



HAL
open science

Ecosystem productivity is associated with bacterial phylogenetic distance in surface marine waters

Pierre E. Galand, Ian Salter, Dimitri Kalenitchenko

► **To cite this version:**

Pierre E. Galand, Ian Salter, Dimitri Kalenitchenko. Ecosystem productivity is associated with bacterial phylogenetic distance in surface marine waters. *Molecular Ecology Notes*, 2015, 24 (23), pp.5785-5795 10.1111/mec.13347 . hal-01205185

HAL Id: hal-01205185

<https://hal.sorbonne-universite.fr/hal-01205185>

Submitted on 25 Sep 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Ecosystem productivity is associated with bacterial phylogenetic distance in**
2 **surface marine waters**

3
4 Pierre E. Galand^{1*}, Ian Salter^{2,3}, Dimitri Kalenitchenko¹

5 ¹ Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Ecogéochimie des
6 Environnements Benthiques (LECOB), Observatoire Océanologique de Banyuls, F-66650,
7 Banyuls sur Mer, France

8 ² Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Océanographie
9 Microbienne (LOMIC), Observatoire Océanologique de Banyuls, F-66650 Banyuls sur Mer,
10 France

11 ³ Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany

12
13 *Correspondence to: Pierre Galand, Observatoire Océanologique de Banyuls, Banyuls sur
14 Mer, France. mail: pierre.galand@obs-banyuls.fr. phone: +33 430192451

15

16

17

18

19

20

21

22

23

24

25 **Abstract**

26 Understanding the link between community diversity and ecosystem function is a
27 fundamental aspect of ecology. Systematic losses in biodiversity are widely acknowledged
28 but the impact this may exert on ecosystem functioning remains ambiguous. There is growing
29 evidence of a positive relationship between species richness and ecosystem productivity for
30 terrestrial macroorganisms, but similar links for marine microorganisms, which help drive
31 global climate, are unclear. Community manipulation experiments show both positive and
32 negative relationships for microbes. These previous studies rely, however, on artificial
33 communities and any links between the full diversity of active bacterial communities in the
34 environment, their phylogenetic relatedness, and ecosystem function remains hitherto
35 unexplored. Here we test the hypothesis that productivity is associated to diversity in the
36 metabolically active fraction of microbial communities. We show in natural assemblages of
37 active bacteria that communities containing more distantly related members were associated
38 with higher bacterial production. The positive phylogenetic diversity–productivity
39 relationship was independent of community diversity calculated as the Shannon index. From
40 our long-term (7-year) survey of surface marine bacterial communities we also found that
41 similarly productive communities had greater phylogenetic similarity to each other, further
42 suggesting that the traits of active bacteria are an important predictor of ecosystem
43 productivity. Our findings demonstrate that the evolutionary history of the active fraction of a
44 microbial community is critical for understanding their role in ecosystem functioning.

45

46

47

48

49

50 **Introduction**

51 The effect of biodiversity on ecosystem functioning is a key topic in ecology pertaining to the
52 current biodiversity crisis and mounting evidence that biodiversity loss reduces ecosystem
53 function (Cardinale *et al.* 2012; Naeem *et al.* 2009). The majority of current research on the
54 biodiversity – ecosystem functioning (BEF) relationship has focused on plants and
55 manipulated plots (Balvanera *et al.* 2006). Although a positive relationship is generally
56 observed (Cardinale *et al.* 2012; Gillman & Wright 2006), negative, U-shaped, and absent
57 relationships have all been reported (Adler *et al.* 2011; Balvanera *et al.* 2006; Gillman &
58 Wright 2006). In the oceans, species richness of macro-organisms appears to enhance
59 ecosystem function (Worm *et al.* 2006). However, it is widely acknowledged that marine
60 ecosystems represent the largest knowledge gap in BEF studies despite providing a number of
61 key services (Naeem 2012).

62 In the context of BEF relationships, ecosystem function is a rather generic concept that
63 requires clarification and perhaps distinction from ecosystem services (e.g. (Worm *et al.*
64 2006)), the latter representing an anthropocentric viewpoint. For plants, ecosystem function
65 has largely been addressed as productivity (Gillman & Wright 2006), which accurately
66 reflects their role in structuring ecosystems. Marine bacterial communities, however, mediate
67 a wide range of biogeochemical pathways that contribute to ecosystem function, including but
68 not limited to metabolic balance through respiration (Del Giorgio *et al.* 1997), nutrient
69 regeneration (Francis *et al.* 2005; Martens-Habbena *et al.* 2009) and the production of
70 climatically active gases (Carini *et al.* 2014; Howard *et al.* 2008). In the present study we
71 define microbial ecosystem function as heterotrophic production estimated from leucine
72 incorporation (Kirchman *et al.* 1985). This facilitates a direct comparison with primary
73 production and provides a rate measurement to establish a conceptual framework linking
74 diversity-productivity relationships in heterotrophic bacterial communities.

75 Marine bacteria provide significant ecosystem services through the breakdown and
76 mineralization of organic matter, which in turn generates climatically active gases and
77 influences trophic energy transfer. Despite the ecological significance of bacterial processes,
78 the importance of the enormous diversity contained within marine bacterial communities for
79 ecosystem productivity remains ambiguous. BEF research on marine microbes is scarce even
80 though they represent good community models to test ecological theory and often follow
81 ecological patterns similar to macroorganisms (Fuhrman 2009; Martiny *et al.* 2006). The BEF
82 relationship for microbes is hypothesised to represent the form of a saturating curve (Naeem
83 2012) and initial experiments based on artificial communities do display positive relationships
84 (Bell *et al.* 2005; Gravel *et al.* 2011; Hodgson *et al.* 2002). However, contrasting results show
85 a negative or no relationship between bacterial richness and productivity in both the natural
86 environment (Obenosterer *et al.* 2010; Reinthaler *et al.* 2005) and controlled experimental
87 settings (Becker *et al.* 2012; Horner- Devine *et al.* 2003). The ambiguity of these findings
88 may reflect a number of confounding factors including evolutionary history (Gravel *et al.*
89 2011) or the dominance effect (Hodgson *et al.* 2002), which says that more diverse
90 communities are more likely to harbour highly productive species.

91 Phylogenetic diversity can be considered as a measure of the ecological differences
92 between species (Cavender- Bares *et al.* 2009; Mouquet *et al.* 2012) and is also a good
93 predictor of ecosystem productivity (Cadotte 2013; Cadotte *et al.* 2008; Gravel *et al.* 2012).
94 However, in order to use phylogenetic diversity to predict ecological function a number of
95 key assumptions should be respected. These assumptions have been recently reviewed and the
96 use of phylogenetic diversity in ecosystem function studies was critically re-examined
97 (Narwani *et al.* 2015). Nevertheless, phylogenetic diversity has recently been proposed as a
98 better index than richness for conservation policy because it takes into account the
99 evolutionary uniqueness of a species, and phylogenetic based conservation can help

100 maintaining a better ecosystem functioning (Mouquet *et al.* 2012; Rolland *et al.* 2012). In
101 studies of ecology the use of evolutionary information could be a good alternative to trait-
102 based approaches of the BEF relationship, which include the phenotypic characteristics of
103 individuals that impact their fitness (Mouquet *et al.* 2012; Srivastava *et al.* 2012). Including
104 estimates of community trait or evolutionary information may help to disentangle the two
105 main drivers of the BEF relationship: the complementary effect, for which high diversity
106 communities efficiently use resources through positive interactions and/or niche partitioning,
107 versus the selection or sampling effect, whereby diverse community are more likely to contain
108 highly productive organisms (Loreau & Hector 2001). In artificial microbial communities a
109 positive relationship between phylogenetic distance and productivity (Jousset *et al.* 2011;
110 Venail & Vives 2013) disappears under certain evolutionary conditions (Gravel *et al.* 2012).

