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1 **Inhibitions dominate but stimulations and growth rescues are not rare among bacterial isolates**  
2 **from grains of forest soil**

3

4

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14

15 **Abstract**

16 Soil is a complex environment made of multiple microhabitats in which a wide variety of microorganisms  
17 co-exist and interact to form dynamic communities. While the abiotic factors that regulate the structure  
18 of these communities are now quite well documented, our knowledge of how bacteria interact with each  
19 other within these communities is still insufficient. Literature reveals so far contradictory results and is  
20 mainly focused on antagonistic interactions.

21 To start filling this gap, we isolated 35 different bacterial isolates from grains of soil assuming that, at  
22 this scale, these bacteria would have been likely interacting in their natural habitat. We tested pairwise  
23 interactions between all isolates from each grain and scored positive and negative interactions. We  
24 compared the effects of simultaneous versus delayed co-inoculations, allowing or not to a strain to  
25 modify first its environment.

26 One hundred fifty seven interactions, either positive or negative were recorded among the 525 possible  
27 one's. Members of the *Bacillus subtilis*, *Pseudomonas*, and *Streptomyces* genera were responsible for  
28 most inhibitions while positive interactions occurred between isolates of the *Bacilliales* order and only in  
29 delayed inoculation conditions. Antagonist isolates had broad spectra abilities to acquire nutrients from  
30 organic and inorganic matter while inhibited isolates tended to have little potentials. Despite an overall  
31 domination of antagonistic interactions (87 %), a third of the isolates were able to stimulate or rescue  
32 the growth of other isolates, suggesting that cooperation between bacteria may be underestimated.

33

34 **Key words:** bacterial interactions, microhabitat, antagonism, beneficial interactions, forest soil

35 **Introduction**

36 Microbial communities are key components of forest soil ecosystems since they perform critical  
37 functions such as nutrient cycling, organic matter decomposition and mineral weathering [1, 2]. The high  
38 heterogeneity in the structure of soils and their colonization by plethora of macro-organisms (plant roots,  
39 insects, worms...) create a multiplicity of habitats that strongly differ from each other in terms of nutrient  
40 availability and physico-chemical properties. For instance, rhizospheres and worm burrows are very rich  
41 in nutrients compared to soil particles that contain little bioavailable nutrients. Both types are colonized  
42 by microorganisms and almost each soil particle form a specific microhabitat that provides unique  
43 environmental conditions [3]. A single gram of soil is expected to be colonized by  $10^6$  to  $10^9$  microbial  
44 cells belonging to several hundred to thousands of microbial species [4, 5]. We start to have extensive  
45 knowledge on the identity of microorganisms that colonize different soils and different habitats in soils  
46 thanks to the recent massive use of amplicon sequencing, and in a lower extent, of the metabolic  
47 activities of these microorganisms through metagenomic and metatranscriptomic sequencing [4]. By  
48 contrast, we still have little information on how these microorganisms interact together. However, biotic  
49 interactions are one of the pillars of microbial community structuring, together with environmental  
50 filtering and random selection [6].

51

52 It is well established that microorganisms inhabiting the same environment compete for space and  
53 resources. For such interactions, bacteria have developed a wide array of strategies, including antibiotic  
54 production, nutrient scavenging, quorum sensing and signal disruption [7, 8]. In addition to antagonism,  
55 numerous cases of synergy through modulation of the environment or production of common goods  
56 have been reported [9]. But questions remain such as how prevalent are those interactions and who are  
57 the species involved in such interactions in soils. Most studies addressing these questions focused on  
58 other types of environments (i.e marine sediments and particles, aquatic systems, food products) that  
59 are very different in terms of physico-chemical properties and thus are colonized by different microbial

60 communities (e.g. [10–14]). The few studies performed on soil bacteria focused on specific groups of  
61 bacteria such as *Streptomyces* [15–18], *Bacillus* [19], ligno-cellulolytic bacteria [20] or arctic soil [21]. In  
62 addition, most of them analysed inhibitory interactions and did not look for potential positive interactions  
63 [18–22]. If all studies indicate that interactions are not randomly distributed and are very likely to  
64 contribute to the organization of soil microbial communities, contradictory results were obtained. The  
65 proportion of interactions deeply varied between studies, ranging from a few per cent of strains that  
66 interact with others [15, 22] to more than 60 % [17]. Furthermore, some found that kin bacteria with  
67 elevated metabolic overlap were more likely to inhibit each others [19, 22] while other found the  
68 opposite correlation [17, 20]. Altogether, these results indicate the existence of important gaps in our  
69 knowledge of how bacteria interact and how prevalent are those interactions.

