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Pierre E. Galand, Ian Salter, Dimitri Kalenitchenko

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

3  
4 Pierre E. Galand<sup>1\*</sup>, Ian Salter<sup>2,3</sup>, Dimitri Kalenitchenko<sup>1</sup>

5 <sup>1</sup> 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 <sup>2</sup> 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 <sup>3</sup> 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

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.

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## 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 (Obernosterer *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- $\mu$ m  
142 pore-size GV Sterivex cartridges (Millipore) from 10 L of seawater after prefiltration through  
143 3- $\mu$ m pore-size polycarbonate filters (Millipore). Filters were stored at –80 °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 bar). Following filtration, samples were processed immediately or  
147 stored at -20°C for a period < 1 week. Upon processing, samples were soaked in 90% acetone  
148 at 4°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 <sup>3</sup>H-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 <sup>3</sup>H-leucine (Perkin Elmer, (SA) 115.4 Ci mmol<sup>-1</sup>) 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<sup>-1</sup>) applied directly  
169 to Sterivex cartridges, and a second incubation after adding proteinase K (20 mg mL<sup>-1</sup>),  
170 followed by extraction using the AllPrep DNA/RNA kit (Qiagen), which gave average DNA  
171 concentrations of 28 ng µL<sup>-1</sup>. 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

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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

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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.

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578 **Figure legends**

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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.

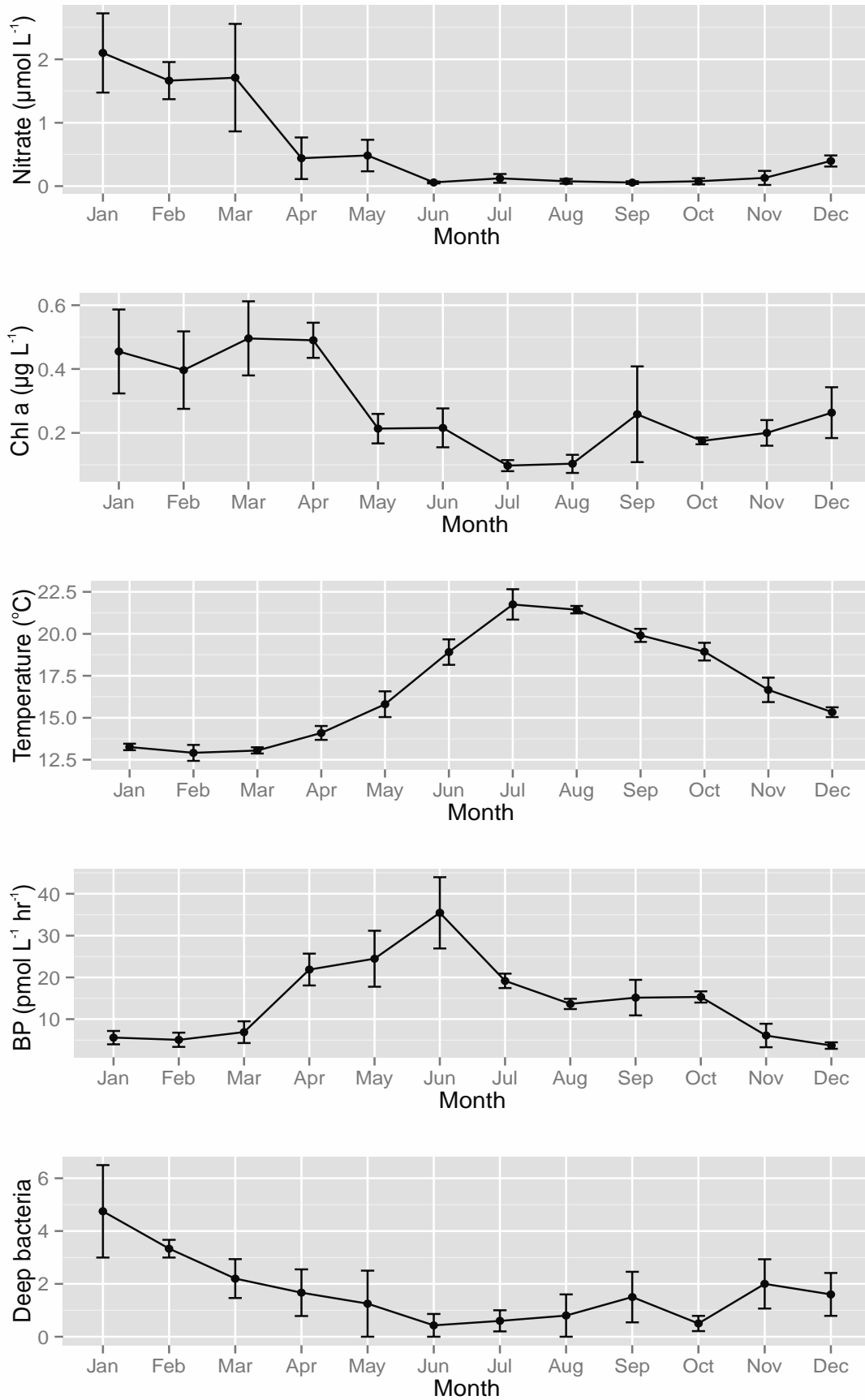
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594 **Figure 3.** Differences in bacterial production in relation to phylogenetic distance between  
595 communities for the active fraction (a) and the standing stock of bacteria (b). Differences in  
596 bacterial production are calculated as Euclidian 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.

