Title

Defining the functional traits that drive bacterial decomposer community productivity

Running title

Functional traits predict community productivity

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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

4

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 OD_{600}

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 CO_2 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

6

120 the cumulative change in absorbance (λ=595nm) 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 $_{336}$=30.3, p<0.001) and carbon source ($F_{6, 336}$=105.8, p<0.001) on productivity and a

217 significant interaction between these factors ($F_{66, 336}$=6.8, p<0.001), suggesting niche

218 differentiation in resource use among the species. It is notable that some species, in

219 particular *Rhodococcus* sp. E31, displayed generalist resource use, being able to grow on

220 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 $_{264}$=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 ($F_{1, 264}$=182.7, p<0.001) and was the best

238 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

241 variation respectively. There were significant positive relationships between the remaining

242 carbon sources and community productivity though these explained less variation than

243 community richness and were not significant when species richness was included in models.

244 The fundamental niche space of community is unlikely to be achieved due to interactions

245 between species such as competition for resources. Therefore to approximate the realised

246 niche space of each community we also analysed the maximum performance per carbon

247 source in a community. Consistent with the analysis of summed performance, maximum

248 performance on β-glucan, arabinoxylan and xylan had significant positive relationships with

249 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

250 respectively) explaining 34%, 23% and 22% of variation respectively. There were significant

251 positive relationships between the maximum performance on lignin ($F_{1, 264}$=7.4, p<0.01),

252 pectin ($F_{1, 264}$=20.8, p<0.001) and galactomannan ($F_{1, 264}$=47.1, p<0.001) and community

253 productivity though these explained less variation than community richness. There was no

254 significant relationship between the maximum ability to degrade filter paper and

255 community productivity (Supplementary Table 2). This suggests that identifying and

256 measuring key functional traits could be a better predictor of community productivity than

257 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

13

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, Krištůfek 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, Gulledge 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 OD_{595} 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.

Productivity (ΔOD_{595})

Carbon source
- Filter paper
- β-glucan
- Xylan
- Arabinoxylan
- Galactomannan
- Pectin
- Lignin