70

71 If the outputs of the interactions highly depend on the identities and functional abilities of the  
72 microorganisms involved, they also vary depending on the environmental conditions. The production of  
73 the secondary metabolites used to scavenge nutrients or to antagonize competitors is a costly process  
74 under tight regulation [7]. Since nutrients are present in limited amounts in soil and vary in quantity and  
75 quality along time, interaction behaviours are likely to change depending on the initial conditions. In the  
76 present work, we tested the impact of delayed inoculation vs co-inoculation on the output of interactions.  
77 The two conditions were seen as a proxy of two events that can occur in natural conditions: sets of  
78 resting bacterial isolates that simultaneously start their growth in response to a nutrient bloom (e.g.  
79 arrival of a root) versus bacterial isolate incomer that reaches a habitat already colonized by bacteria.  
80 We hypothesized that more inhibitions of higher intensities should occur in delayed conditions than in  
81 co-inoculation because of nutrient depletion and of the accumulation of antagonistic compounds.  
82 However, this should only concern bacterial antagonists with broad activities that constitutively produce  
83 such compounds. In addition, we expected to obtain more positive interactions in delayed conditions as  
84 it gives time for bacteria to modify their environment, provide common goods and eventually create new

85 niches. To test these hypotheses, we isolated bacteria from grains of forest soil, a scale at which they  
86 were likely to interact in natural conditions. We chose to work with bacteria isolated from two different  
87 microhabitats, bulk soil and rhizosphere, in order to increase the amount of possible interactions. We  
88 identified bacterial isolates on the basis of their 16S rDNA sequence and characterized their potential  
89 functional abilities. Then pairwise interactions between isolates from the same grains of soil were  
90 performed in co-inoculation and delayed inoculation conditions to investigate the type of interactions  
91 occurring, their frequencies and intensities.

92

### 93 **Material & methods**

94 Soil samples were collected from a 40 years old mixed forest dominated by oaks (*Quercus robur*) and  
95 beeches (*Fagus sylvatica*) at the edge of the Champenoux site of the MOS research network in October  
96 2016 (N 48.7215350, E 6.3408050, site description in [23]). Two oaks located three meters apart were  
97 chosen and the litter was removed in between. Then, a cube of 15 x 15cm of topsoil was extracted with  
98 a clean shovel and wrapped in a sterile bag and then taken to the laboratory in an icebox. The cube was  
99 then opened in the laboratory to access parts that have not been potentially contaminated by the  
100 shovel or during transportation. Three distinct zones, separated from each other by at least 5 cm and  
101 colonised by roots considered to be metabolically active because they also harboured turgescient  
102 ectomycorrhizae, were chosen to collect the grains of soil. In each area, one grain (approx. 5 mg) of soil  
103 adhering to a root (rhizosphere) and one grain of bulk soil (5 cm away from any root) were collected  
104 under a binocular using a sterile clamp to isolate bacteria (n=6). Root samples (1cm long) were taken in  
105 the same time to confirm by molecular identification that roots belonged to *Quercus robur* (see  
106 supplemental methods)

107

108 *Isolation of bacterial strains from grains of soil*

109 In order to isolate a broad diversity of bacteria from each grain of soil, two isolation methods were used:  
110 one based on non-selective medium that favours the growth of aerobic soil bacteria and one that  
111 specifically selects for sporulating *Streptomyces* because i) they are important active members of soil  
112 and rhizosphere communities [24], ii) they are expected to be important interactants in communities  
113 [18], iii) only spores can be kept over long period of time in laboratory libraries.

