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Title

Defining the functional traits that drive bacterial decomposer community productivity

Running title

Functional traits predict community productivity

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Conflict of Interest

The authors declare no conflict of interest.

Subject Category

We suggest that this article is best suited to the “Microbial population and community ecology” category.

1 **Abstract**

2 Microbial communities are essential to a wide range of ecologically and industrially
3 important processes. To control or predict how these communities function, we require a
4 better understanding of the factors which influence microbial community productivity.
5 Here, we combine functional resource use assays with a biodiversity-ecosystem functioning
6 (BEF) experiment to determine whether the functional traits of constituent species can be
7 used to predict community productivity. We quantified the abilities of 12 bacterial species
8 to metabolise components of lignocellulose and then assembled these species into
9 communities of varying diversity and composition to measure their productivity growing on
10 lignocellulose, a complex natural substrate. A positive relationship between diversity and
11 community productivity was caused by a selection effect whereby more diverse
12 communities were more likely to contain two species that significantly improved community
13 productivity. Analysis of functional traits revealed that the observed selection effect was
14 primarily driven by the abilities of these species to degrade β -glucan. Our results indicate
15 that by identifying the key functional traits underlying microbial community productivity we
16 could improve industrial bioprocessing of complex natural substrates.

17

18

19 **Introduction**

20 Microbial communities underpin the functioning of natural ecosystems (Soliveres *et al.*,
21 2016) and the efficiency of a wide range of industrial bioprocesses (e.g. waste bioreactors)
22 (Widder *et al.*, 2016; Cydzik-Kwiatkowska and Zielińska, 2016). The form of the biodiversity-
23 ecosystem functioning (BEF) relationship is therefore an important property of microbial
24 communities both in nature and the simpler communities used in a range of industrial

25 bioprocesses. Several studies have identified positive BEF relationships for microbial
26 community productivity (Bell *et al.*, 2005; Gravel *et al.*, 2011), stability (Awasthi *et al.*, 2014),
27 micropollutant degradation (Johnson *et al.*, 2015) and resistance to invasion (Elsas *et al.*,
28 2012), suggesting that for a range of functions microbial community performance improves
29 with increasing species richness. Positive BEF relationships can arise via the
30 complementarity effect, whereby diverse communities use more of the available resource
31 space through niche differentiation or facilitation (Salles *et al.*, 2009; Singh *et al.*, 2015), or
32 the selection effect (also termed the sampling effect), whereby diverse communities are
33 more likely to contain species which have a large impact on community functioning (Awasthi
34 *et al.*, 2014; Hooper *et al.*, 2005; Langenheder *et al.*, 2012, 2010). Both complementarity
35 and selection effects depend on the functional traits of constituent species and several
36 studies have now shown functional diversity to be a better predictor of community function
37 than phylogenetic diversity (Krause *et al.*, 2014; Mokany *et al.*, 2008; Salles *et al.*, 2009).
38 However, for many ecologically and biotechnologically important microbial communities it is
39 still unclear how the functional traits of individual species scale-up to determine the
40 performance of a diverse community.

41

42 One of the most important ecosystem functions microbial communities perform is the
43 decomposition of plant material and subsequent nutrient cycling (McGuire and Treseder,
44 2010; Van Der Heijden *et al.*, 2008). Understanding the decomposition of plant material also
45 has important industrial relevance. Plant biomass (collectively referred to as lignocellulose)
46 is the most abundant raw material on Earth (Pauly and Keegstra, 2008). It is typically
47 composed of approximately 40-50% cellulose, 20-40% hemicellulose and 20-35% lignin
48 which together form a complex, recalcitrant structure (Himmel *et al.*, 2007; Liao *et al.*,

49 2016). The high sugar content and abundance of lignocellulose make it a promising
50 substrate for biofuel production (Naik *et al.*, 2010). However, lignin is highly recalcitrant to
51 enzymatic attack causing a bottleneck in the efficient conversion of lignocellulose to biofuels
52 reducing cost-effectiveness (Jorgensen *et al.*, 2007; Naik *et al.*, 2010). Understanding how
53 natural microbial communities (e.g. in soils (Lynd *et al.*, 2002), compost (Lopez-Gonzalez *et*
54 *al.*, 2014) or termite guts (Brune, 2014)) achieve efficient lignocellulose degradation could
55 inform both the prediction of nutrient cycling in natural systems and the design of efficient
56 microbial communities for industrial processes (Wei *et al.*, 2012). Both biodiversity and the
57 presence of certain species have been shown to influence the rate of decomposition by
58 bacterial communities (Bell *et al.*, 2005; Bonkowski and Roy, 2005; Langenheder *et al.*, 2012)
59 but the mechanisms which determine community decomposition performance remain
60 poorly understood (McGuire and Treseder, 2010). A key question therefore is to what
61 extent community functioning is predictable from the combined functional traits of
62 constituent species?

63

64 Using culturable bacterial strains isolated from compost we performed a random partition
65 design BEF experiment (Bell *et al.* 2009) to test the contributions of species richness and
66 composition to productivity of communities when grown on wheat straw. Although using
67 only the culturable fraction of the community is likely to overlook some functionally
68 important species in the natural community, culturability is a key feature of microbes that
69 could feasibly be used in industrial bioprocessing. Next we tested how the functional traits
70 of individual species shaped the productivity of these communities to determine the extent
71 to which community productivity was predictable from the functional traits of the
72 constituent species and to determine the contribution of each functional trait to overall

73 productivity. We quantified the functional resource use traits of each species by their ability
74 to utilise a range of known components of lignocellulose (i.e. cellulose, hemicellulose, pectin
75 and lignin).

76

77 **Materials and methods**

78 *Bacterial isolates*

79 Bacterial strains used in this study were isolated from wheat straw compost enrichment
80 cultures (700 ml M9 minimal media (22 mM KH_2PO_4 , 42 mM Na_2HPO_4 , 19 mM NH_4Cl , 1 mM
81 MgSO_4 , 0.09 mM CaCl_2 , 9 mM NaCl), 1% (w/v) wheat straw compost, 5% (w/v) milled wheat
82 straw). Cultures were grown on an orbital shaker (150rpm) at 30°C for eight weeks. The
83 enrichment culture process will have favoured those species able to grow at 30°C in a well
84 aerated environment which are required characteristics for further experiments. As a result
85 the isolated bacteria used in this study do not provide a full representation of the complex
86 microbial community present in compost, but do represent a diverse collection of naturally
87 coexisting isolates that could potentially be used in industrial bioprocessing. Each week
88 serial dilutions were prepared and spread onto nutrient agar, potato dextrose agar and M9
89 minimal media containing 1.5% (w/v) agar and 1% (w/v) milled wheat straw. Single colonies
90 that appeared morphologically distinct on agar plates (Supplementary Table 1) were
91 assayed for activity against carboxymethylcellulose (CMC) and xylan (both from Sigma-
92 Aldrich, Dorset, UK) using Congo red staining assays (Teather and Wood, 1982). Species with
93 activity against CMC and/or xylan were identified by 16S rRNA gene sequencing (16S
94 sequences were deposited in GenBank under the accession numbers KX527645-KX527656).
95 The twelve species included in this study were chosen as they represent phylogenetic or

96 functional diversity based on 16S rRNA sequences and CMC and xylan assays
97 (Supplementary Figure S1 and Table 1).