111 In the present study we use a long-term microbial time-series from Mediterranean
112 surface waters (MOLA, Microbial Observatory of Laboratoire Arago) to test the hypotheses
113 that (i) productivity is associated to diversity in the metabolically active fraction of bacterial
114 communities and (ii) phylogenetic diversity is a better predictor of bacterial productivity than
115 comparative measures of community diversity. The basis of our hypotheses reflects two
116 fundamental facets of microbial ecosystems. First, a significant fraction of microbial diversity
117 may be present as inactive or dormant cells (Lennon and Jones 2011), thereby limiting their
118 contribution to ecosystem function. Active and inactive microbial communities have also
119 been shown to have different phylogenetic structure (DeAngelis & Firestone 2012). Second,
120 the breakdown of complex organic substrates relies on complementary microbial consortia
121 possessing a wide range of traits. Addressing these questions with natural bacterial
122 communities should negate any uncertainties associated to artificial selection of bacteria
123 grown on culture media. Further, the use of data obtained from a microbial observatory
124 provides a robust framework for the study, as repeated measures from a single location

125 strongly limits possible bias associated to fluctuating conditions in nature. Pyrosequencing of
126 the 16S ribosomal RNA genes and 16S ribosomal RNA was carried out to compare the entire
127 bacterial assemblage, referred to as “standing stock”, to the “active fraction”. Parallel to
128 measurements of phylogenetic structure within bacterial communities, the incorporation of
129 tritiated-leucine was used as a direct rate estimate of community productivity.

130

131 **Materials and Methods**

132 **Sample collection**

133 Seawater was collected at approximately monthly resolution from July 2004 to April 2011 at
134 3-5 m depth at the MOLA (Microbial Observatory of Laboratoire Arago) station off Banyuls
135 sur Mer (42°27'205 N – 03°32'565 E). Occasionally samples were not collected due to poor
136 weather conditions. A subset of 41 field samples were selected for the diversity/production
137 comparison, as production was not always measured during the course of the MOLA
138 monitoring. Seasonal patterns of community similarity and environmental conditions were
139 followed on 57 samples. As described earlier (Salter *et al.* 2015), the water sampled with a
140 12-L Niskin bottle was kept in 10-L high-density polyethylene carboys in the dark until being
141 processed in the laboratory (within 1.5 h). The microbial biomass was collected on 0.22- μm
142 pore-size GV Sterivex cartridges (Millipore) from 10 L of seawater after prefiltration through
143 3- μm pore-size polycarbonate filters (Millipore). Filters were stored at $-80\text{ }^{\circ}\text{C}$ until nucleic
144 acid extraction. In-situ temperature and salinity were obtained using a Seabird CTD SBE9/11.
145 Chlorophyll a concentrations were measured from one liter of seawater collected on a GF/F
146 filter at low pressure ($<0.2\text{ bar}$). Following filtration, samples were processed immediately or
147 stored at $-20\text{ }^{\circ}\text{C}$ for a period < 1 week. Upon processing, samples were soaked in 90% acetone
148 at $4\text{ }^{\circ}\text{C}$ for a period of approximately 12-16 h and processed within 2 h. Filters were soaked in

149 acetone for 24 h at 4°C. Fluorescence was measured before and after acidification to correct
150 for phaeopigments.

151

152 **Bacterial production**

153 Bacterial production was determined by ³H-leucine incorporation using the centrifugation
154 method (Smith & Azam 1992). Subsamples (1.5 mL; three replicates and two blanks killed
155 with 50% of trichloroacetic acid (TCA)) were incubated for 2 h in the dark at in situ
156 temperature with a mixture of ³H-leucine (Perkin Elmer, (SA) 115.4 Ci mmol⁻¹) and non-
157 radioactive leucine at final concentrations of 7 and 13 nM, respectively. Incubations were
158 stopped by the addition of TCA to a final concentration of 5%. After a centrifugation at
159 13,300Xg for 15 min, the supernatant was discarded, and 0.5 mL of 5% TCA were added.
160 This step was applied twice with a second centrifugation for 5 min. Ethanol (0.5 mL of 70%)
161 were added prior to the last centrifugation for 5 min. The supernatant was discarded, and 1
162 mL of PCS liquid scintillation cocktail was added. The radioactivity incorporated into
163 bacterial cells was measured with a LS 6500 Beckman liquid scintillation counter.

164

165 **Nucleic acid extraction and pyrosequencing**

166 The present study relies on the sequence data originally published in the study by Salter and
167 colleagues (2015). The nucleic acid extraction method followed (Hugoni *et al.* 2013) and
168 consisted of cell lysis with freshly prepared lysozyme solution (20 mg mL⁻¹) applied directly
169 to Sterivex cartridges, and a second incubation after adding proteinase K (20 mg mL⁻¹),
170 followed by extraction using the AllPrep DNA/RNA kit (Qiagen), which gave average DNA
171 concentrations of 28 ng μL⁻¹. The RNA samples were tested for the presence of contaminating
172 genomic DNA by PCR and then reverse-transcribed with random primers using the
173 SuperScript III Reverse Transcriptase kit (Invitrogen). The amplification of the V1–V3 region

174 of the 16S rRNA gene was performed by a commercial laboratory (Research and Testing
175 Laboratory, Lubbock, TX) with universal bacterial primers 28F (TTTGATCNTGGCTCAG)
176 and 519R (GTNTTACNGCGGCKGCTG), followed by pyrosequencing using a Roche 454
177 GS-FLX system with titanium chemistry. All sequences have been submitted to the sequence
178 read archive (SRA) under the Bioproject accession number: PRJNA235253 (Salter *et al.*
179 2015).

180

181 **Sequence data analyses**

182 Sequences were analyzed as described earlier (Blanquer *et al.* 2013). Briefly, sequences were
183 first filtered by removing low quality reads, then trimmed to remove reads having $\geq 3\%$ of
184 bases with Phred values < 27 (0.2% per-base error probability). This is recommended to
185 ensure that when clustering at 97%, the influence of erroneous reads is minimized (Huse *et al.*
186 2010). Sequences were then clustered at a 97% threshold using the Uclust algorithm (Edgar
187 2010). Sequences from each OTU were classified by comparison to the Greengenes database
188 (DeSantis *et al.* 2006). Sequence analyses were conducted with Pyrotagger (Kunin &
189 Hugenholtz 2010). Sequences affiliated to chloroplasts were removed but cyanobacterial
190 sequences were kept. For diversity analysis, all samples were randomly re-sampled to the size
191 of the sample containing fewest sequences ($n = 446$) using Daisy Chopper (Gilbert *et al.*
192 2009). Resampling allows a comparison of bacterial communities without bias associated to
193 varying sampling size.

194

195 **Diversity measures**

196 The Shannon diversity index (H) was calculated using the software PAST v2.17 (Hammer *et*
197 *al.* 2001). In the calculation of phylogenetic diversity, 300 bp long representative sequences
198 for each OTU were aligned using MUSCLE (Edgar 2004) and the alignment was then cleaned

199 to remove non overlapping sequence regions. A phylogenetic tree was constructed using
200 FastTree (Price *et al.* 2010).