114 To do so, each grain of soil was weighted then suspended in 1 mL of 0.9% NaCl and mixed with a vortex  
115 3x 1 min to separate bacterial cells from soil particles. Each solution was divided in two subsamples that  
116 were treated independently. Serial dilutions of first subsamples were plated on 1/10 strength tryptic soy  
117 agar (1/10<sup>th</sup> TSA, see supplemental methods) as a non-selective medium with low level of nutrients [25].  
118 All agar plates were incubated at 25°C for 10 days. All colonies showing different morphotypes (colour,  
119 shape, smooth vs rough...) were picked and plated on new 1/10<sup>th</sup> TSA plates. Each isolate from the  
120 1/10 TSA plate was re-streaked on 1/10 TSA 3 times for purification purposes. Initial plates were further  
121 incubated for 8 additional days to look for potential slow growers. Then, all the bacterial isolated were  
122 cryopreserved at -80 °C in 20% glycerol.

123 Second subsamples were subjected to a heat treatment of 50°C for 1 hour as described by Bontemps  
124 et al. [26] and then serial dilutions were inoculated on SIM agar (see supplemental methods). Plates  
125 were incubated at 25°C for 10 days. Sporulating *Streptomyces*-like strains were selected and inoculated  
126 on soy flour medium (SFM, see supplemental methods) for purification. After 7 days of growth at 25°C,  
127 the lawn of spores were collected with sterile water. The spore solutions were vortexed, filtered using  
128 sterile cotton, centrifuged (2400 g, 10 min, 4°C), suspended in 20% glycerol, and stored at -80°C. Initial  
129 plates were further incubated for 8 additional days to look for potential slow growers. The spore  
130 concentration (CFU/mL) was determined by dilution and plating.

131

132 *Molecular identification of bacterial isolates and phylogenetic analysis*

133 For the non-actinomycete bacteria, DNA extraction was performed using Wizard genomic DNA  
134 purification kit using manufacturer recommendations (Promega). The 16S rRNA gene sequence of  
135 these bacterial strains was amplified by PCR with the primers 16S\_27F (5'- AGAGTTTGAT  
136 CCTGGCTCAG -3') and 16S\_907R (5'- CCGTCAATTC ATTTGAGTTT -3') using Phusion high fidelity  
137 Taq polymerase (Fisher Scientific) following PCR conditions described in [25]. For *Streptomyces*-like  
138 isolates, 16S rRNA gene PCR amplification was done using 100% DMSO-cell suspension mix [27] and  
139 the StrepB forward (5'- ACAAGCCCTGGAAACGGGGT-3') and StrepF reverse (5'-  
140 ACGTGTGCAGCCCAAGACA-3') primers as described by Rintala et al. [28]. Lengths of PCR products  
141 were checked with 1% agarose gel then purified with QIAquick PCR purification kit (Qiagen) following  
142 manufacturer protocol. PCR products were then sequenced by Sanger sequencing method by Eurofins  
143 company (Germany). After verification of the chromatograms, forward and reverse sequences were  
144 assembled using CLC Main Workbench (CLC bio) and taxonomical affiliations of bacterial isolates were  
145 determined using the Ribosomal Database Project (RDP-II) classifier with 95% confidence threshold  
146 [29]. Phylogenetic trees were then built using the NGPhylogeny.fr pipeline [30]. Sequences were  
147 aligned with MUSCLE algorithm, alignments were curated with GBLOCKS and phylogeny inferred with  
148 PhyML using GTR as substitution model. All sequences are available on Genbank database under the  
149 accession numbers MK734019 to MK734040 (bulk soil isolates – Grain 1) and MK801941-MK801965  
150 (rhizosphere isolates – Grain 2).

151

#### 152 *Preparation of bacterial inocula*

153 Non-actinobacteria were grown from glycerol stock on 1/10 TSA plates at 25°C for 48 h. Then 2-3  
154 bacterial colonies were picked, suspended in 10 mL of tryptic soy broth (TSB) and grown at 25°C under  
155 agitation of 200 rpm for 48 h. The bacterial cultures were spun down and the pellets were washed with  
156 1X phosphate buffered saline (PBS) 3 times and resuspended in 1X PBS to obtain a suspension of



157  $A_{600nm} = 0.6$ . For actinobacteria, spore solutions were diluted to reach an approximate of  $10^8$  cfu/mL and  
158 heat-shock treated (50°C, 10 min) to induce spore germination before further inoculation [31].