98

99 *Biodiversity ecosystem functioning experiment*

100 Communities for the BEF experiment were designed using the random partition design
101 described by Bell et al. (2009). Species were randomly divided into communities with
102 species richness levels of 1, 2, 3, 4, 6 and 12 species with each isolate represented an equal
103 number of times at each richness level. This process was repeated to produce 12
104 monocultures, 66 two-isolate communities, 58 three-isolate communities, 63 four-isolate
105 communities, 68 six-isolate communities and one twelve isolate community. Each
106 community was replicated five times to give a total of 1340 communities. The twelve
107 species were grown for two days in 5 ml nutrient broth at on an orbital shaker (150rpm) at
108 30°C. Cultures were harvested by centrifugation, washed and suspended in M9 minimal
109 media and left at room temperature for 2h to metabolise remaining nutrients before OD₆₀₀
110 was standardised to 0.1 to ensure similar starting densities. Deep well plates containing 380
111 µl M9 minimal media with 1% (w/v) milled wheat straw per well were inoculated with a
112 total of 120 µl cultures, e.g. monocultures were inoculated with 120 µl single species culture
113 whereas the 12 species community was inoculated with 10 µl of each culture. The
114 MicroResp system was used to measure respiration of cultures (Campbell *et al.*, 2003).
115 Briefly, each well in the deepwell plate is sealed to a microplate well containing indicator
116 dye which changes colour in response to CO₂ concentration. Microplates containing
117 indicator gel were replaced every 24h to prevent cultures becoming anaerobic. Community
118 productivity was estimated as cumulative respiration (Armitage, 2016; Tiunov and Scheu,
119 2005). Specifically, cultures were grown for 7 days at 30°C and productivity was measured as

120 the cumulative change in absorbance ($\lambda=595\text{nm}$) of the indicator gel immediately before
121 and after being sealed to deep well cultures plates. The change in OD of the indicator gel
122 from control wells containing no inoculum was used to account for atmospheric CO_2
123 concentration. Note that due to the presence of particles of wheat straw in the growth
124 medium it was not possible to measure change in microbial biomass by absorbance.

125

126 *Functional trait assays*

127 To quantify the fundamental niche of each species, growth assays were performed on
128 several polysaccharides present in lignocellulose. Hemicellulose substrates included xylan
129 (Sigma-Aldrich), arabinoxylan (P-WAXYL, Megazyme, Bray, Ireland) and galactomannan (P-
130 GALML, Megazyme); cellulose substrates included β -glucan (P-BGBL, Megazyme) and
131 Whatman filter paper; additional substrates included pectin (Sigma-Aldrich) and Kraft lignin
132 (Sigma-Aldrich). Cultures were prepared as described for the BEF experiment. These
133 cultures (5 μl) were used to inoculate 495 μl of M9 minimal media with 0.2% (w/v) of each
134 carbon source or one 6mm sterile filter paper disc in 96-well deepwell plates. Cultures were
135 replicated six times and several blank wells containing no inoculum were included as
136 negative controls. Cultures were grown for 7 days at 30°C and the MicroResp system was
137 used to measure culture respiration as described above.

138

139 *Statistical analysis*

140 The biodiversity and ecosystem functioning relationship was analysed using the linear model
141 method described by Bell et al. (2009). The species coefficients provided by this method give
142 a measure of the effect of each species on community productivity relative to an average
143 species: values of >1 indicate an above average contribution while values of <1 indicate a

144 below average contribution to community productivity. To assess the effect of *Paenibacillus*
145 sp. A8 and *C. flavigena* D13 on community productivity, communities containing both
146 species, *Paenibacillus* sp. A8 only, *C. flavigena* D13 only or neither of these species were
147 compared using analysis of variance (ANOVA) followed by post hoc Tukey tests. Linear
148 models were used to compare the ability of species richness and the presence or absence of
149 *Paenibacillus* sp. A8 and *C. flavigena* D13 to predict community productivity.

150

151 To standardise measures of functional traits across diverse substrates, performance on each
152 substrate was normalised by dividing by the maximum observed respiration on that
153 substrate. For each bacterial isolate we can then calculate its fundamental niche (along the
154 carbon degradation axis) by summing performance on all substrates. To estimate the niche
155 space of each community we used the community niche (CN) metric described by Salles et
156 al. (2009), which sums the maximal performance on each substrate:
157 $CN = \sum_{i=1}^7 \max_{j=1}^n (P_{ij})$, where P_{ij} is the performance of species j on carbon source i and n
158 is the number of species in each community.

159

160 The ability of each functional trait to predict community productivity was analysed by
161 summing performance of all species in a community on each carbon source to give a
162 measure of the total fundamental niche space of that community. To approximate the
163 realised niche space of communities we also assessed the ability of the maximum
164 performance on each carbon source in a community to predict community productivity; this
165 metric assumes that the species best able to grow on a given carbon source in a community
166 dominates consumption of that carbon source providing a conservative estimate of realised
167 niche. Linear regressions were used to analyse how well CN and functional trait

168 performance predicted community productivity. It is important to note that because all
169 species can grow on several carbon sources, summing functional trait use may act as a proxy
170 of species richness. To control for this effect we analysed whether summed community
171 functional traits remained significant when fitted to the residuals of the species richness
172 model (i.e. community productivity predicted by species richness). Competing models were
173 compared using the Akaike information criterion (AIC).

174

175 **Results**

176 *Biodiversity-ecosystem function relationship*

177 We observed a positive relationship between species richness and community productivity
178 ($F_{1, 264} = 60.1$; $p < 0.001$, Figure 1) with species richness explaining 19% of variation in
179 productivity. As highlighted by the variance in productivity within species richness levels,
180 species identity also had a significant effect on community productivity ($F_{12, 254} = 45.3$,
181 $p < 0.001$). The linear model coefficient for each species provides the estimated contribution
182 of that species to community productivity relative to an average species (Bell *et al.*, 2009).
183 Two species, *Paenibacillus* sp. A8 and *C. flavigena* D13, made significantly greater
184 contributions to community function relative to an average species ($F_{1, 254} = 73.1$, $p < 0.001$
185 and $F_{1, 254} = 256.3$, $p < 0.001$ respectively, Supplementary Figure S2). Of the remaining species,
186 the contribution of *Rheinheimera* sp. D14A and *Stenotrophomonas* sp. D12, did not
187 significantly differ from the average species while the remaining eight species made
188 significantly below average contributions to community functioning (Supplementary Figure
189 S2).

190

191 To further investigate the effects of *Paenibacillus* sp. A8 and *C. flavigena* D13, the
192 productivity of communities containing either one, both or neither of these species was
193 compared. Communities that contained both *Paenibacillus* sp. A8 and *C. flavigena* D13 were
194 significantly more productive than communities containing either one or neither of these
195 species (post-hoc Tukey tests, $p < 0.001$, Figure 1). The productivity of communities
196 containing both *Paenibacillus* sp. A8 and *C. flavigena* D13 did not significantly differ across
197 species richness levels suggesting additional species within these communities are not
198 contributing to community productivity ($F_{1, 28} = 0.42$, $p > 0.05$, green line Figure 1).
199 Communities containing only *C. flavigena* D13 were more productive than those containing
200 only *Paenibacillus* sp. A8 (post-hoc Tukey test, $p < 0.001$), while communities which did not
201 contain these species were significantly less productive than communities containing either
202 one of these species (post-hoc Tukey test, $p < 0.001$). These results indicate that the positive
203 BEF relationship is predominantly driven by the selection effect, i.e. more diverse
204 communities are more likely to contain the highly performing species *Paenibacillus* sp. A8
205 and *C. flavigena* D13 and are therefore more productive.

206

207 *Quantification of functional traits*

208 To determine if differences in productivity could be explained by the functional traits of
209 species we assayed the ability of species to utilise various components of lignocellulose. All
210 species were able to grow to varying degrees on the labile substrates, hemicellulose (xylan,
211 arabinoxylan and galactomannan) and pectin, whereas growth on recalcitrant substrates (β -
212 glucan, filter paper and lignin) was less universal (Figure 2). This pattern is consistent with
213 the hypothesis that functional groups that degrade recalcitrant substrates are not as
214 common as those that degrade labile substrates (Schimel and Gulledge, 1998; Waldrop and

215 Firestone, 2004). A linear model revealed significant main effects of both species ($F_{11, 216}$
216 $_{336}=30.3$, $p<0.001$) and carbon source ($F_{6, 217}$
217 $_{336}=105.8$, $p<0.001$) on productivity and a
218 significant interaction between these factors ($F_{66, 219}$
219 $_{336}=6.8$, $p<0.001$), suggesting niche
220 differentiation in resource use among the species. It is notable that some species, in
221 particular *Rhodococcus* sp. E31, displayed generalist resource use, being able to grow on
222 recalcitrant substrates like lignin as well as on the more labile substrates.