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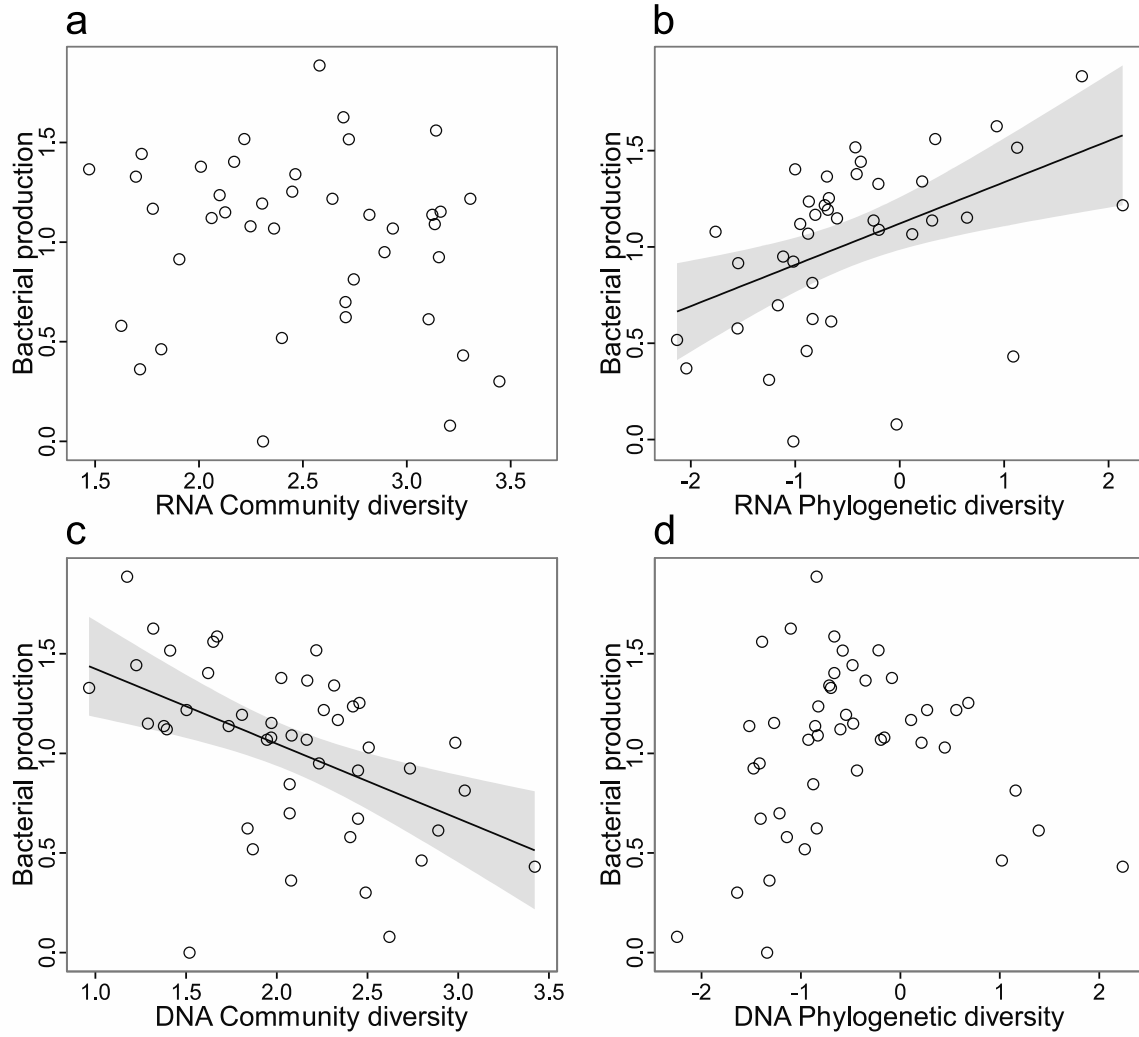
**Figure 4.** Similarity in recurring bacterial assemblies and temporal trends in community diversity. Panels (a) and (c) show correlograms of bacterial assemblies as a function of time lag for the active fraction (a) and the standing stock (c). Positive and negative correlation values show similar and 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).