A — R²=0.22, p<0.001 — Productivity (ΔOD₅₉₅) vs Community niche

B — R²=0.41, p<0.001 — Productivity (ΔOD₅₉₅) vs Cumulative growth on β-glucan

**C** — R²=0.34, p<0.001 — Productivity (ΔOD₅₉₅) vs Maximum growth on β-glucan
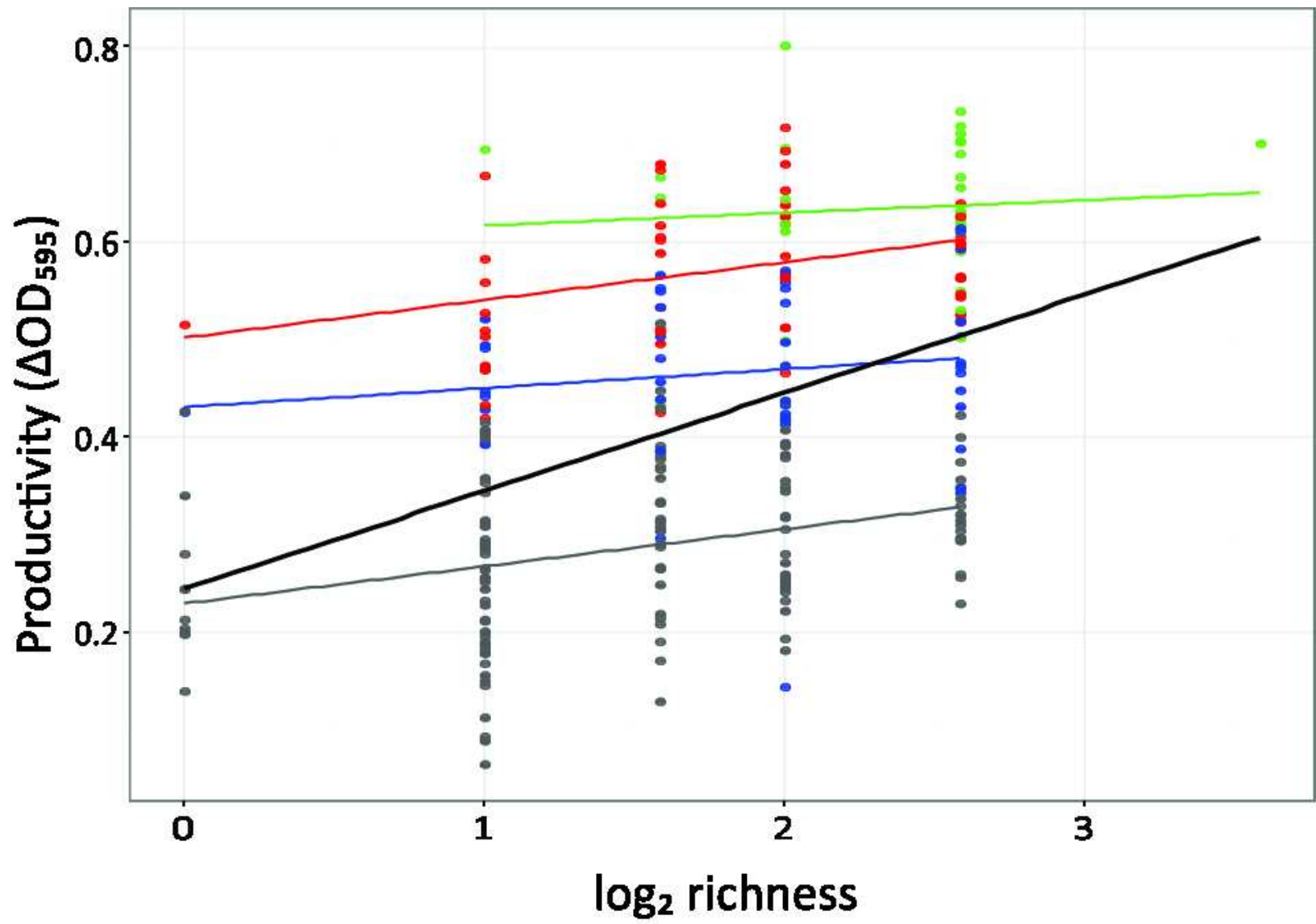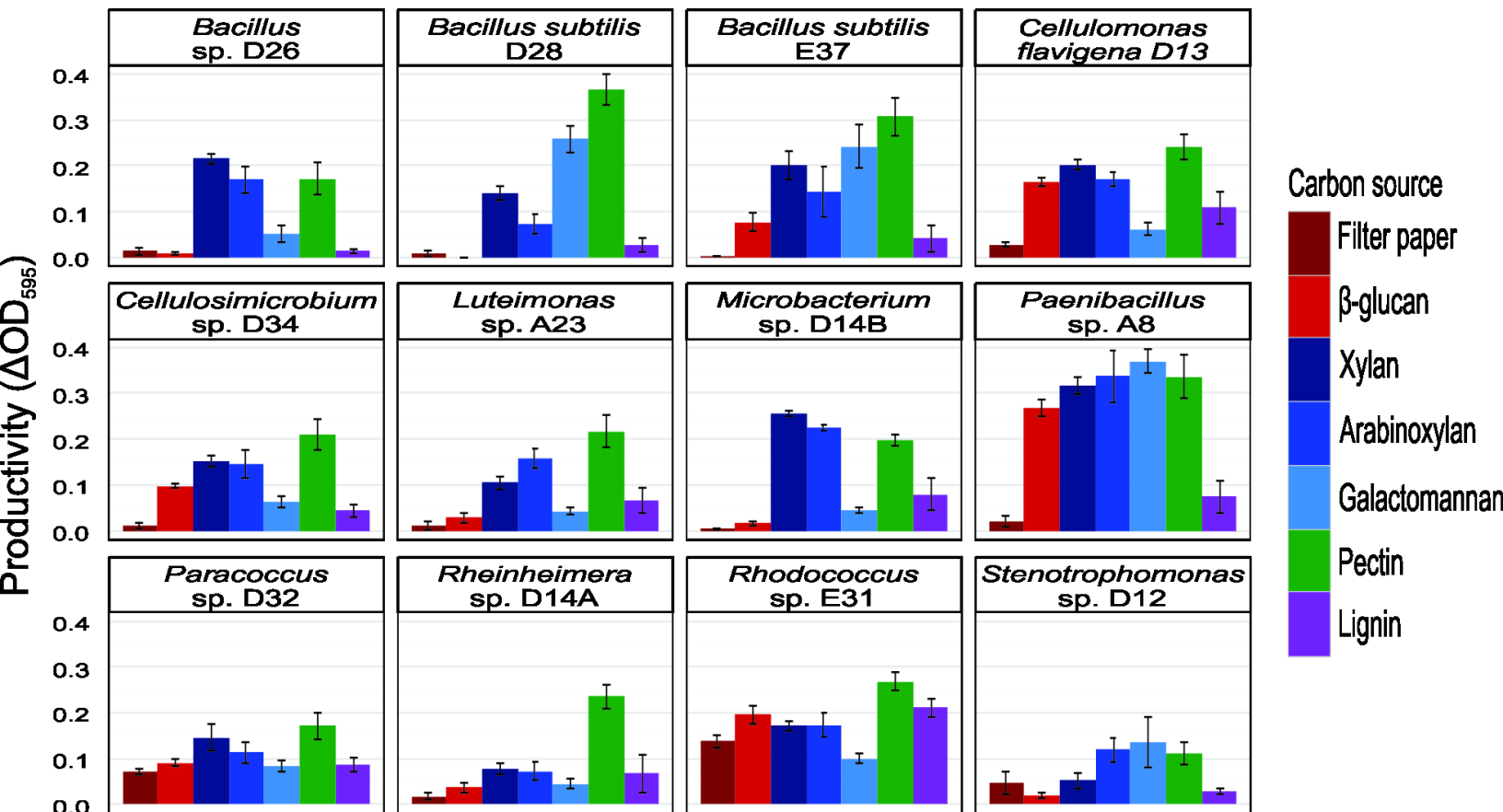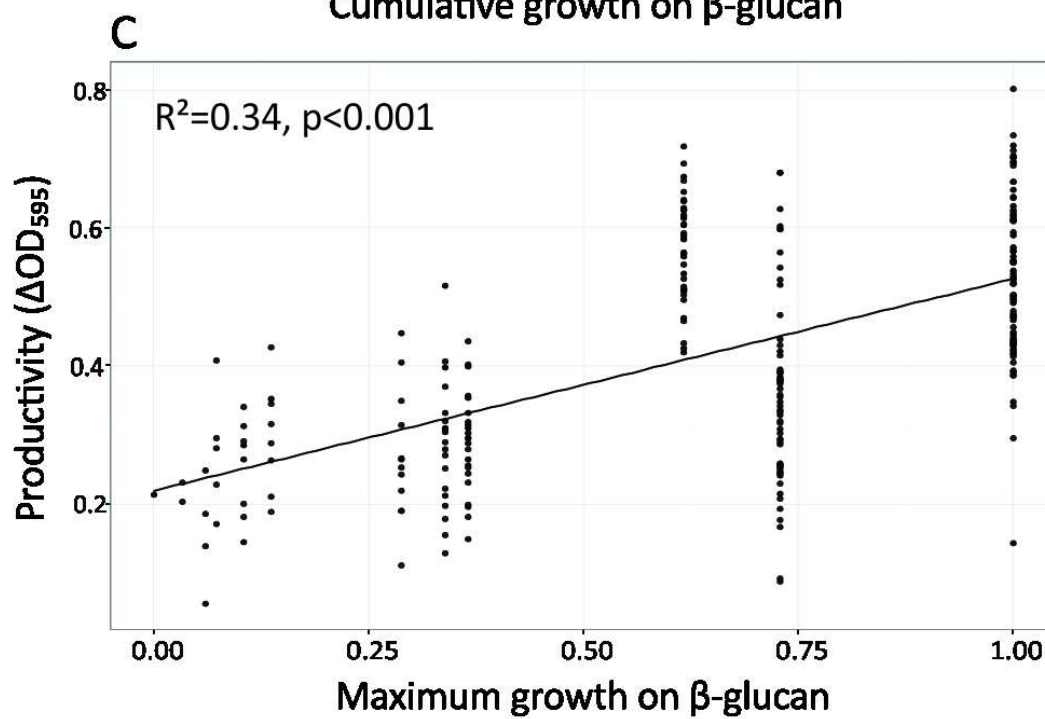