221

222 *Community productivity and functional traits*

223 To determine if the functional niche of communities could be used to predict productivity
224 we calculated community niche as described by Salles et al. (2009). This index sums the
225 maximum growth achieved by a constituent species on each substrate. We found a
226 significant positive relationship between community niche and community productivity ($F_{1, 227}$
227 $_{264}=73.31$, $p<0.001$, Figure 3a). Similar to the results of Salles et al. (2009), community niche
228 explained more variation in community productivity than species richness (22% and 19%
229 respectively).

230

231 When calculating community niche, each functional trait is weighted equally despite
232 differences in the abundances of substrates in wheat straw lignocellulose, e.g. cellulose
233 constitutes 40-50% whereas pectin only constitutes 1-2%. To determine which functional
234 traits were important for predicting community productivity we summed the growth of
235 constituent species on each carbon source used in functional trait assays to calculate the
236 total fundamental niche of that community. The summed activity on β -glucan had a
237 significant positive relationship with productivity ($F_{1, 238}$
238 $_{264}=182.7$, $p<0.001$) and was the best
239 predictor of community productivity, explaining 41% of variation (Figure 3b). The ability to

239 utilise arabinoxylan and xylan also had significant positive relationships with productivity ($F_{1, 264}=105.8$, $p<0.001$ and $F_{1, 264}=98.6$, $p<0.001$ respectively), explaining 29% and 27% of
240 variation respectively. There were significant positive relationships between the remaining
241 carbon sources and community productivity though these explained less variation than
242 community richness and were not significant when species richness was included in models.
243 The fundamental niche space of community is unlikely to be achieved due to interactions
244 between species such as competition for resources. Therefore to approximate the realised
245 niche space of each community we also analysed the maximum performance per carbon
246 source in a community. Consistent with the analysis of summed performance, maximum
247 performance on β -glucan, arabinoxylan and xylan had significant positive relationships with
248 productivity ($F_{1, 264}=134.8$, $p<0.001$, $F_{1, 264}=76.2$, $p<0.001$ and $F_{1, 264}=74.5$, $p<0.001$
249 respectively) explaining 34%, 23% and 22% of variation respectively. There were significant
250 positive relationships between the maximum performance on lignin ($F_{1, 264}=7.4$, $p<0.01$),
251 pectin ($F_{1, 264}=20.8$, $p<0.001$) and galactomannan ($F_{1, 264}=47.1$, $p<0.001$) and community
252 productivity though these explained less variation than community richness. There was no
253 significant relationship between the maximum ability to degrade filter paper and
254 community productivity (Supplementary Table 2). This suggests that identifying and
255 measuring key functional traits could be a better predictor of community productivity than
256 either species richness or community niche.

258

259 **Discussion**

260 Understanding the factors that influence microbial community productivity has potentially
261 important ecological and industrial applications (Widder *et al.*, 2016). The ability of
262 community niche to predict functioning in well-defined media has been demonstrated

263 previously (Salles *et al.*, 2009). Here, we define for communities growing in complex
264 undefined media, the key functional resource use traits that predict decomposer
265 community productivity. Crucially, functional resource use traits explained more variation in
266 productivity than either species richness or measures of community niche. Indeed, a single
267 function, the ability to degrade β -glucan, explained a larger proportion of variation than
268 community niche. This key functional trait was shared by two dominant strains which were
269 shown to significantly increase the productivity of communities.

270

271 As with several previous BEF studies (Awasthi *et al.*, 2014; Bell *et al.*, 2009; Gravel *et al.*,
272 2011), we identified a positive relationship between species richness and community
273 productivity. By analysing the effect of community composition we found that the presence
274 of two highly functioning species, *Paenibacillus* sp. A8 and *C. flavigena* D13, significantly
275 increased community productivity suggesting this positive BEF relationship is driven by the
276 selection effect. To determine if the dominance of these two species could be explained by
277 their functional traits, we compared the ability of these species to utilise the various carbon
278 sources used in functional trait assays to the other species. With the exception of
279 *Rhodococcus* sp. E31, *Paenibacillus* sp. A8 and *C. flavigena* D13 were the highest performing
280 species on β -glucan (Figure 2). The ability to utilise β -glucan may suggest these species are
281 able to metabolise the cellulose portion of wheat straw in addition to the more labile
282 hemicellulose and pectin fractions. Interestingly, when the productivity of communities
283 containing either one, both or neither of these species is compared across each day of the
284 experiment (Supplementary Figure S3), it is noticeable that communities containing neither
285 of these species have very low productivity during the later days of the experiment. A
286 possible explanation is that easily-accessible labile substrates are being used within the first

287 two days of growth after which only recalcitrant and inaccessible substrates remain. The
288 ability to degrade cellulose would allow *Paenibacillus* sp. A8 and *C. flavigena* D13 to
289 maintain higher levels of growth when labile substrates become depleted.

290

291 Interestingly, *Paenibacillus* sp. A8 and *C. flavigena* D13 have similar functional traits which
292 would indicate they occupy overlapping niche space and may be in direct competition with
293 each other. However, communities containing both these species were significantly more
294 productive than communities containing only one or neither suggesting complementarity or
295 facilitation effect between these species, i.e. they are able to exploit a wider niche space
296 when grown together potentially because they each produce enzymes or by-products that
297 improve the overall community productivity. Wohl et al. (2004) found a similar result
298 whereby functionally redundant cellulose degrading bacteria were more productive in
299 communities than in monoculture.

300

301 The ability of species within communities to utilise β -glucan was a better predictor of
302 community productivity than measures of community niche or species richness. The
303 significance of this activity is consistent with the composition of wheat straw lignocellulose,
304 which is made up of 40-50% cellulose. Interestingly, functional trait assays revealed that
305 *Rhodococcus* sp. E31 achieved the second highest growth on β -glucan but this species did
306 not significantly increase community productivity compared to an average species
307 (Supplementary Figure S2). In addition, *Rhodococcus* sp. E31 was able to utilise lignin as well
308 as the more labile hemicellulose substrates (Figure 2). It might have reasonably been
309 expected that as lignin is the major contributing factor to recalcitrance, species able to
310 degrade it would increase community productivity by increasing accessibility of

311 saccharification enzymes to cellulose. The limited contribution of *Rhodococcus* sp. E31 to
312 community productivity may be explained in part by structural differences between Kraft
313 lignin used in functional trait assays and native lignin present in lignocellulose (Vishtal and
314 Kraslawski, 2011). Alternatively, although able to achieve efficient degradation of all
315 substrates in monoculture growth assays, *Rhodococcus* sp. E31 may be outcompeted in
316 communities and unable to achieve the functional potentials revealed by trait assays.
317 Recalcitrant substrates may require more energy expensive breakdown pathways than labile
318 substrates (Lynd *et al.*, 2002) which may put species that are specialised to degrade such
319 substrates, e.g. *Rhodococcus* sp. E31, at a competitive disadvantage in communities.
320 Measuring the abundance of species in each community would allow us to better determine
321 the functional traits present in communities assuming that enzyme expression does not
322 differ between monoculture and communities. Alternatively, it may be possible to match
323 functional traits to community productivity by comparing the transcriptome and proteome
324 of focal communities, although any such approach is necessarily limited by the correct
325 annotation of functional genes and/or proteins.