159

#### 160 *Assessment of potential functional abilities*

161 In order to evaluate the abilities of the bacterial isolates to obtain nutrients from soil organic and  
162 inorganic sources of C, N, P and Fe, agar tests and fluorogenic assays were performed. We evaluated  
163 the potential ability of isolates to achieve hydrolysis of cellulose with endo and exo-enzymes,  
164 proteolysis, phytate degradation, production of inorganic and organic phosphatases, and iron  
165 scavenging by siderophores. Detailed protocols are provided in supplemental methods

166

#### 167 *Pairwise bacterial interactions*

168 The ability of bacterial isolates to interact within each other was assessed using two complementary  
169 approaches: i) simultaneous co-inoculation and ii) delayed co-inoculation. Interactions were made on  
170 modified Murashige & Skoog (mMS) agar plate (Murashige and Skoog, 1962, described in  
171 supplemental methods) in which sucrose was replaced by glucose as most bacteria cannot use sucrose  
172 as a source of carbon. This medium was chosen with the aim to later test the impact of interactions on  
173 plant development.

174 First, 5  $\mu$ L of bacterial or spore suspensions ( $A_{600nm} = 0.6$  or  $10^8$  CFU/mL) were streaked on mMS agar  
175 along the centre of a square petri dish and then 5  $\mu$ L of bacterial or spore suspension ( $A_{600nm} = 0.6$  or  
176  $10^8$  CFU/mL) were cross-streaked at around 5 mm away from the centre to produce a 5 cm long streak  
177 either immediately after the deposition of the central streak (co-inoculation, Supplemental Fig. 1A) or 48  
178 h after the inoculation of the central streak (delayed-inoculation, Supplemental Fig. 1B). Plates were  
179 incubated 5 days at 25°C. Interactions (effect of the central streak on the side streaks) were determined  
180 by measuring the length of the bacterial streaks on the sides by comparison to the initial inoculation  
181 streak (5 cm long). Combinations were done from bacteria originating from the same grain only.

182 Negative controls consisted of single species streak inoculation against 1X PBS. Self-interactions were  
183 also tested. For each method, three independent assays were analysed.

184

#### 185 *Data visualisation and statistical analyses*

186 Negative interactions were scored between 0 and 4 on the basis on the length of the streak after 5 days  
187 of growth compared to the initial size of the streak (5 cm). Scores were coded as follows: 0 – full growth,  
188 1 – up to 20 % of reduction of growth, 2 – 20 to 40 %, 3 – 40 to 60%, 4 – more than 60 % of growth  
189 reduction. An interaction was considered to be positive when the length and/or width of the streak was  
190 longer or larger than the one of the streak in the negative control. Positive interactions were recorded as  
191 binary data: no effect / positive effect. Raw data are provided in Supplemental Table 1.

192 Interaction networks between isolates from the same grain were constructed after transformation of  
193 interaction records into 5 categories: no visible interaction, one-way and mutual inhibitions and  
194 stimulation, respectively. Networks were then constructed with yFiles hierarchical layout option of  
195 Cytoscape v3.7.2 [33].

196 To measure the level of interaction of each isolate as a sender and as a receiver, the proportions of  
197 isolates that were affected by a sender and by which it was affected as a receiver were calculated for  
198 each grain and for each inoculation condition.

199 Sender and receiver degrees were calculated to evaluate the properties of the network and to  
200 characterize relationships between sender and receiver abilities of the different strains. The sender  
201 degree of an isolate was considered to be the number of isolates it inhibits while the receiver degree  
202 was the number of isolates that inhibit the isolate [13].

203 Effects of type of inoculation (co-inoculation vs delayed) or grain origin on the proportion of interactions,  
204 proportions of inhibitions, distribution of intensities of interactions and sender/receiver degree  
205 distributions were tested with  $X^2$  tests ( $p < 0.05$ ).

206

## 207 **Results**

### 208 *Classification of microorganisms isolated from grains of soil*

209 Bacterial colonies were successfully isolated from two grains out of six: one collected from bulk soil  
210 (further referred as “grain 1”) and one collected from rhizosphere (further referred as “grain 2”). One to 6  
211 colonies were obtained from the four other grains and were not further considered. A total of 137 and  
212 154 colonies were isolated from the two selected grains after 10 days of growth. No more isolate was  
213 obtained after 8 additional days of growth. Twenty-two and 25 had different morphotypes. Out of these,  
214 10 and 15 isolates were considered to be different based on 16S rRNA sequences and functional  
215 potential abilities and were further considered to study bacteria-bacteria interactions (data not shown).  
216 The 25 isolates were affiliated to 3 Phyla, including 18 Firmicutes (72%), six Actinobacteria (24%) and  
217 one Proteobacteria (4%) (Fig. 1A). At the genus level, *Bacillus* spp. and *Streptomyces* spp. were the  
218 most abundant isolates. All isolates except one (*Pseudomonas* sp. A17) were Gram positive. Isolates  
219 belonging to the same species were found in the two communities: *Streptomyces olivochromogenes*,  
220 *Streptomyces sioayensis* and *Bacillus drentensis*. *Bacillus* isolates were affiliated to four clades (Suppl.  
221 Fig. 2).