201 Phylogenetic diversity (PD) (Faith 1992) is the most common measure of phylogenetic
202 diversity but since the number of taxa in a sample affects PD, and because the number of taxa
203 varied significantly between our samples, we computed a standardized measure of
204 phylogenetic diversity. The standardized effect size (SES) of the phylogenetic diversity is
205 equivalent to -1 times the Nearest Relative Index (NRI) (Webb *et al.* 2002). It was calculated
206 as:

$$SES_{MPD} = \frac{MPD_{observed} - mean(MPD_{randomizations})}{sd(MPD_{randomizations})}$$

207
208 where MPD is the mean pairwise phylogenetic distance among taxa within a community
209 weighted by taxa abundance (Webb *et al.* 2008). We chose to weight MPD by taxa abundance
210 to account for the structure of environmental marine bacteria communities, which are
211 characterized few abundant taxa and a very large number of rare taxa. The observed
212 phylogenetic diversity is compared to the average (mean) phylogenetic diversity in a
213 randomly generated community (null model) and divided by the standard deviation (sd) of
214 phylogenetic distances in the null model. The null model randomizes community data matrix
215 with the independent swap algorithm maintaining species occurrence frequency and sample
216 species richness (Kembel 2009). For comparison, we also computed the unweighted MPD.

217 Positive SES values indicate greater phylogenetic distance among co-occurring
218 species than expected by chance while negative values indicate small phylogenetic distance.
219 Phylogenetic diversity was computed using the Picante package (Kembel *et al.* 2010) in R.

220

221 **Statistics**

222 Linear relationships between productivity and diversity were tested using ordinary least
223 squares regression (OLS) models and the statistical significance of models described with F
224 statistics. All statistical computations were performed in R. A Breusch-Pagan test for
225 heteroskedasticity was conducted on the residuals to verify that the assumption of
226 homoscedasticity was met, and residuals were tested for normality by examining the quantiles
227 of a standard normal distribution against the corresponding quantiles of the observed data (Q-
228 Q plot). The most homoscedastic and normally distributed residuals were found in linear
229 models using a log transformation of bacterial production. If linear models were not
230 significant polynomial quadratic functions were tested to detect possible curvilinear
231 relationships. Productivity-diversity relationships were considered to be non-significant when
232 model fits to either linear or curvilinear regressions were not significant.

233 In order to test if there was a correlation between the phylogenetic structure of a
234 community and the level of bacterial production we measured the phylogenetic distance
235 between each community (sample) by quantifying the mean phylogenetic distance between
236 each OTU in one community and its closest relative in a second community. Distances were
237 computed using R with the `comdistnt` function of the `picante` package (Webb *et al.* 2008).
238 Similarly, to measure similarity between community composition (beta diversity) and
239 bacterial production we also calculated the Bray-Curtis index based on abundance data and
240 the Sorensen index based on presence-absence data. Differences in bacterial heterotrophic
241 production between communities were calculated as a Euclidian distance. A mantel test (999
242 permutations) was used to determine whether community composition similarity followed
243 differences in bacterial production.

244 We use canonical correspondence analysis (CCA) to explore the relationship between
245 community composition and the following environmental parameters: salinity, chlorophyll a,
246 nitrate, nitrite, ammonium, phosphate and silicate. The community data matrices were

247 converted using a Hellinger transformation prior to analysis and environmental data were log
248 transformed (Legendre & Gallagher 2001). The significance of the CCA results was tested by
249 permutation test. Analyses were conducted in R with the vegan package.

250 In order to detect possible seasonal patterns in bacterial community composition we
251 carried out an autocorrelation analysis to look at the similarity between Bray-Curtis values,
252 calculated between each bacterial communities, as a function of time, in number of days,
253 which separates two samples. The Lomb periodogram algorithm implemented in Past v2.17
254 (Hammer *et al.* 2001) was used to detect if there were seasonal patterns of community
255 diversity and to identify the frequency of the pattern when detected.

256

257 **Results**

258 A total of 66 705 16S rDNA and 57 524 rRNA sequences were obtained after
259 performing quality filtering and yielded a total of 2222 OTUs. SAR11 sequences were the
260 most abundant, with an average contribution of 78 and 45% in the rDNA and rRNA fractions,
261 followed by Cyanobacteria. The detailed seasonal patterns are described in Salter *et al.* 2015.
262 Overall, the oligotrophic Northwestern Mediterranean was characterized by a marked
263 seasonality with a peak of bacterial production that closely succeeded chlorophyll a maxima
264 and preceded temperature maxima. In winter, microbes from deeper layers were introduced to
265 surface waters (Figure 1).

266 Through the analysis of a seven year monthly time-series of surface bacterial
267 communities we observed no linear and no unimodal (quadratic) relationship between the
268 diversity of active bacteria communities, calculated as the Shannon index, and bacterial
269 production ($F_{1,39}=0.60$, $P=0.44$) (Figure 2a). Considering different metrics of diversity, like
270 OTU richness, did not influence the results (Figure S1a, Table S1). In contrast, the
271 phylogenetic diversity of the active communities, calculated as average observed

272 phylogenetic diversity between taxa, was positively correlated to bacterial production
273 ($F_{1,39}=10.86$, $P=0.003$)(Figure 2b, Table S1). When phylogenetic diversity was not weighted
274 by taxa abundance, there was no relationship between production and phylogenetic diversity
275 (Figure S2).

276 The diversity (Shannon's index) of the standing stock was negatively correlated to
277 bacterial production ($F_{1,44}=13.87$, $P<0.01$)(Figure 2c) and the phylogenetic diversity was not
278 associated to bacterial production (Figure 2d).

279 We also investigated if active community composition was related to ecosystem
280 productivity and found a positive relationship between difference in bacterial production and
281 phylogenetic distance between active communities ($r\text{-Mantel}=0.24$, $P<0.01$) (Figure 3a). The
282 similarity in community composition of the active fraction, calculated as the Bray-Curtis or
283 Sorensen index, was also associated to differences in bacterial production ($r\text{-Mantel}=0.34$,
284 $P<0.01$)(Figure S3). The association did not hold for the standing stock of Bacteria (Figure
285 3b).

286 We examined how predictable the bacterial community composition was from year to
287 year in the surface Mediterranean Sea by calculating a Bray-Curtis distance between each pair
288 of samples and then conducting an autocorrelation analysis (Fig. 4a, c). For the active fraction
289 (rRNA), we observed a highly reproducible seasonal composition. Samples taken one year
290 apart had the most similar community composition, shown by the highest correlation values,
291 and communities separated by 6 months were the most different as shown by the lowest
292 correlation values (Figure 4a). Inversely, the standing stock (rRNA gene copies) patterns of
293 high and low correlation values didn't have a significant regular frequency, which indicates
294 that communities did not exhibit regular patterns of community similarity (Figure 4c). As for
295 community diversity, the active fraction did not show a significant regular seasonal pattern
296 (Figure 4b) while the standing stock did (Figure 4d, Figure S4).

297 Finally, overall, active bacteria appear to have a stronger association to environmental
298 conditions than the standing stock (Table S2).