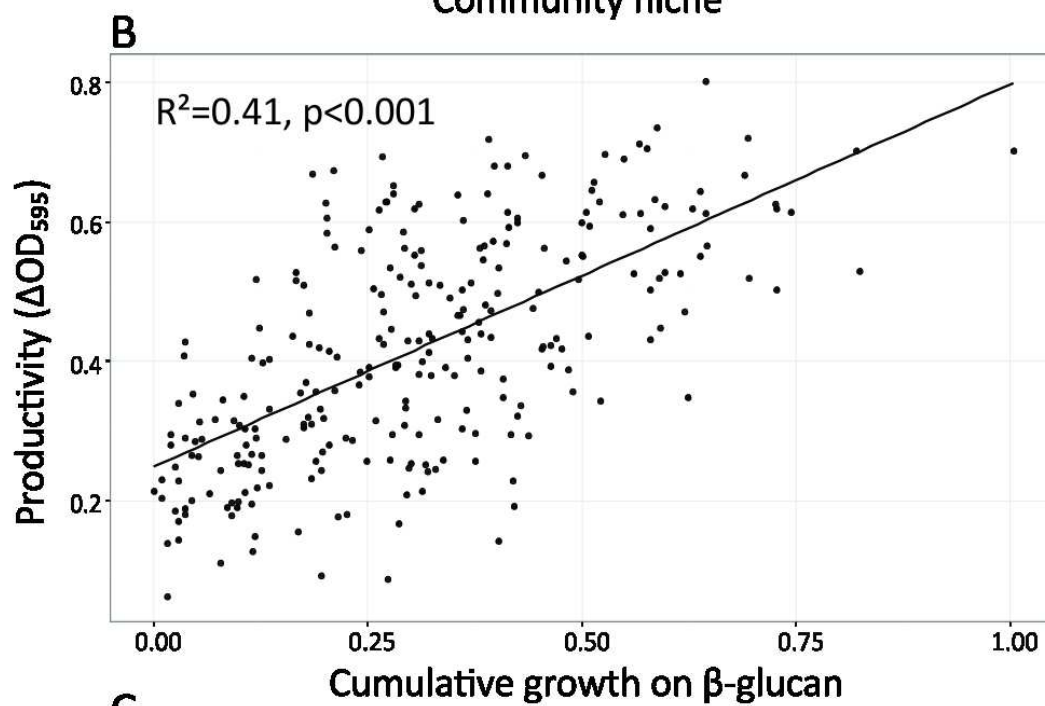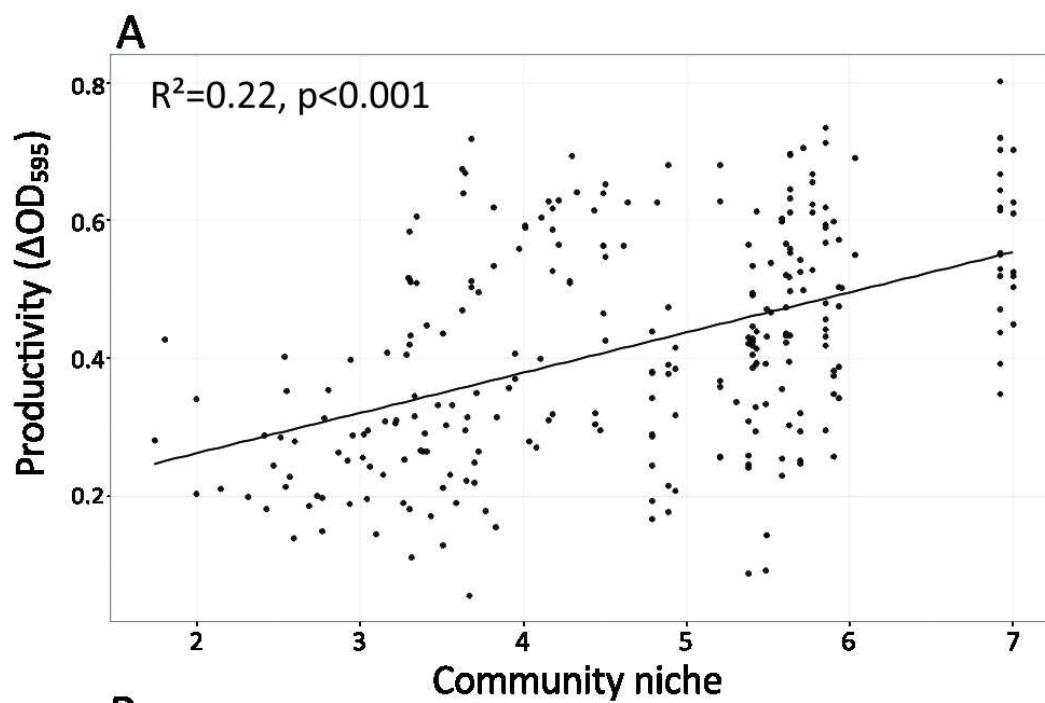
Supplementary information