326

327 Rivett *et al.* (2016) found that the ability of species to degrade labile resources could be
328 explained by metabolic plasticity whereas the ability to degrade more recalcitrant
329 substrates required evolutionary adaptation. Species best adapted to utilise the accessible
330 labile substrates may be able to dominate communities during initial growth stages but as
331 labile substrates become depleted, species able to adapt to utilise the remaining recalcitrant
332 substrates will become more dominant in communities. When comparing the contribution
333 of species across each day of the BEF experiment, we found that the contribution of species
334 did not noticeably differ throughout the seven days of growth. *Paenibacillus* sp. A8

335 significantly improved community productivity relative to the average species on each day
336 while *C. flavigena* D13 made a significantly higher contribution than the average species
337 from day two onwards (Supplementary Figure 3). The presence of *Rheinheimera* sp. D14A
338 made a significantly above average contribution to community productivity on day one of
339 the experiment, though for the remaining six days the contribution of this species did not
340 significantly differ from that of an average species. Of the remaining 9 species, contributions
341 remained lower than or did not significantly differ from the average species throughout the
342 7 days. The ability of *C. flavigena* D13 and *Paenibacillus* sp. A8 to efficiently degrade both
343 recalcitrant and labile substrates may allow them to outcompete other species before they
344 are able to adapt to utilise recalcitrant substrates. Allowing the species used here a period
345 of evolutionary adaptation to the wheat straw substrate may increase their ability to
346 degrade recalcitrant substrates and alter the dominance hierarchy within these
347 communities and is an interesting topic for future study.

348

349 In conclusion, we have identified key functional traits that define the productivity of
350 communities degrading lignocellulose. We found that the degradative abilities of
351 communities against β -glucan, arabinoxylan and xylan were able to predict community
352 productivity more effectively than either measures of community niche or species richness.
353 Furthermore, we found that two species, *Paenibacillus* sp. A8 and *C. flavigena* D13, made
354 greater than average contributions to community productivity suggesting a key role for the
355 selection effect in driving the observed positive BEF relationship. Our results suggest that,
356 using simple experiments, it is possible to identify the important functional traits and
357 species that drive microbial community productivity on complex natural substrates like
358 wheat straw, potentially simplifying efforts to predict the functioning of natural

359 communities and the assembly of highly performing communities for biotechnological
360 industrial applications.

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Conflict of Interest

The authors declare no conflict of interest.

- 361 Armitage DW. (2016). Time-variant species pools shape competitive dynamics and
362 biodiversity–ecosystem function relationships. *Proc R Soc B* **283**: 20161437.
- 363 Awasthi A, Singh M, Soni SK, Singh R, Kalra A. (2014). Biodiversity acts as insurance of
364 productivity of bacterial communities under abiotic perturbations. *ISME J* **8**: 2445–2452.
- 365 Bell T, Lilley AK, Hector A, Schmid B, King L, Newman JA, *et al.* (2009). A Linear Model
366 Method for Biodiversity–Ecosystem Functioning Experiments. *Am Nat* **174**: 836–849.
- 367 Bell T, Newman JA, Silverman BW, Turner SL, Lilley AK. (2005). The contribution of species
368 richness and composition to bacterial services. *Nature* **436**: 1157–1160.
- 369 Bonkowski M, Roy J. (2005). Soil Microbial Diversity and Soil Functioning Affect Competition
370 among Grasses in Experimental Microcosms. *Oecologia* **143**: 232–240.
- 371 Brune A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nat Rev Microbiol* **12**:
372 168–180.
- 373 Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM. (2003). A Rapid Microtiter
374 Plate Method To Measure Carbon Dioxide Evolved from Carbon Substrate Amendments so
375 as To Determine the Physiological Profiles of Soil Microbial Communities by Using Whole
376 Soil. *Appl Environ Microbiol* **69**: 3593–3599.
- 377 Cydzik-Kwiatkowska A, Zielińska M. (2016). Bacterial communities in full-scale wastewater
378 treatment systems. *World J Microbiol Biotechnol* **32**: 66.
- 379 Elsas JD van, Chiurazzi M, Mallon CA, Elhottová D, Křišťůfek V, Salles JF. (2012). Microbial
380 diversity determines the invasion of soil by a bacterial pathogen. *Proc Natl Acad Sci* **109**:
381 1159–1164.
- 382 Gravel D, Bell T, Barbera C, Bouvier T, Pommier T, Venail P, *et al.* (2011). Experimental niche
383 evolution alters the strength of the diversity-productivity relationship. *Nature* **469**: 89-
384 U1601.
- 385 Himmel ME, Ding S-Y, Johnson DK, Adney WS, Nimlos MR, Brady JW, *et al.* (2007). Biomass
386 recalcitrance: Engineering plants and enzymes for biofuels production. *Science* **315**: 804–
387 807.
- 388 Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, *et al.* (2005). Effects of
389 Biodiversity on Ecosystem Functioning: A Consensus of Current Knowledge. *Ecol Monogr* **75**:
390 3–35.
- 391 Johnson DR, Helbling DE, Lee TK, Park J, Fenner K, Kohler H-PE, *et al.* (2015). Association of
392 Biodiversity with the Rates of Micropollutant Biotransformations among Full-Scale
393 Wastewater Treatment Plant Communities. *Appl Environ Microbiol* **81**: 666–675.
- 394 Jorgensen H, Kristensen JB, Felby C. (2007). Enzymatic conversion of lignocellulose into
395 fermentable sugars: challenges and opportunities. *Biofuels Bioprod Biorefining-Biofpr* **1**:
396 119–134.

397 Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S, *et al.* (2014).
398 Trait-based approaches for understanding microbial biodiversity and ecosystem functioning.
399 *Aquat Microbiol* **5**: 251.

400 Langenheder S, Bulling MT, Prosser JI, Solan M. (2012). Role of functionally dominant
401 species in varying environmental regimes: evidence for the performance-enhancing effect of
402 biodiversity. *BMC Ecol* **12**: 14.

403 Langenheder S, Bulling MT, Solan M, Prosser JI. (2010). Bacterial Biodiversity-Ecosystem
404 Functioning Relations Are Modified by Environmental Complexity. *PLoS ONE* **5**: e10834.

405 Liao JC, Mi L, Pontrelli S, Luo S. (2016). Fuelling the future: microbial engineering for the
406 production of sustainable biofuels. *Nat Rev Microbiol* **advance online publication**. e-pub
407 ahead of print, doi: 10.1038/nrmicro.2016.32.

408 Lopez-Gonzalez JA, Vargas-Garcia M del C, Lopez MJ, Suarez-Estrella F, Jurado M, Moreno J.
409 (2014). Enzymatic characterization of microbial isolates from lignocellulose waste
410 composting: chronological evolution. *J Environ Manage* **145**: 137–46.

411 Lynd LR, Weimer PJ, Zyl WH van, Pretorius IS. (2002). Microbial Cellulose Utilization:
412 Fundamentals and Biotechnology. *Microbiol Mol Biol Rev* **66**: 739–739.

413 McGuire KL, Treseder KK. (2010). Microbial communities and their relevance for ecosystem
414 models: Decomposition as a case study. *Soil Biol Biochem* **42**: 529–535.

415 Mokany K, Ash J, Roxburgh S. (2008). Functional identity is more important than diversity in
416 influencing ecosystem processes in a temperate native grassland. *J Ecol* **96**: 884–893.

417 Naik SN, Goud VV, Rout PK, Dalai AK. (2010). Production of first and second generation
418 biofuels: A comprehensive review. *Renew Sustain Energy Rev* **14**: 578–597.

419 Pauly M, Keegstra K. (2008). Cell-wall carbohydrates and their modification as a resource for
420 biofuels. *Plant J* **54**: 559–568.

421 Salles JF, Poly F, Schmid B, Roux XL. (2009). Community niche predicts the functioning of
422 denitrifying bacterial assemblages. *Ecology* **90**: 3324–3332.

423 Schimel JP, Gullede J. (1998). Microbial community structure and global trace gases. *Glob*
424 *Change Biol* **4**: 745–758.