299

300 **Discussion**

301 The linear increase of productivity with phylogenetic diversity (Figure 2) suggests that
302 assemblages containing more distantly related species are associated to higher community
303 productivity, maybe through more efficient utilization of growth resources. Assuming that
304 trait dissimilarity is correlated with evolutionary time (Cadotte *et al.* 2009; Connolly *et al.*
305 2011), bacteria with distant common ancestors are more likely to be ecologically different.
306 Through competitive exclusion any such ecological differentiation would promote distinct
307 species, or groupings of species with reduced niche overlap (e.g. (Spehn *et al.* 2005; Tilman
308 *et al.* 2001)) that can utilize a wide range of substrates associated with enhanced community
309 productivity. Our findings from a natural environment adds to recent reports from artificial
310 bacterial communities showing that higher functional dissimilarity can increase ecosystem
311 functioning through a better use of resources, especially in complex resource environments
312 (Jousset *et al.* 2011; Venail & Vives 2013). Alternatively, a phylogenetically diverse
313 community may reflect functional complementarity between species collaborating for an
314 efficient use of resources (Cavender- Bares *et al.* 2009). Our data did not help us separate
315 these hypotheses as we could not specifically identify taxa always associated to high
316 phylogenetic diversity scenarios (not shown). Nevertheless, such positive interactions are
317 especially relevant for microbial communities in which substrates are often used through a
318 cascade of commensal or mutualistic organisms and consumption of secondary metabolites.
319 Alternatively to these two hypotheses, the causation could go the other direction and more
320 productive communities may select for bacteria that are more phylogenetically diverse.

321 In the oligotrophic Northwestern Mediterranean, the peak of heterotrophic bacterial
322 production closely succeeds chlorophyll a maxima and precedes temperature maxima,
323 indicating that substrates derived from the decaying phytoplankton promote high levels of
324 community productivity (Figure 1). The production of a wide range of organic carbon
325 substrates from zooplankton grazing and phytoplankton lysis (e.g. (Van Wambeke 1994))
326 may be the initial trigger for facilitating the development of a phylogenetically diverse
327 assemblage. The presence of diverse assemblages would fit the recent genome streamlining
328 theory, which suggests a more efficient use of nutrients in microbial communities composed
329 of different but highly connected microorganisms (Giovannoni *et al.* 2014). Many microbial
330 pathways may leak metabolites that can escape the cell and become available to other
331 members of the community (Morris *et al.* 2012). The prevalence of genome streamlining in
332 the oceans together with specialization in resource utilization (Swan *et al.* 2013) are
333 additional arguments for the presence of interdependent microorganisms within marine
334 communities.

335 In turn, the negative phylogenetic diversity values associated to low productivity times
336 can be interpreted as communities structured by environmental filtering. The environment
337 selects a subset of ecologically similar taxa able to thrive under specific environmental
338 conditions. In the Northwestern Mediterranean, such filter could be the lower winter
339 temperature, the higher nitrogen availability or other factors that we did not measure.
340 However, the temporal resolution (monthly) of our observations within a natural ecosystem
341 renders it challenging to identify mechanisms of causality between phylogenetic diversity and
342 productivity.

343 Another important ecological question is whether the relationship between
344 phylogenetic diversity and community production is due to a strong co-variation with
345 community richness (Mouquet *et al.* 2012). Co-variation is not supported by our data showing

346 that bacterial assemblage diversity was not correlated to productivity (Figure 2). In contrast,
347 manipulation experiments frequently describe positive relationships between diversity and
348 productivity (Bell *et al.* 2005; Gravel *et al.* 2011; Hodgson *et al.* 2002). We infer that the
349 biodiversity – ecosystem functioning (BEF) relationship observed in a natural system is
350 influenced by complex interactions taking place between individuals and environmental
351 factors that cannot be accurately reproduced using artificial communities in a laboratory
352 setting. The unique effect of phylogenetic diversity suggests that increased productivity can
353 be explained by complementary rather than sampling effect. Sampling a deep branching or a
354 clustered community, at similar richness level, would in theory result in a similar probability
355 of sampling more productive taxa.

356 The positive relationship between differences in bacterial production and phylogenetic
357 distance between communities (Figure 3) indicates that similar assemblages of active bacteria
358 are associated to comparable levels of community production, and specifically that the
359 communities that were the most different had distinct productivity levels. In support of our
360 other findings these results show that the identity of the active bacteria composing bacterial
361 assemblages, and consequently the ecological traits of individuals rather than community
362 diversity, is essential for predicting productivity. Reports of relationships between community
363 composition and ecosystem function for natural bacterial communities are rare. They show
364 only weak or no coupling between communities and ecosystem function, thus implying a
365 certain degree of functional redundancy (Frossard *et al.* 2011; Langenheder *et al.* 2005).
366 Opposingly, based on our findings of a highly reproducible seasonal composition of active
367 communities we argue that active bacterial communities in a defined ecosystem exhibit little
368 functional redundancy and that the non-active members of a community (e.g. standing stock)
369 mask the relationship between composition and function. Active bacteria appear to have a
370 stronger association to biogeochemical forcing than the standing stock further suggesting that

371 ecosystem productivity is associated to a specific pool of active bacteria that could respond
372 predictably to seasonality in environmental conditions.

373 It should be noted that there is still some ambiguity as to whether 16S rRNA is a
374 reliable metric of metabolically active cells. The correlation between rRNA copy number and
375 real time activity can be inconsistent in environmental samples and rRNA has been suggested
376 to represent a protein synthesis potential rather than a direct indicator of metabolic state
377 (Blazewicz *et al.* 2013). Our previous work on the MOLA microbial Observatory data shows,
378 however, a correlation between SAR11 RNA/DNA ratio and single cell activity measured by
379 fluorescence in-situ hybridisation coupled with microautoradiography (MICRO-CARD-FISH)
380 (Salter *et al.* 2015). It supports the idea that RNA sequence data might be a useful metric for
381 tracking the general metabolic activity of these communities.

382 Contrary to our observations on active bacteria, the diversity of the standing stock was
383 negatively correlated to bacterial production. In the surface waters of the Mediterranean this
384 negative relationship reflects a physical process with significant ecological consequences. In
385 winter, wind-induced breakdown of water column stratification introduces microbes from
386 deeper layers (Salter *et al.* 2015). This seasonal vertical mixing occurs each year and although
387 it enhances diversity of the standing stock, as deep Bacteria are probably not active at the
388 surface, it does not result in a predictable community composition or a systematic increase in
389 diversity of active bacterial communities. This result from our off shore Mediterranean site
390 contrasts with earlier reports of strongly predictable patterns of bacterial community
391 composition (Chow *et al.* 2013; Fuhrman *et al.* 2006).

392 The contrasting patterns of standing stock and active communities reflect the
393 specificity of microbial communities that are frequently composed of inactive organisms able
394 to survive long periods of with reduced activity or in a dormant stage (Lennon & Jones 2011).
395 We argue that the existence of a large number of microorganisms that don't contribute to

396 ecosystem productivity underpins a fundamental dichotomy between micro- and macro-
397 ecology. These features of microbial community diversity and productivity need to be taken
398 into account when using microbes as model communities and transferring ecological theories
399 to microorganisms.

400 In summary, our results present strong support for a positive relationship between
401 phylogenetic diversity, independent of community diversity, and productivity in natural
402 communities, and stress the importance of community structure for predicting ecosystem
403 function. We also emphasize that the community diversity-productivity relationship observed
404 for the standing stock of bacteria was different from the one expressed by the active fraction,
405 and argue that active microorganisms need to be targeted in ecological studies, especially in
406 dynamic ecosystems, for an unbiased comparison of micro- and macro-ecology. Using this
407 approach in the future should lead to a better understanding of oscillations in the ocean
408 services provided by marine microbial communities and improve the predictive capacity of
409 models linking environmental and anthropogenic change to community diversity and
410 productivity.

