Microeukaryotes
Incidence-based beta-diversity (±SE)
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
Aug 14
Aug 18
Aug 22
Aug 26
Aug 30
Sep 03
Sep 07
Sep 11
Sep 15
Sep 19
Sampling occasion




A
B
Dry period
Wet period
Bacteria
Microeukaryotes
Mantel correlation
βRCbray vs. environmental distance
βRCbray vs. spatial distance
0.2
0.1
0.0
-0.1
Aug 14
Aug 18
Aug 22
Aug 26
Aug 30
Sep 03
Sep 07
Sep 11
Sep 15
Sep 19
Sampling occasion
significant
non-significant