425 Singh M, Awasthi A, Soni SK, Singh R, Verma RK, Kalra A. (2015). Complementarity among
426 plant growth promoting traits in rhizospheric bacterial communities promotes plant growth.
427 *Sci Rep* **5**: 15500.

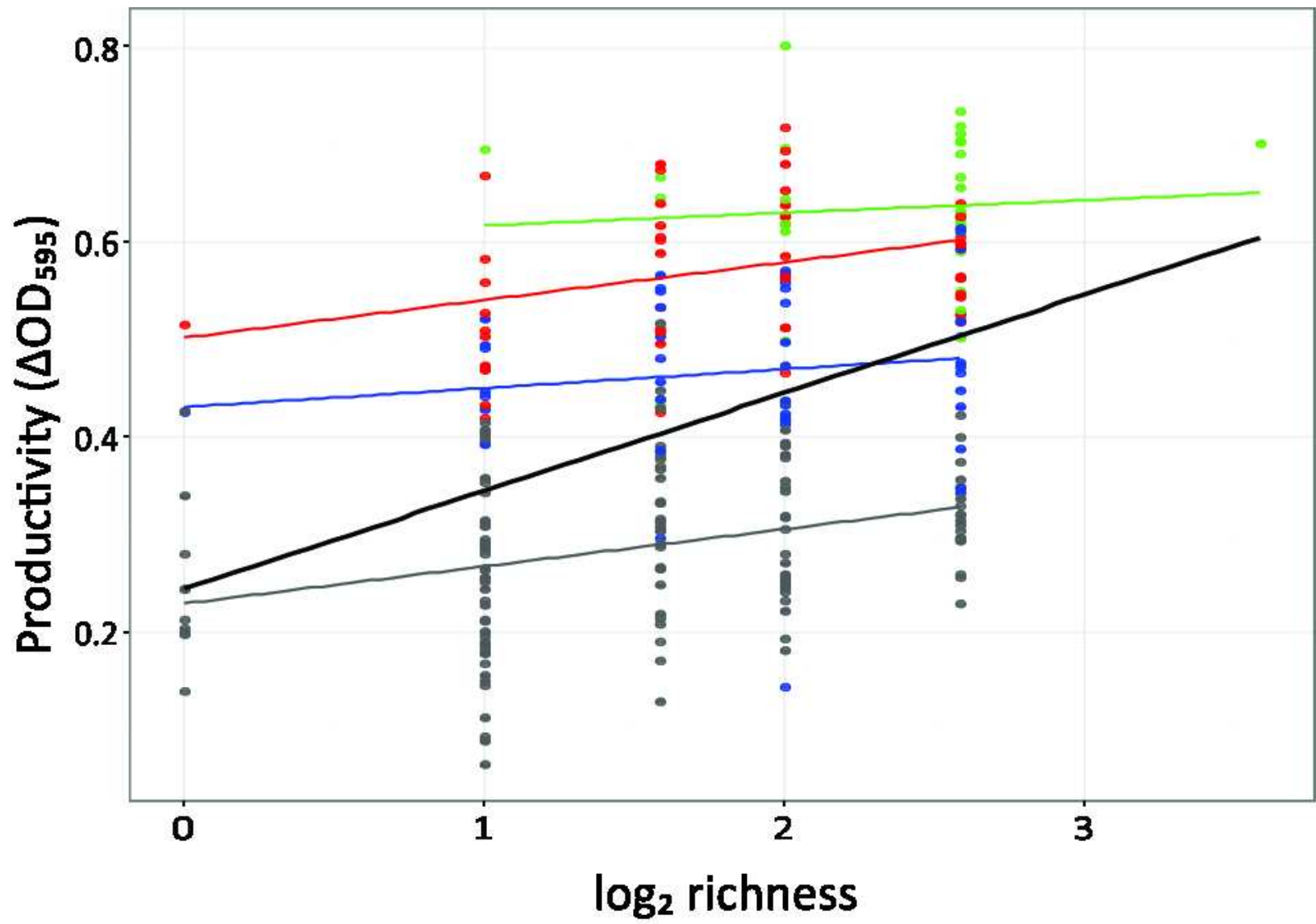
428 Soliveres S, van der Plas F, Manning P, Prati D, Gossner MM, Renner SC, *et al.* (2016).
429 Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature*
430 **536**: 456–459.

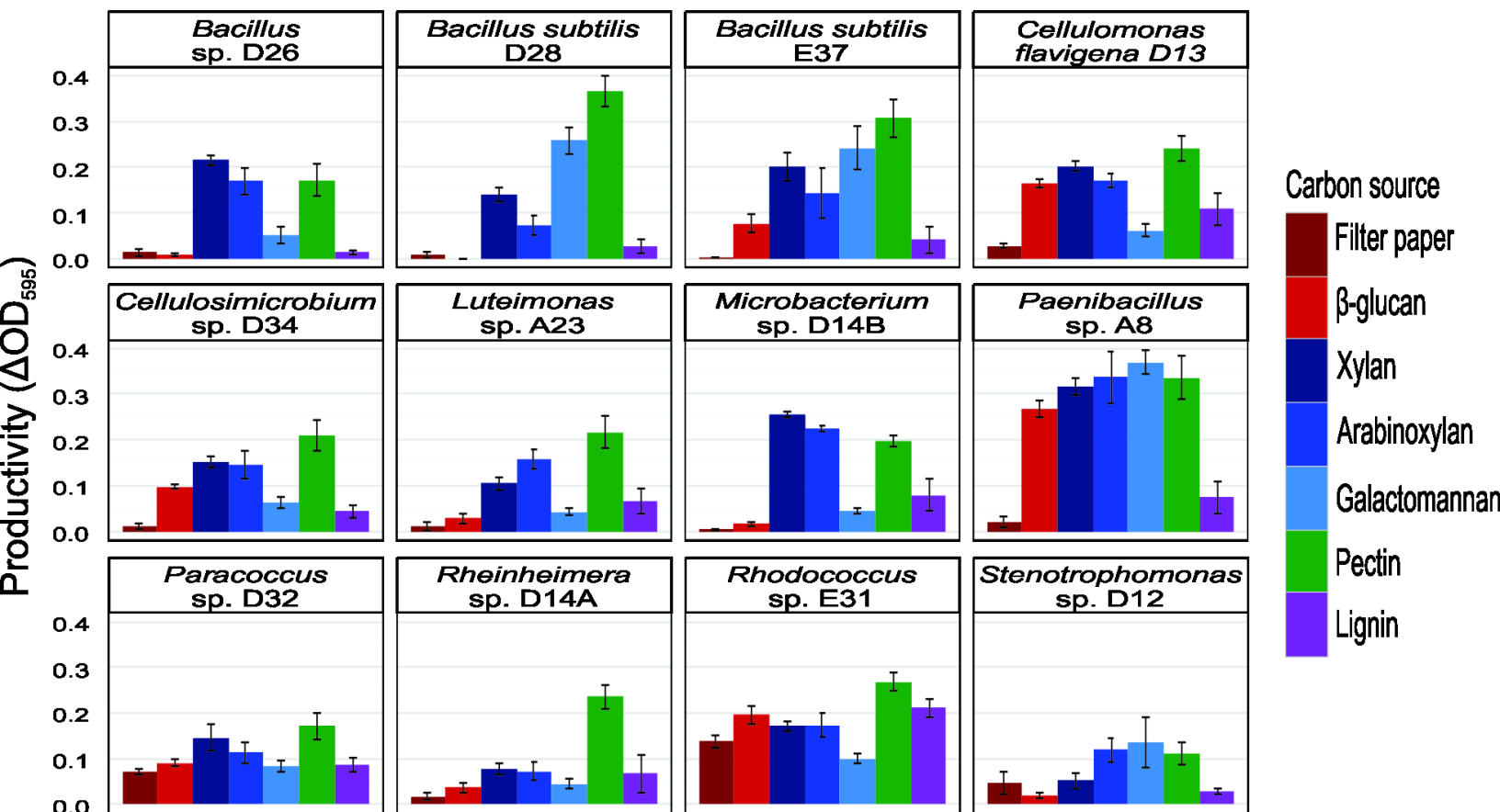
- 431 Teather RM, Wood PJ. (1982). Use of Congo red-polysaccharide interactions in enumeration
432 and characterization of cellulolytic bacteria from the bovine rumen. *Appl Environ Microbiol*
433 **43**: 777–780.
- 434 Tiunov AV, Scheu S. (2005). Facilitative interactions rather than resource partitioning drive
435 diversity-functioning relationships in laboratory fungal communities. *Ecol Lett* **8**: 618–625.
- 436 Van Der Heijden MGA, Bardgett RD, Van Straalen NM. (2008). The unseen majority: soil
437 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* **11**:
438 296–310.
- 439 Vishtal A, Kraslawski A. (2011). Challenges in industrial applications of technical lignins.
440 *Bioresources* **6**: 3547–3568.
- 441 Waldrop MP, Firestone MK. (2004). Microbial Community Utilization of Recalcitrant and
442 Simple Carbon Compounds: Impact of Oak-Woodland Plant Communities. *Oecologia* **138**:
443 275–284.
- 444 Wei H, Tucker MP, Baker JO, Harris M, Luo Y, Xu Q, *et al.* (2012). Tracking dynamics of plant
445 biomass composting by changes in substrate structure, microbial community, and enzyme
446 activity. *Biotechnol Biofuels* **5**: 20.
- 447 Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT, *et al.* (2016). Challenges in
448 microbial ecology: building predictive understanding of community function and dynamics.
449 *ISME J.* e-pub ahead of print, doi: 10.1038/ismej.2016.45.
- 450 Wohl DL, Arora S, Gladstone JR. (2004). Functional Redundancy Supports Biodiversity and
451 Ecosystem Function in a Closed and Constant Environment. *Ecology* **85**: 1534–1540.