411

412 **Acknowledgments**

413 The work of P.E.G. was supported by the Agence Nationale de la Recherche (ANR) through
414 the projects MICADO (ANR-11-JSV7-003-01) and EUREKA (ANR-14-CE02-0004-01). I.S.
415 was jointly supported by a LEFE-CYBER grant and LOMIC 500 micro-project grant. We
416 thank Cyrielle Tricoire, Eric Maria and Louise Oriol for assisting with the collection and
417 analysis of samples for the physical and chemical parameters over the time-series. We extend
418 our acknowledgments to all the researchers that were involved in working with the time-series
419 over the years.

420

421 **References**

- 422 Adler PB, Seabloom EW, Borer ET, *et al.* (2011) Productivity is a poor predictor of plant species richness.
 423 *Science*, **333**, 1750-1753.
- 424 Balvanera P, Pfisterer AB, Buchmann N, *et al.* (2006) Quantifying the evidence for biodiversity effects on
 425 ecosystem functioning and services. *Ecology Letters*, **9**, 1146-1156.
- 426 Becker J, Eisenhauer N, Scheu S, Jousset A (2012) Increasing antagonistic interactions cause bacterial
 427 communities to collapse at high diversity. *Ecology Letters*, **15**, 468-474.
- 428 Bell T, Newman JA, Silverman BW, Turner SL, Lilley AK (2005) The contribution of species richness and
 429 composition to bacterial services. *Nature*, **436**, 1157-1160.
- 430 Blanquer A, Uriz MJ, Galand PE (2013) Removing environmental sources of variation to gain insight on
 431 symbionts vs. transient microbes in High and Low Microbial Abundance sponges. *Environmental*
 432 *Microbiology*, **15**, 3008-3019.
- 433 Blazewicz SJ, Barnard RL, Daly RA, Firestone MK (2013) Evaluating rRNA as an indicator of microbial
 434 activity in environmental communities: limitations and uses. *ISME Journal*, **7**, 2061-2068.
- 435 Cadotte MW (2013) Experimental evidence that evolutionarily diverse assemblages result in higher productivity.
 436 *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 8996-9000.
- 437 Cadotte MW, Cardinale BJ, Oakley TH (2008) Evolutionary history and the effect of biodiversity on plant
 438 productivity. *Proceedings of the National Academy of Sciences of the United States of America*, **105**,
 439 17012-17017.
- 440 Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH (2009) Using phylogenetic, functional and trait diversity
 441 to understand patterns of plant community productivity. *PLoS ONE*, **4**, e5695.
- 442 Cardinale BJ, Duffy JE, Gonzalez A, *et al.* (2012) Biodiversity loss and its impact on humanity. *Nature*, **486**, 59-
 443 67.
- 444 Carini P, White AE, Campbell EO, Giovannoni SJ (2014) Methane production by phosphate-starved SAR11
 445 chemoheterotrophic marine bacteria. *Nature communications*, **5**.
- 446 Cavender- Bares J, Kozak KH, Fine PV, Kembel SW (2009) The merging of community ecology and
 447 phylogenetic biology. *Ecology Letters*, **12**, 693-715.
- 448 Chow C-ET, Sachdeva R, Cram JA, *et al.* (2013) Temporal variability and coherence of euphotic zone bacterial
 449 communities over a decade in the Southern California Bight. *The ISME journal*, **7**, 2259-2273.
- 450 Connolly J, Cadotte MW, Brophy C, *et al.* (2011) Phylogenetically diverse grasslands are associated with
 451 pairwise interspecific processes that increase biomass. *Ecology*, **92**, 1385-1392.
- 452 DeAngelis KM, Firestone MK (2012) Phylogenetic clustering of soil microbial communities by 16S rRNA but
 453 not 16S rRNA genes. *Applied and Environmental Microbiology*, **78**, 2459-2461.
- 454 Del Giorgio PA, Cole JJ, Cimleris A (1997) Respiration rates in bacteria exceed phytoplankton production in
 455 unproductive aquatic systems. *Nature*, **385**, 148-151.
- 456 DeSantis TZ, Hugenholtz P, Larsen N, *et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database
 457 and workbench compatible with ARB. *Applied and Environmental Microbiology*, **72**, 5069-5072.
- 458 Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity.
 459 *BMC Bioinformatics*, **5**, 113.
- 460 Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26**, 2460-2461.
- 461 Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1-10.
- 462 Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-
 463 oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy*
 464 *of Sciences of the United States of America*, **102**, 14683-14688.
- 465 Frossard A, Gerull L, Mutz M, Gessner MO (2011) Disconnect of microbial structure and function: enzyme
 466 activities and bacterial communities in nascent stream corridors. *The ISME journal*, **6**, 680-691.
- 467 Fuhrman JA (2009) Microbial community structure and its functional implications. *Nature*, **459**, 193-199.
- 468 Fuhrman JA, Hewson I, Schwalbach MS, *et al.* (2006) Annually reoccurring bacterial communities are
 469 predictable from ocean conditions. *Proceedings of the National Academy of Sciences of the United*
 470 *States of America*, **103**, 13104-13109.
- 471 Gilbert JA, Field D, Swift P, *et al.* (2009) The seasonal structure of microbial communities in the Western
 472 English Channel. *Environmental Microbiology*, **11**, 3132-3139.
- 473 Gillman LN, Wright SD (2006) The influence of productivity on the species richness of plants: a critical
 474 assessment. *Ecology*, **87**, 1234-1243.
- 475 Giovannoni SJ, Cameron Thrash J, Temperton B (2014) Implications of streamlining theory for microbial
 476 ecology. *ISME J*, **8**, 1553-1565.
- 477 Gravel D, Bell T, Barbera C, *et al.* (2011) Experimental niche evolution alters the strength of the diversity-
 478 productivity relationship. *Nature*, **469**, 89-92.

479 Gravel D, Bell T, Barbera C, *et al.* (2012) Phylogenetic constraints on ecosystem functioning. *Nature*
480 *communications*, **3**, 1117.

481 Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and
482 data analysis. *Palaeontologia Electronica*, **4**, 9.

483 Hodgson DJ, Rainey PB, Buckling A (2002) Mechanisms linking diversity, productivity and invasibility in
484 experimental bacterial communities. *Proceedings of the Royal Society B: Biological Sciences*, **269**,
485 2277-2283.

486 Horner-Devine MC, Leibold MA, Smith VH, Bohannan BJ (2003) Bacterial diversity patterns along a gradient
487 of primary productivity. *Ecology Letters*, **6**, 613-622.

488 Howard EC, Sun S, Biers EJ, Moran MA (2008) Abundant and diverse bacteria involved in DMSP degradation
489 in marine surface waters. *Environmental Microbiology*, **10**, 2397-2410.

490 Hugoni M, Taib N, Debroas D, *et al.* (2013) Structure of the rare archaeal biosphere and seasonal dynamics of
491 active ecotypes in surface coastal waters. *Proceedings of the National Academy of Sciences of the*
492 *United States of America*, **110**, 6004-6009.

493 Huse SM, Welch DM, Morrison HG, Sogin ML (2010) Ironing out the wrinkles in the rare biosphere through
494 improved OTU clustering. *Environmental Microbiology*, **12**, 1889-1898.