222 Potential functional abilities to obtain carbon, nitrogen, phosphorous and iron from organic and inorganic  
223 sources were measured as a proxy to evaluate the level of specialization of bacterial isolates (Fig. 1B).  
224 Isolates deeply varied in their potential abilities: some such as *S. olivochromogenes* showed broad  
225 spectra activities while other like *Bacillus* sp. had very little activities.

226

### 227 *Pairwise interactions between bacteria isolated from the same grain of soil*

228 Among the 25 isolates studied, four did not grow on mMS medium when inoculated alone (*B. lentus* B6,  
229 *Sporocarcina* sp. B29, *Staphylococcus* sp. B42 and R9) but the growth of two isolates (B6 and R9) was  
230 rescued by the presence of other isolates (“B” refers to isolates from grain 1 and “R” to those isolated  
231 from grain 2). In these conditions, 560 potential interactions could be occur between isolates of each

232 grain, when taking into account inhibition, stimulation and rescue of growth and when taking into  
233 account both co- and delayed inoculations (Table 1, Suppl. Table 1). Of these, a total of 160 bacterial  
234 interactions were recorded: 45 occurred between isolates of Grain 1 and 115 between isolates of Grain  
235 2. One-way and reciprocal interactions, both antagonistic and beneficial, have been observed (Table 1,  
236 Fig. 2). However, one-way interactions were dominant and represented 72 % of the interactions. Only  
237 two mutually beneficial interactions (*Bacillus* sp. R1- *Paenibacillus* sp. R2 & *Bacillus* sp. R24- *Bacillus*  
238 *drentensis* R39) were recorded while a total of 23 mutual antagonisms were observed (Table 1, Fig. 2).  
239 Those later included few cases of self-inhibition processes in isolates from two species: *S.*  
240 *olivochromogenes* and *B. subtilis*. Beneficial interactions represented 15 % of all interactions recorded.  
241 All bacterial isolates interacted with at least one other bacterial isolate, either by having an effect on  
242 another isolate, or by being affected in its own growth. However, interactions were not evenly distributed  
243 among strains leading to a hierarchical organization of interaction networks with some strains being  
244 mainly “inhibitors” while others were being mainly “inhibited” and/or “stimulated” (Fig. 2).  
245 Few isolates had no visible effect on any other tested isolates no matter the condition (*B. drentensis*  
246 R25 & R28). Eight among the eleven isolates having inhibitory activities were responsible for most of  
247 the inhibitions measured (Fig. 2). Those belonged to the genera *Streptomyces*, *Pseudomonas* and to  
248 the species *B. subtilis*.  
249 Conversely, nine isolates had stimulatory effects and one (*Bacillus* sp. R1) showed both inhibitory and  
250 stimulatory activities depending on the recipient isolate considered (Fig. 2). Most beneficial isolates  
251 promoted the growth of several other isolates (Fig. 2). In addition, the growth of *Bacillus lentus* B6, an  
252 isolate that was not able to grow alone on mMS medium, was rescued when inoculated with delay with  
253 *Bacillus* sp. B2. Similarly, the isolate *Staphylococcus* sp. R9 was only able to grow after being  
254 inoculated with delay with *Paenibacillus* sp. R34 or *Bacillus* sp. isolates R1 and R23.  
255