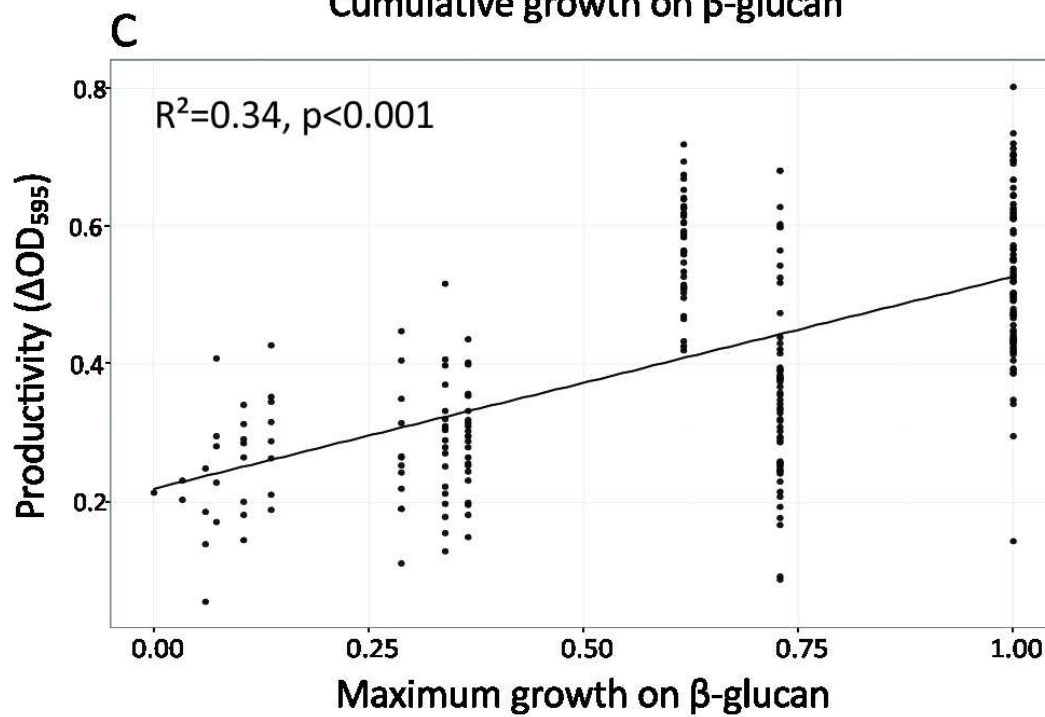
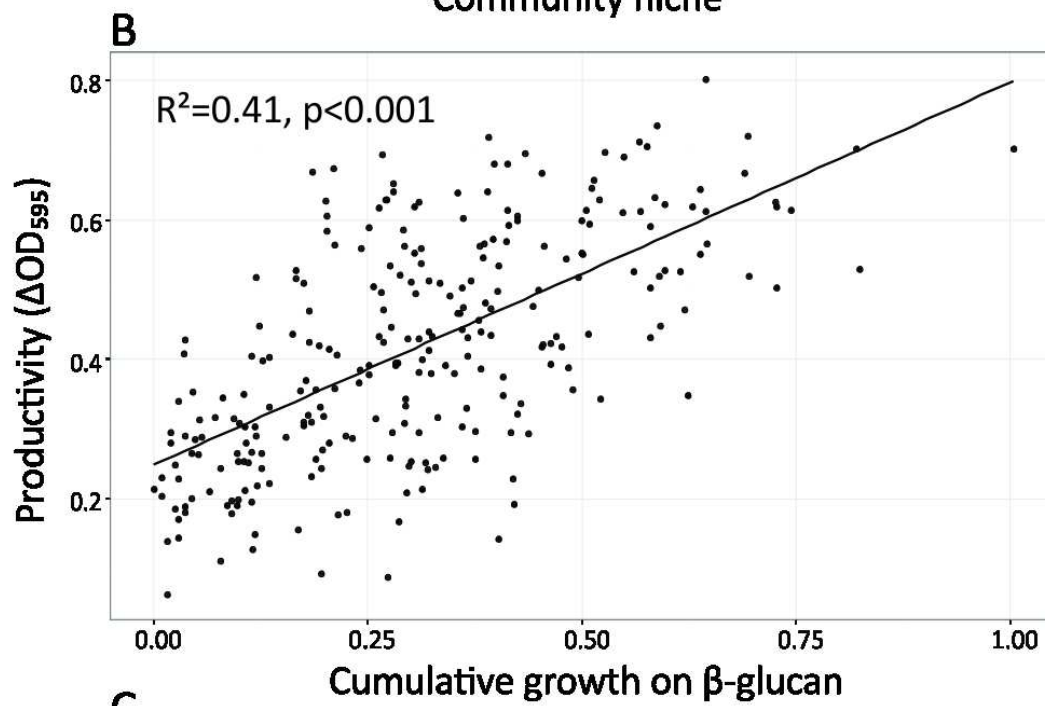
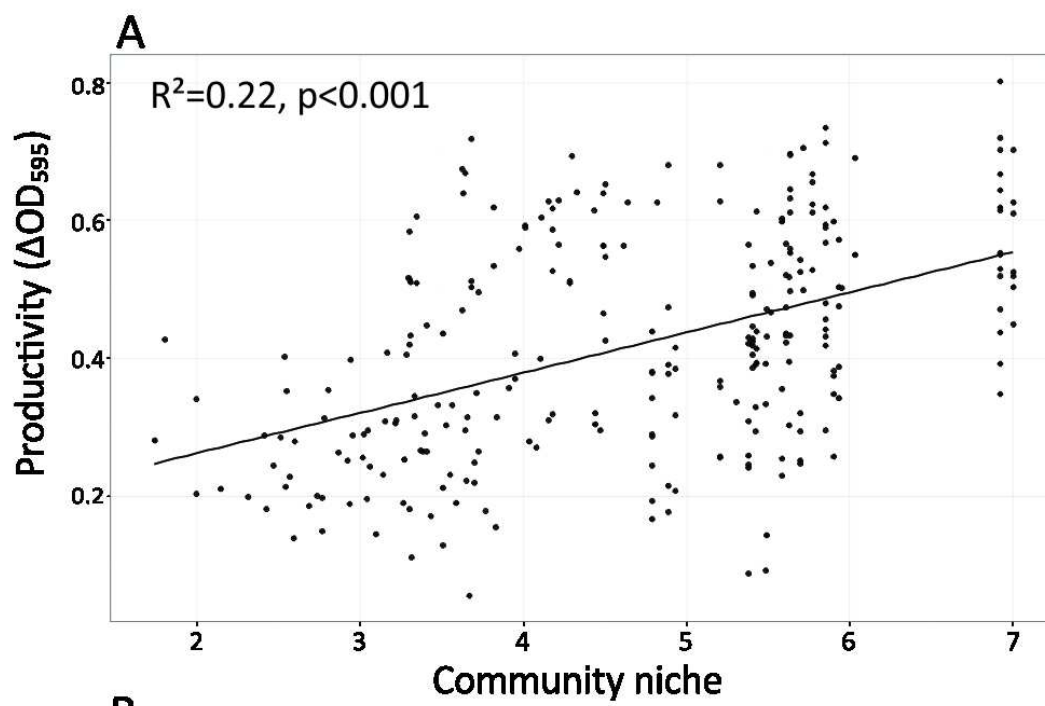
Figure 1 Relationship between community productivity and species richness. Black line shows linear regression for all data points ($F_{1, 264}=60.1$, $R^2=0.19$, $p<0.001$). Each point is the mean productivity of five replicate communities. Points are coloured by the presence or absence of *Cellulomonas flavigena* D13 and *Paenibacillus* sp. A8 and linear regressions between community productivity and species richness are shown for each of these groups: green points represent communities containing both these species ($F_{1, 28}=0.42$, $p>0.05$); red points represent communities containing *C. flavigena* D13 ($F_{1, 50}=4.43$, $p<0.05$); blue points represent communities containing *Paenibacillus* sp. A8 ($F_{1, 50}=1.01$, $p>0.05$); grey points represent communities containing neither of these species ($F_{1, 129}=60.1$, $p<0.001$). Productivity is measured as the cumulative change in OD₅₉₅ of MicroResp indicator plate after 7 days growth.