495 Jousset A, Schmid B, Scheu S, Eisenhauer N (2011) Genotypic richness and dissimilarity opposingly affect
496 ecosystem functioning. *Ecology Letters*, **14**, 537-545.

497 Kembel SW (2009) Disentangling niche and neutral influences on community assembly: assessing the
498 performance of community phylogenetic structure tests. *Ecology Letters*, **12**, 949-960.

499 Kembel SW, Cowan PD, Helmus MR, *et al.* (2010) Picante: R tools for integrating phylogenies and ecology.
500 *Bioinformatics*, **26**, 1463-1464.

501 Kirchman D, K'nees E, Hodson R (1985) Leucine incorporation and its potential as a measure of protein
502 synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology*, **49**, 599-
503 607.

504 Kunin V, Hugenholtz P (2010) PyroTagger: A fast, accurate pipeline for analysis of rRNA amplicon
505 pyrosequence data. *The Open Journal*, **1**.

506 Langenheder S, Lindström ES, Tranvik LJ (2005) Weak coupling between community composition and
507 functioning of aquatic bacteria. *Limnology and Oceanography*, **50**, 957-967.

508 Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data.
509 *Oecologia*, **129**, 271-280.

510 Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy.
511 *Nature Reviews Microbiology*, **9**, 119-130.

512 Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature*, **412**,
513 72-76.

514 Martens-Habbena W, Berube PM, Urakawa H, de La Torre JR, Stahl DA (2009) Ammonia oxidation kinetics
515 determine niche separation of nitrifying Archaea and Bacteria. *Nature*, **461**, 976-979.

516 Martiny JBH, Bohannan BJM, Brown JH, *et al.* (2006) Microbial biogeography: putting microorganisms on the
517 map. *Nature Reviews Microbiology*, **4**, 102.

518 Morris JJ, Lenski RE, Zinser ER (2012) The Black Queen Hypothesis: Evolution of Dependencies through
519 Adaptive Gene Loss. *mBio*, **3**.

520 Mouquet N, Devictor V, Meynard CN, *et al.* (2012) Ecophylogenetics: advances and perspectives. *Biological*
521 *reviews*, **87**, 769-785.

522 Naeem S (2012) Ecological consequences of declining biodiversity: a biodiversity–ecosystem function (BEF)
523 framework for marine systems. In: *Marine Biodiversity and Ecosystem Functioning: Frameworks,*
524 *methodologies, and integration* (eds. Solan M, Aspden RJ, Paterson DM), p. 256. Oxford University
525 Press, Oxford.

526 Naeem S, Bunker DE, Hector A, Loreau M, Perrings C (2009) *Biodiversity, ecosystem functioning, and human*
527 *wellbeing. An ecological and economic perspective*. Oxford University Press, Oxford.

528 Narwani A, Matthews B, Fox J, Venail P (2015) Using phylogenetics in community assembly and ecosystem
529 functioning research. *Functional Ecology*, **29**, 589-591.

530 Obernosterer I, Lami R, Larcher M, *et al.* (2010) Linkage between bacterial carbon processing and the structure
531 of the active bacterial community at a coastal site in the NW Mediterranean Sea. *Microbial Ecology*,
532 **59**, 428-435.

533 Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large
534 alignments. *PLoS ONE*, **5**, e9490.

535 Reinthaler T, Winter C, Herndl GJ (2005) Relationship between bacterioplankton richness, respiration, and
536 production in the southern North Sea. *Applied and Environmental Microbiology*, **71**, 2260-2266.

537 Rolland J, Cadotte MW, Davies J, *et al.* (2012) Using phylogenies in conservation: new perspectives. *Biology*
538 *Letters*, **8**, 692-694.

539 Salter I, Galand PE, Fagervold SK, *et al.* (2015) Seasonal dynamics of active SAR11 ecotypes in the
540 oligotrophic Northwest Mediterranean Sea. *ISME Journal*, **9**, 347-360.
541 Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in
542 seawater using 3H-leucine. *Marine Microbial Food Webs*, **6**, 107-114.
543 Spehn E, Hector A, Joshi J, *et al.* (2005) Ecosystem effects of biodiversity manipulations in European
544 grasslands. *Ecological Monographs*, **75**, 37-63.
545 Srivastava DS, Cadotte MW, MacDonald AAM, Marushia RG, Mirotnick N (2012) Phylogenetic diversity
546 and the functioning of ecosystems. *Ecology Letters*, **15**, 637-648.
547 Swan BK, Tupper B, Sczyrba A, *et al.* (2013) Prevalent genome streamlining and latitudinal divergence of
548 planktonic bacteria in the surface ocean. *Proceedings of the National Academy of Sciences*, **110**, 11463-
549 11468.
550 Tilman D, Reich PB, Knops J, *et al.* (2001) Diversity and productivity in a long-term grassland experiment.
551 *Science*, **294**, 843-845.
552 Van Wambeke F (1994) Influence of phytoplankton lysis or grazing on bacterial metabolism and trophic
553 relationships. *Microbial Ecology*, **27**, 143-158.
554 Venail PA, Vives MJ (2013) Phylogenetic distance and species richness interactively affect the productivity of
555 bacterial communities. *Ecology*, **94**, 2529-2536.
556 Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community
557 structure and trait evolution. *Bioinformatics*, **24**.
558 Webb CO, Ackerly DD, McPeck MA, Donoghue MJ (2002) Phylogenies and Community Ecology. *Annual*
559 *Review of Ecology and Systematics*, **33**, 475-505.
560 Worm B, Barbier EB, Beaumont N, *et al.* (2006) Impacts of biodiversity loss on ocean ecosystem services.
561 *Science*, **314**, 787-790.
562
563

564 **Data Accessibility:**

565 DNA sequences: NCBI SRA PRJNA235253

566 Final OTU table, alignment file, tree file, environmental data and phylogenetic distance

567 matrix: Dryad: doi:10.5061/dryad.dh3st

568

569 **Author Contributions:** P.E.G and I.S. designed the study and wrote the paper. P.E.G. and

570 D.K. analysed data. All authors discussed the results and commented on the manuscript.

571

572

573

574

575

576

577

578 **Figure legends**

579

580 **Figure 1.** Average values and standard errors for environmental data. Nitrate, Chlorophyll a
581 (Chl a), temperature, bacterial production (BP, leucine incorporation) and deep bacteria
582 (expressed as number of SAR202 and SAR406 sequences) presented by month during the
583 seven years survey.

584 **Figure 2.** Relationship between bacterial production and the community and phylogenetic
585 diversity of active bacteria (RNA, upper panel) and standing stock (DNA, lower panel).
586 Bacterial production is measured as the rate of tritiated-leucine incorporation ($\text{pmol L}^{-1} \text{h}^{-1}$)
587 and plotted following logarithmic transformation. Community diversity (a, c) is expressed as
588 the Shannon diversity index and phylogenetic diversity (b, d) as standardized effect size
589 (SES) of the phylogenetic diversity. SES corresponds to the average observed phylogenetic
590 diversity in a community compared to the average phylogenetic diversity in a randomly
591 generated community. The black lines represent linear fit to the data and the grey shading
592 shows the 95% confidence interval.