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628 Figure 1

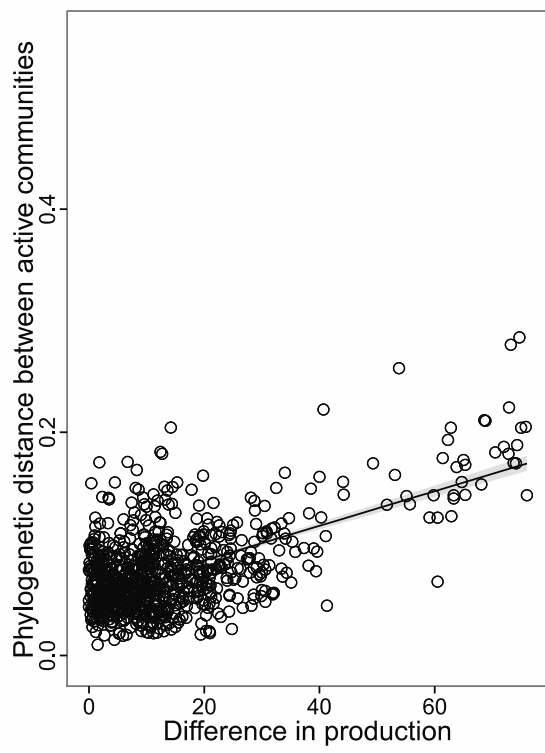




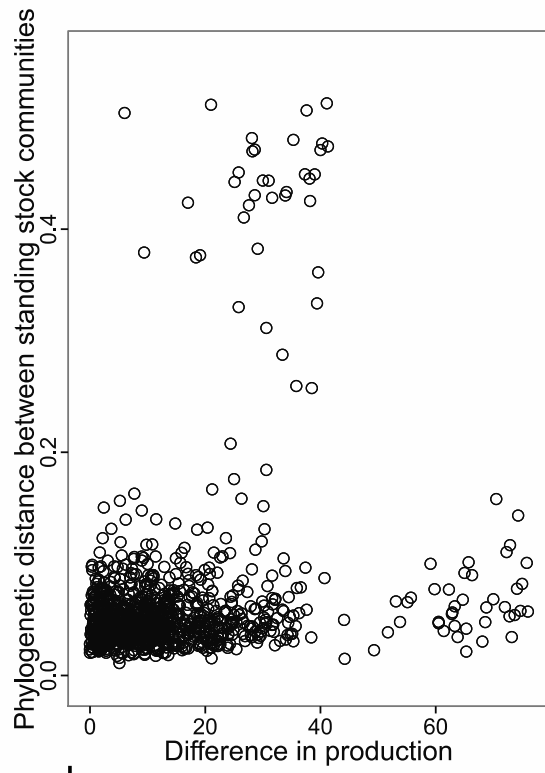
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630 Figure 2