Figure 2 Productivity of species grown on each carbon source. Filter paper and β -glucan represent cellulose like substrates (red); xylan, arabinoxylan and galactomannan represent hemicelluloses (blue). Productivity is measured as the cumulative change in OD of MicroResp indicator plates over 7 days.

Figure 3 Relationship between community productivity and (A) community niche, (B) cumulative ability of constituent species to utilise β -glucan and (C) maximum ability of constituent species to utilise β -glucan. Higher community niche indicates communities can utilise more resources more efficiently. The ability of constituent species to utilise β -glucan was calculated from their ability to grow on this substrate in functional trait assays (Figure 2). Each point represents the mean productivity of five replicate communities.









































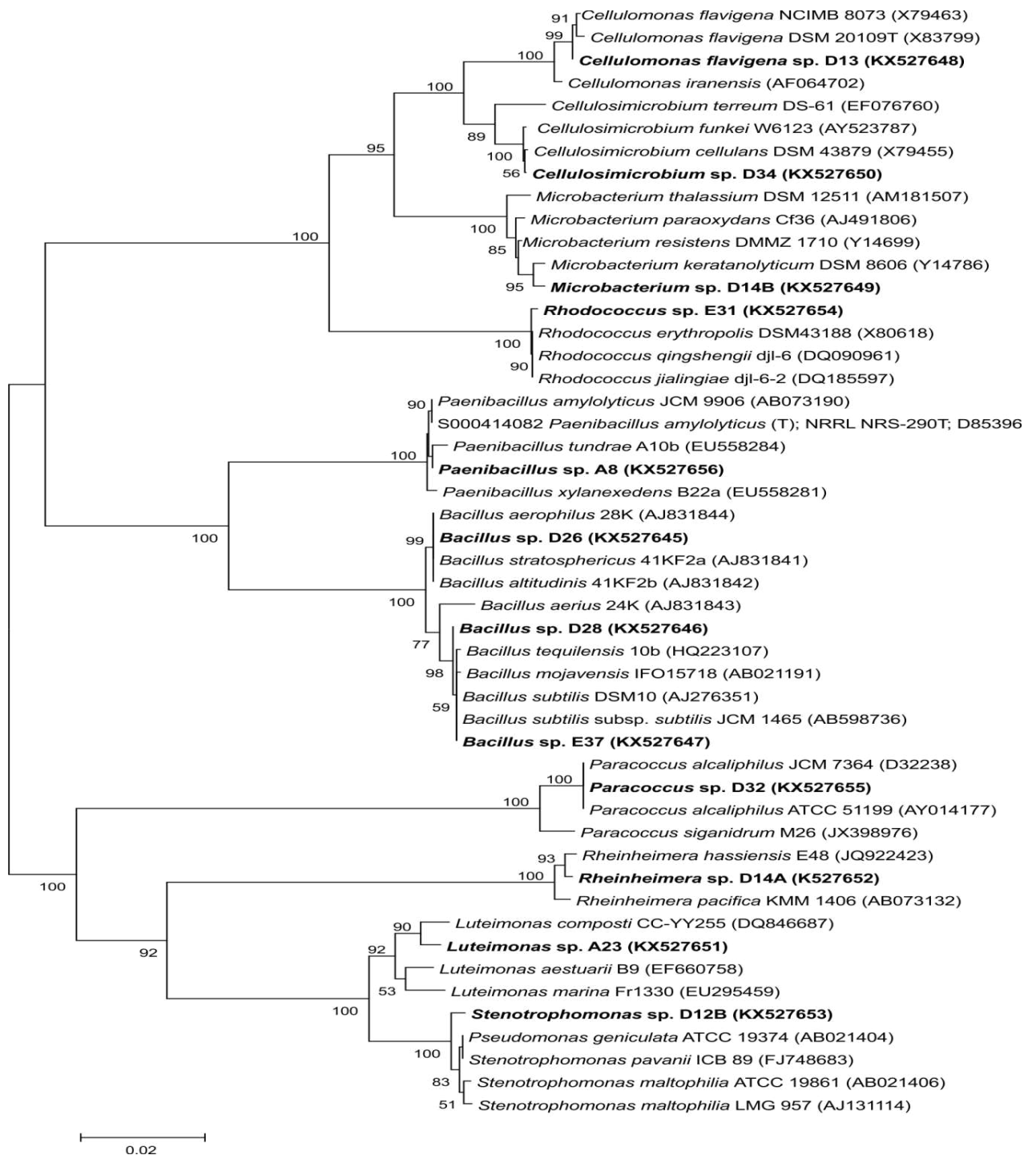


Supplementary information

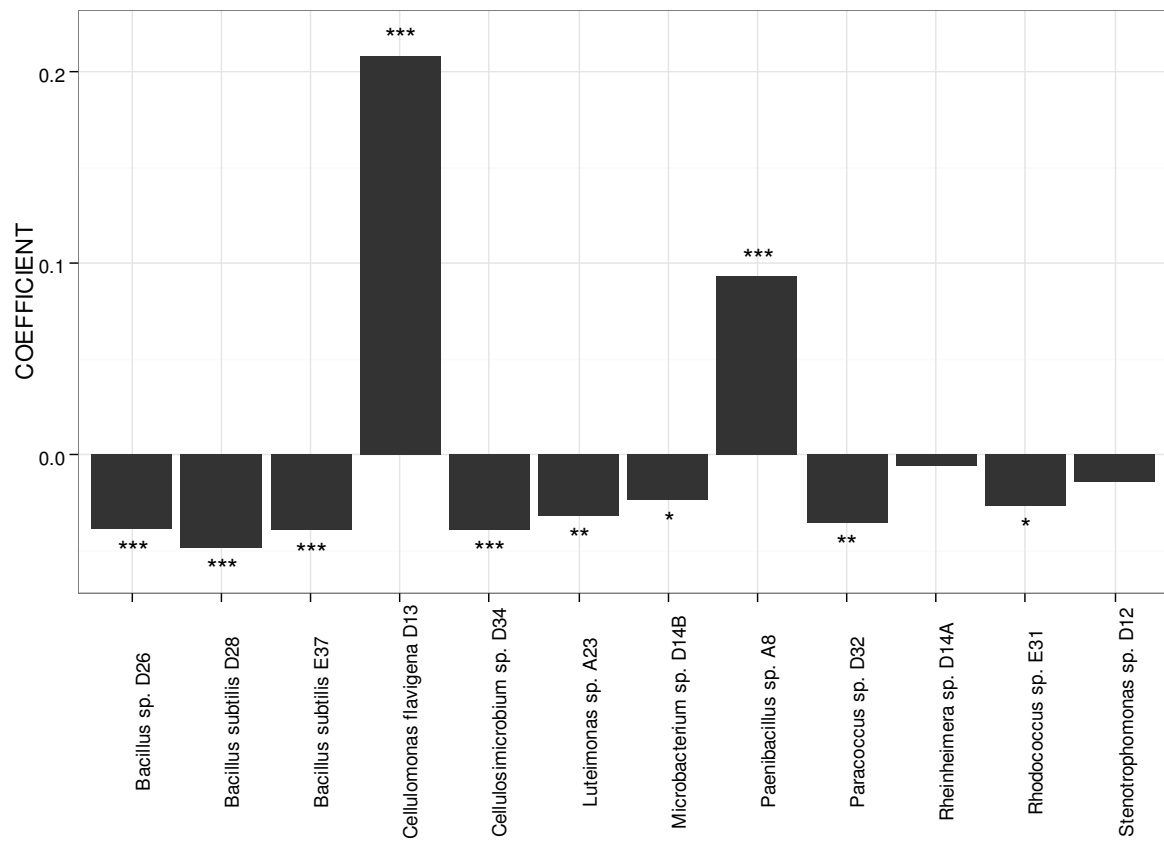
Supplementary Table 1 – Colony morphology and activity of isolates on xylan and carboxymethylcellulose (CMC) plate assays. Clear halos indicate enzymatic activity against xylan or CMC.

Isolate	Colony morphology	Xylan activity assay	CMC activity assay
<i>Cellulomonas flavigena</i> sp. D13			
<i>Cellulosimicrobium</i> sp. D34			
<i>Microbacterium</i> sp. D14B			
<i>Rhodococcus</i> sp. E31			
<i>Paenibacillus</i> sp. A8			
<i>Bacillus</i> sp. D26			
<i>Bacillus</i> sp. D28			
<i>Bacillus</i> sp. E37			
<i>Paracoccus</i> sp. D32			
<i>Rheinheimera</i> sp. D14A			
<i>Luteimonas</i> sp. A23			
<i>Stenotrophomonas</i> sp. D12B			

Supplementary Figure S1 – Neighbour-joining phylogenetic tree based on bacterial 16S rRNA gene partial sequences. Sequences were aligned using the SILVA Incremental Aligner (SINA) and analysed by MEGA6. Isolates from this study are highlighted in bold with accession numbers provided in brackets. Bootstrap values representing percentage of 1000 replicates are shown at nodes.