256 When analysing the behaviours of isolates as receivers, all isolates except one (*Pseudomonas* sp. B17)  
257 were inhibited in at least one interaction. Most isolates were inhibited by at least three other isolates  
258 (Fig. 2). *Bacillus* sp. B2 and *B. drentensis* B28 were inhibited by most other isolates originating from the  
259 same grain. However, the intensities of inhibitions tended to be weak: 49 % of inhibitions reduced the  
260 growth of the colony by 20% or less while only 12.5 % of the inhibitions reduced the growth by more  
261 than 60 % in average (Suppl. Fig 3A). Mutual inhibitions tended to be weak but were not all balanced: in  
262 some cases intensities of inhibitions were similar between interacting strains (e.g. *S. olivochromogenes*  
263 R4 vs *S. sioayensis* R27) while in other cases one strain inhibited more the other strain (e.g. *S.*  
264 *olivochromogenes* B14 vs *B. subtilis* B34 & B36) (Suppl. Table 1).  
265 There was a negative relationship between susceptibility and antagonizing ability: isolates that were  
266 inhibited by many isolates (high receiver degree) tended to inhibit few isolates (low sender degree, Fig.  
267 3, group A). By contrast, isolates with high antagonistic activities (high sender degree) were also  
268 inhibited by other isolates, but in a lower extent (Fig. 3, group B). An inverse relationship between  
269 receivers and donors was also visible for stimulatory activities: isolates that were stimulated had little  
270 positive effects on other isolates (Fig. 3, group C) while isolates that were enhancing the growth of other  
271 did not benefit from others (Fig. 3, group D).

272

### 273 *Comparison of behaviours between grains*

274 Communities of isolates differed between the two grains in terms of size (8 vs 15 growing isolates  
275 including rescues), species composition and potential functional abilities. Twice more interactions were  
276 observed among isolates of grain 1 compared to grain 2 in co-inoculation conditions, as indicated by  
277 interaction densities (0.33 vs 0.19,  $X^2(df=1, N=280)= 6.2149$ ,  $p = 0.01$ , Table 1). This difference was  
278 lost when comparing interaction densities between the two grains in delayed-inoculation conditions  
279 (0.31 vs 0.36,  $X^2(df=1, N=280)= 0.5233$ ,  $p = 0.47$ ). However, proportions of beneficial interactions were  
280 8 fold higher in grain 2 than grain 1 ( $X^2(df=1, N=280)= 6.0764$ ,  $p = 0.01$ , Table 1).

281 There were no significant differences between grains when considering the proportion of receivers ( $X^2$ ,  
282  $p>0.05$ , Table 1), the intensities of the inhibition ( $X^2$   $p>0.05$ , Suppl. Fig. 3A) or the percentage of  
283 receivers being affected by senders ( $X^2$   $p>0.05$ , Suppl. Fig. 3B).

284 Differences between grains also appeared when looking for links between the phylogeny of the isolates  
285 and their effects or sensitivity towards other isolates (Table 2). While *Bacilli* isolates from grain 1  
286 inhibited the growth of half of other *Bacilli*, *Bacilli* from grain 2 had almost no activity towards other  
287 *Bacilli*. Similarly, reciprocal inhibitions differed depending on the origin of the isolates: all *Streptomyces*  
288 isolates from grain 2 inhibited each other growth while such reciprocal interactions did not occur  
289 between *Streptomyces* isolates from grain 1. Instead *S. olivochromogenes* and *B. subtilis* isolates  
290 inhibited each other's growth.

291

#### 292 *Effects of inoculation timing on the output of the interactions*

293 No difference between the number of isolates inhibited/inhibiting was recorded between the two  
294 conditions (co-inoculation vs delayed inoculation): 52 % of isolates had inhibitory activities in both  
295 conditions and 90.4 vs 95.2 isolates were inhibited by another isolate. However the number of  
296 inhibitions slightly increased in delayed conditions (62 vs 74 interactions, Table 1). Furthermore  
297 intensities of inhibitions tended to be stronger in delayed vs co-inoculation condition; (Fig. 4A,  $X^2(df=3,$   
298  $N=136)=24.7944$ ,  $p<0.01$ ). Only 14% of the interactions did not change in intensity between the two  
299 conditions (Fig. 4B & C). Most changes of behaviours were due to an increase of the intensity of the  
300 inhibition (27% of changes) and new inhibitions in delayed co-inoculation (21%). These new inhibitions  
301 were produced by isolates that already inhibited other strains; in other words delayed inoculation led to  
302 a few strains inhibiting even more strains. Only a single strain that did not have any inhibitory activity in  
303 co-inoculation condition started to inhibit another strain in delayed conditions (*Bacillus* sp. R1). No new  
304 mutual inhibition was detected but one was lost between the two conditions (*B. subtilis* B34 vs B36).