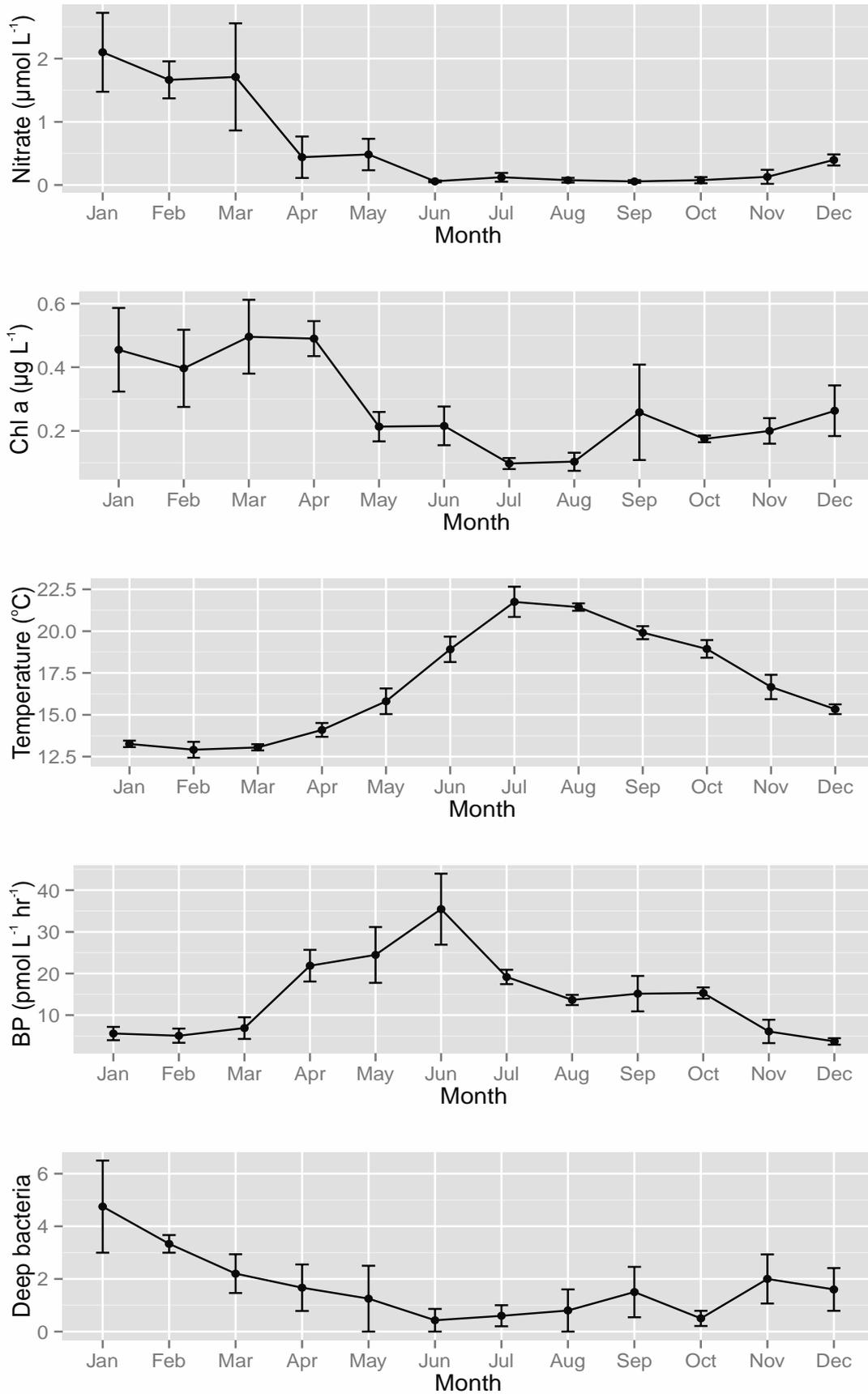
593

594 **Figure 3.** Differences in bacterial production in relation to phylogenetic distance between
595 communities for the active fraction (a) and the standing stock of bacteria (b). Differences in
596 bacterial production are calculated as Euclidian distance between each pair of samples and
597 phylogenetic distance as the mean distance between taxa in a community and their nearest
598 phylogenetic neighbour in the second community. The grey shading shows the 95%
599 confidence interval. The use of Bray-Curtis or Sorensen index for community similarity gave
600 the same result (Figure S3). The grey shading shows the 95% confidence interval.

601

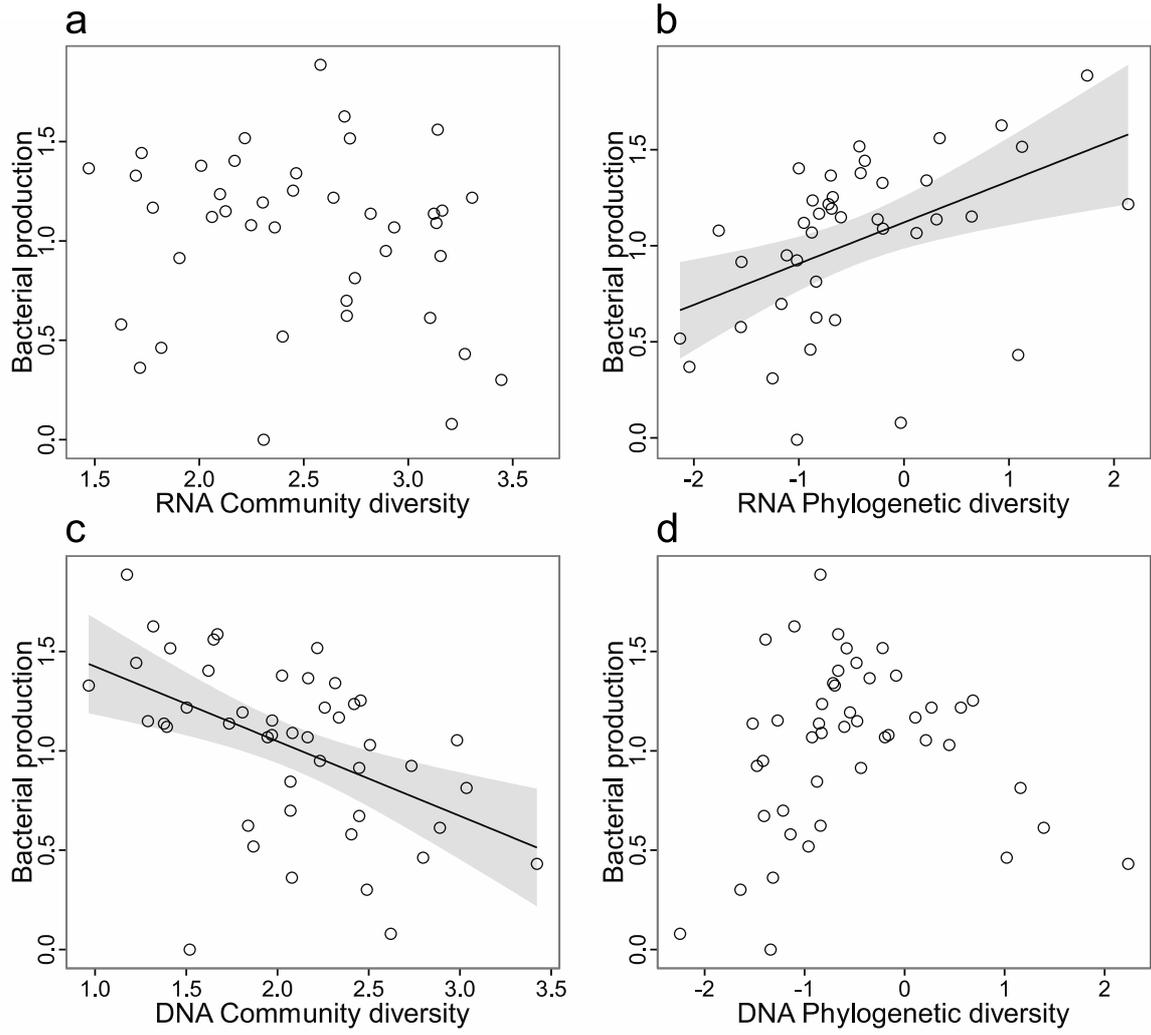
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626