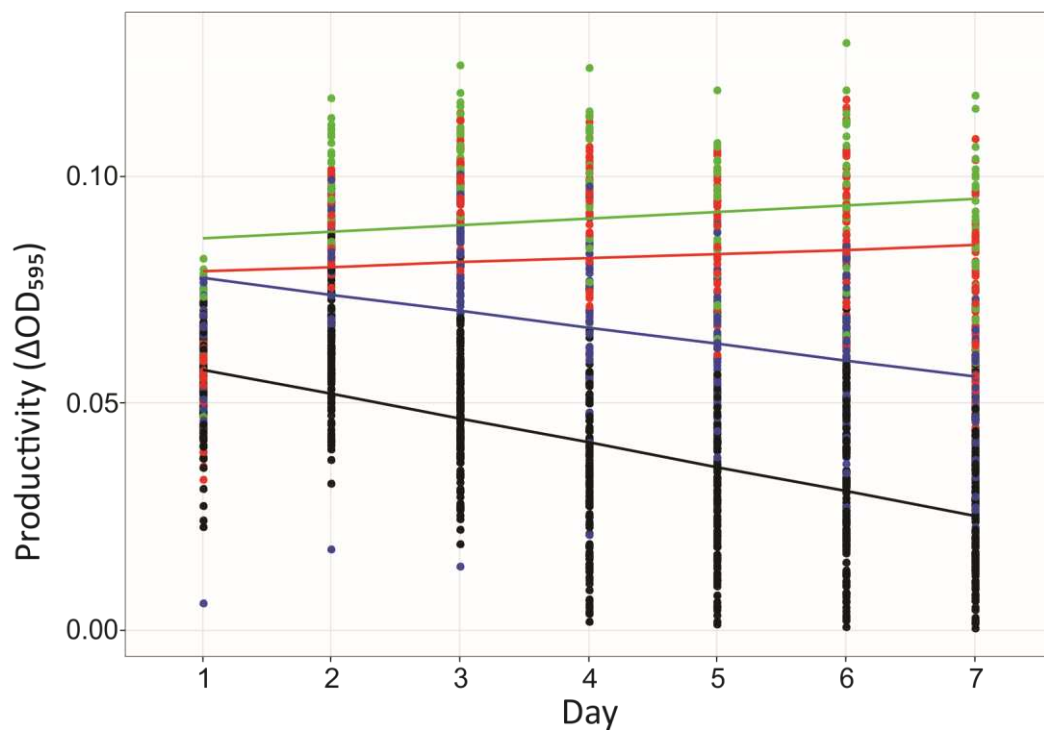


Supplementary Figure S2 – Linear model coefficients for each species in the BEF experiment. Positive or negative coefficients indicate species contribute more or less to community productivity than an average species (Bell et al, 2009).



Supplementary Figure S3 – Productivity of communities on each day of the BEF experiment.

Points represent mean of five replicate communities and are coloured by the presence of *Paenibacillus* sp. A8 (blue), *C. flavigena* D13 (red), both these species (green) or neither of these species (black). Productivity is the change in OD₅₉₅ of MicroResp indicator plates after 24h.



Supplementary Table 2 – Comparison of R^2 and AIC values between linear models with community productivity as the dependent variable. The relationship between cumulative (sum) functional traits and maximum functional traits for each carbon source are shown. Cumulative functional traits are checked for significance when the variation explained by diversity is removed from the model to ensure these variables are not acting as a proxy of diversity.

Explanatory variable		R^2	AIC	Significance when diversity variation removed from model
Species richness		0.19	-266	NA
Community niche		0.22	-277	NA
β -glucan	Sum	0.41	-352	$p < 0.001$
	Max	0.34	-322	NA
Arabinoxylan	Sum	0.28	-302	$p < 0.05$
	Max	0.22	-279	NA
Xylan	Sum	0.27	-297	$p < 0.05$
	Max	0.22	-278	NA
Galactomannan	Sum	0.18	-266	$p > 0.05$
	Max	0.15	-255	NA
Filter paper	Sum	0.04	-223	$p > 0.05$
	Max	n.s.	-212	NA
Pectin	Sum	0.20	-272	$p > 0.05$
	Max	0.07	-232	NA
Lignin	Sum	0.18	-264	$p > 0.05$
	Max	0.02	-219	NA