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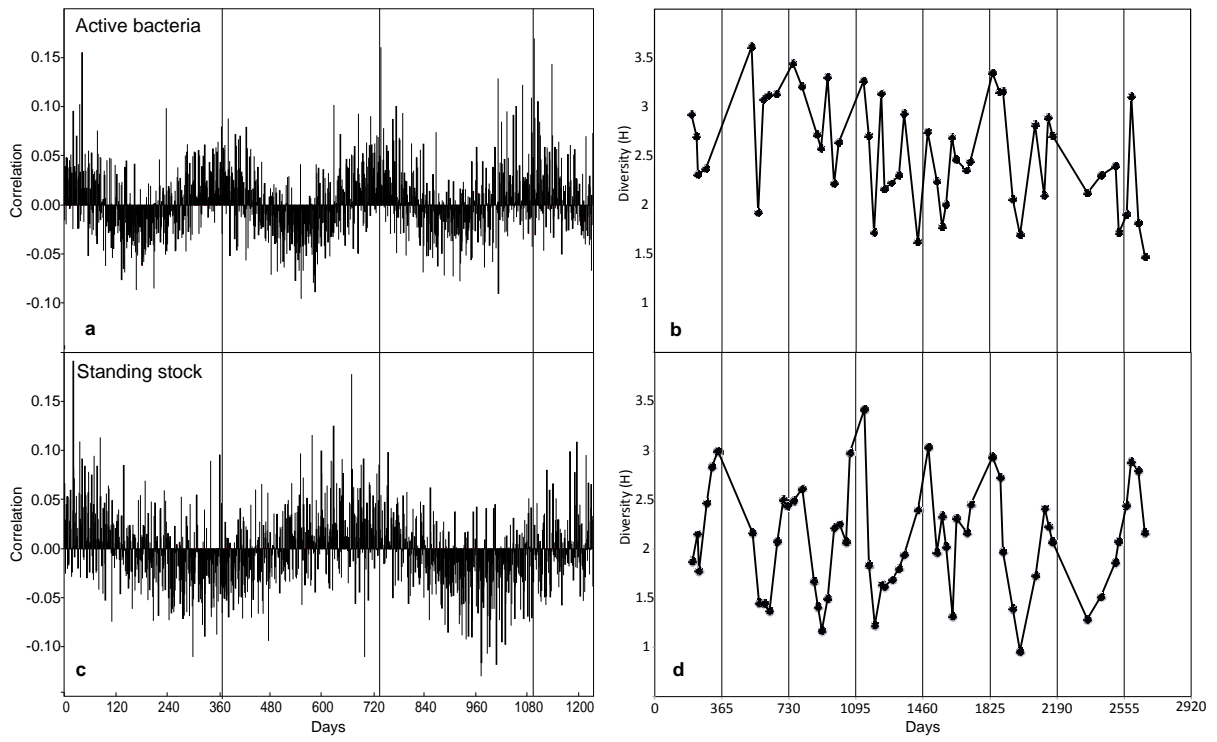


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Figure 3

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Figure 4