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
Cellulomonas flavigena sp. D13			
Cellulosimicrobium sp. D34			
Microbacterium sp. D14B			
Rhodococcus sp. E31			
Paenibacillus sp. A8			
Bacillus sp. D26			
Bacillus sp. D28			
Bacillus sp. E37			
Paracoccus sp. D32			
Rheinheimera sp. D14A			
Luteimonas sp. A23			
Stenotrophomonas 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.
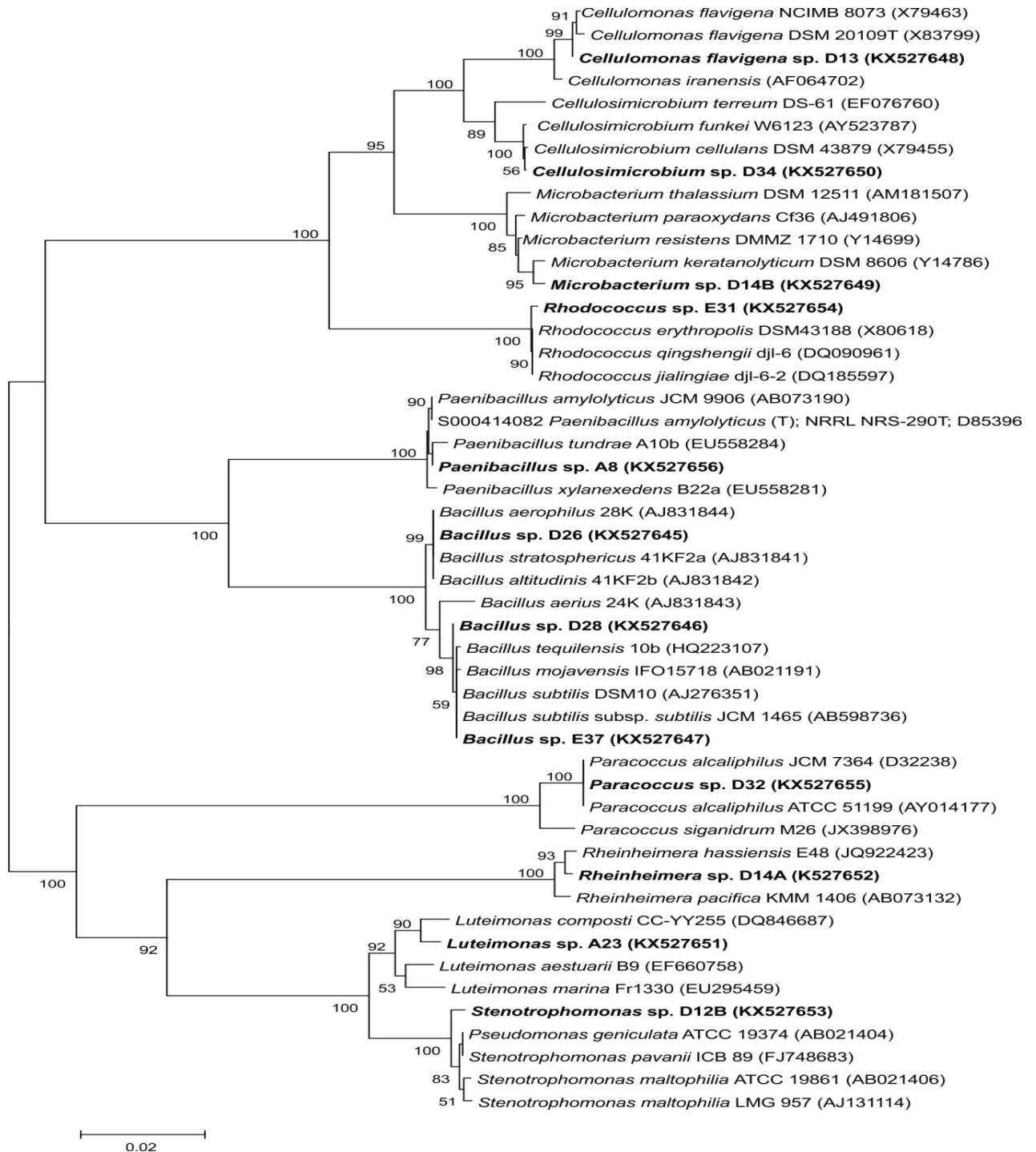
Supplementary Figure S2 – Linear model coefficients for each species in the BEF experiment. Positive or negative coefficients indicate species contribute more or less to community productivity than an average species (Bell et al, 2009).
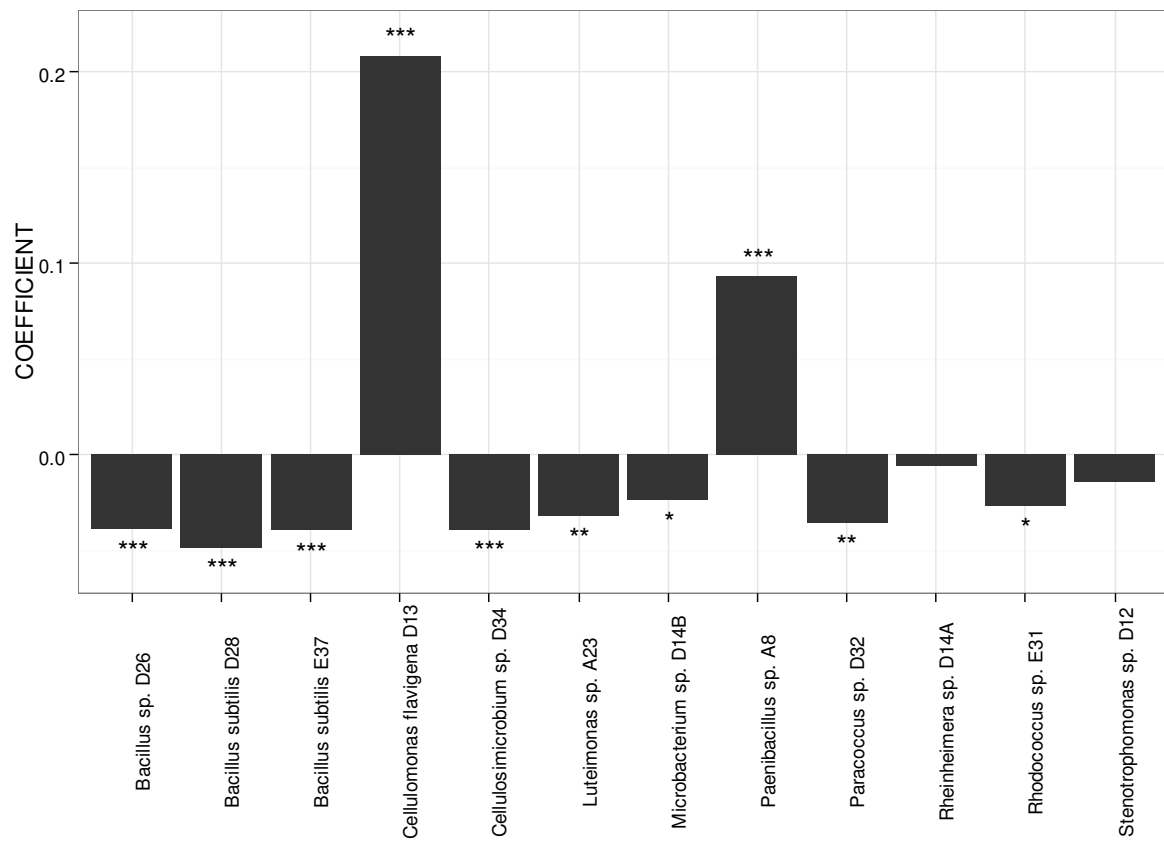