Figure 4. Similarity in recurring bacterial assemblies and temporal trends in community diversity. Panels (a) and (c) show correlograms of bacterial assemblies as a function of time lag for the active fraction (a) and the standing stock (c). Positive and negative correlation values show similar and dissimilar communities, respectively. Panels (b) and (d) show the seasonal trends of community diversity, expressed as the Shannon Index for the active fraction (b) and the standing stock (d). For the active fraction, the highest correlation values for community composition were for samples taken one year apart and the lowest for the communities separated by 6 months. The standing stock did not exhibit regular pattern of community similarity. As for community diversity, the active fraction did not show a significant regular seasonal pattern while the standing stock did (Figure S4).



627

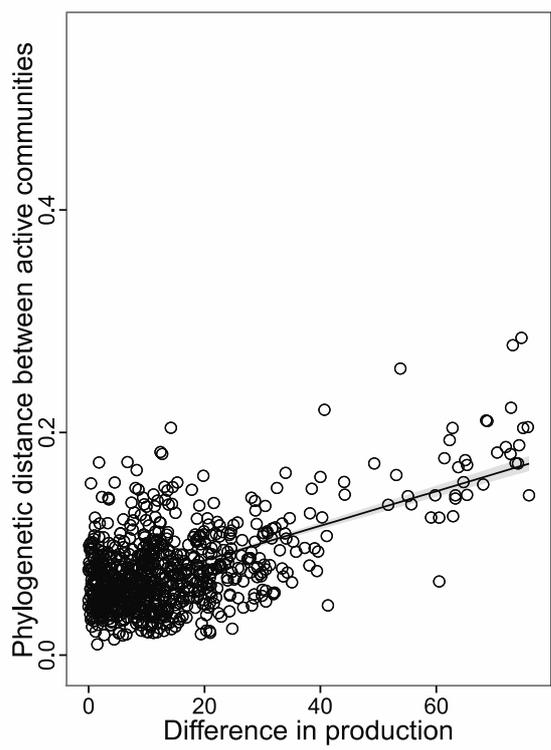
628 Figure 1



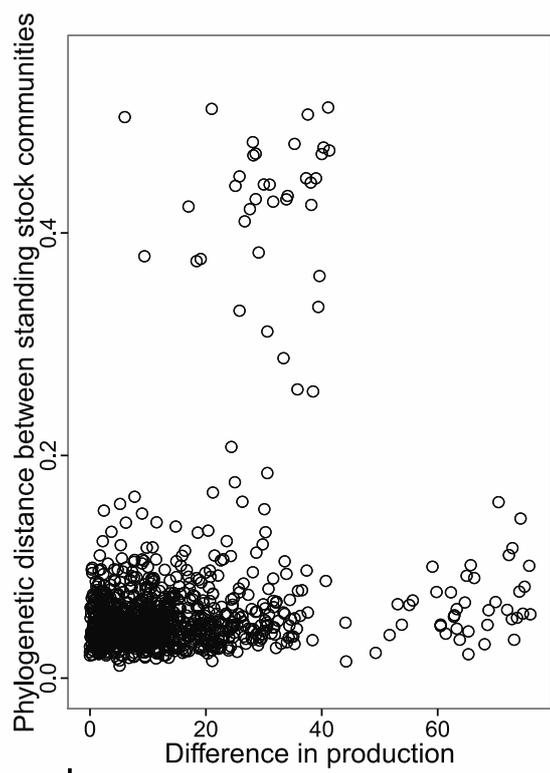
629

630 Figure 2

631



a

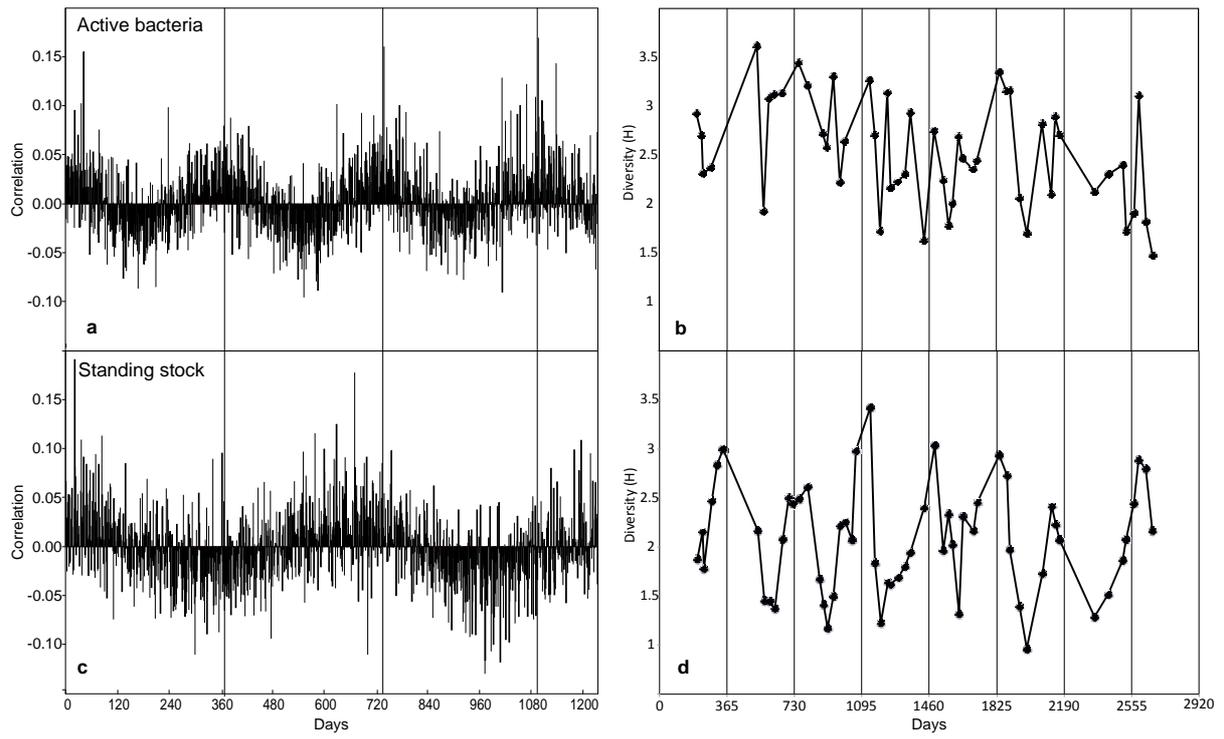


b

Figure 3

632
 633
 634
 635
 636
 637
 638
 639
 640
 641
 642
 643
 644
 645
 646
 647
 648
 649
 650
 651
 652
 653
 654
 655
 656
 657
 658
 659

660



661
662

Figure 4