Supplementary Figure S3 – Productivity of communities on each day of the BEF experiment. Points represent mean of five replicate communities and are coloured by the presence of Paenibacillus sp. A8 (blue), C. flavigena D13 (red), both these species (green) or neither of these species (black). Productivity is the change in OD_{595} of MicroResp indicator plates after 24h.
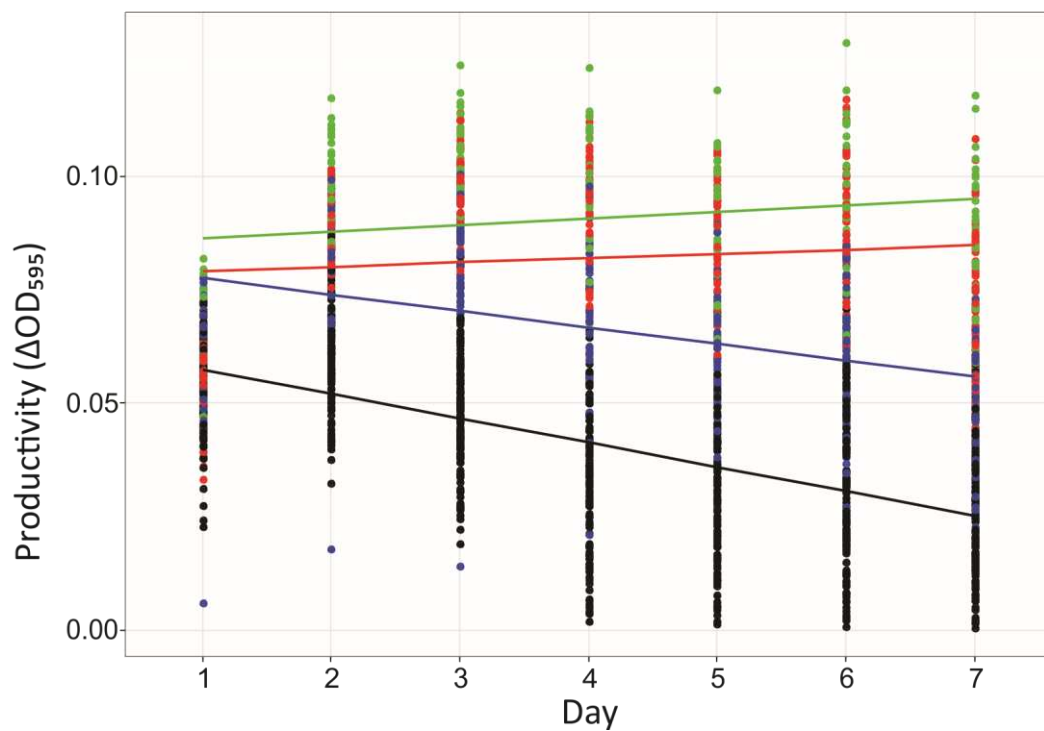
Supplementary Table 2 – Comparison of R^2 and AIC values between linear models with community productivity as the dependent variable. The relationship between cumulative (sum) functional traits and maximum functional traits for each carbon source are shown. Cumulative functional traits are checked for 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