305 Interestingly, eight per cents of the interactions were specific to the co-inoculation condition. *Bacillus* sp.  
306 R24 and R33, and *S. sioayensis* B9 only showed inhibitory activities in co-inoculation conditions. Finally,  
307 six per cents were stronger in co-inoculation condition compared to delayed inoculation. Those were  
308 very specific of pairs of interacting isolates. For instance; *Pseudomonas* sp. B17 inhibited more the  
309 growth of *B. drentensis* B9 in co-inoculation condition than delayed while it inhibited more the growth of  
310 *B. subtilis* B36 in delayed condition than in co-inoculation conditions (Fig. 4B).

311 Change of behaviours between growth conditions differed depending on the sender/receiver pairs. For  
312 instance, *B. subtilis* B34 inhibited the growth of B2, B9, B14, B28 and B36 but antagonism toward B36  
313 and B14 occurred only in co-inoculation and delayed conditions, respectively, while the growth of B2  
314 and B9 was more inhibited by B34 in delayed condition and the inhibition of B28 was similar in the two  
315 conditions (Fig. 4B).

316 It is noteworthy that intensities of reciprocal interactions changed between the two conditions but those  
317 changes were not balanced between interacting strains in most cases. Four different patterns were  
318 found: i) the intensity of the inhibition was stronger for the pair (*S. olivochromogenes* R4 vs *S.*  
319 *sioayensis* R27); ii) a balanced inhibition when co-inoculated, and one inhibiting less in delayed  
320 condition (e.g. *S. olivochromogenes* R4 vs *S. sioayensis* R15), iii) a balanced inhibition when co-  
321 inoculated, and one inhibiting more in delayed condition (*S. sioayensis* R15 vs *S. sioayensis* R27), iv) a  
322 full inversion in which the one that inhibited more in co-inoculation inhibits less in delayed condition and  
323 conversely (e.g. *S. olivochromogenes* B14 vs *B. subtilis* B34 and B36). Lastly self-inhibition loops were  
324 not anymore detected in delayed conditions in *B. subtilis* isolates while they were conserved in *S.*  
325 *olivochromogenes* strains.

326 Altogether these different changes of behaviours between co-inoculation and delayed inoculation had  
327 an impact on the architecture of the network of interactions. The general shape was conserved and the  
328 same isolates occupied higher positions by keeping higher sender degrees, leading to an asymmetric  
329 network. Sender-receiver asymmetry was similar in both cases: sender degree distribution was broad

330 and bi-modal while receiver degree was centred and uni-modal (Fig. 5). Despite this conservation of the  
331 general properties of the network, it was modified locally: intermediate nodes that were both inhibited  
332 and inhibitors changed of status by either loosing their inhibitory activities in delayed conditions (e.g.  
333 *Bacillus* sp. R24 and R33) or by gaining some (e.g. *Bacillus* sp. B28).

334 More striking was the apparition of positive interactions between strains in delayed inoculation  
335 conditions. Those later represented 23% of all interactions in delayed inoculation condition.  
336 Interestingly, sender and receiver distributions were similar ( $X^2$  (df=4, N=45)= 5.6568, p=0.12). Both had  
337 a similar bi-modal distribution that was different from the inhibitory sender-receiver degree distributions  
338 (Fig. 5).

339 Growth promotion occurred between isolates belonging to the same order (*Bacilliales*) but not to the  
340 same species or genus (Fig. 2). Similarly, mutual stimulation of growth occurred between strains  
341 belonging to different genera: *Paenibacillus* R2 – *Bacillus* sp R1. and *B. drentensis* R39 vs *Bacillus* sp.  
342 R24.

343

## 344 **Discussion**

345

346 Bacteria are metabolically versatile and possess a large toolbox to interact with other microbes [34]. Our  
347 ability to predict how microorganisms react in natural environments such as soils is very limited, as our  
348 knowledge of when, how and with whom microorganisms interact is still poor. To get insights into how  
349 bacteria that colonized the same microhabitats could interact, we isolated cultivable bacteria from grains  
350 of soil and selected all strains that showed a divergent phenotype. Interactions captured in this work are  
351 only representative of the activities of a small group of soil bacterial members but these later are well  
352 known kestones species in soil and rhizospheres where many can have plant growth promoting  
353 activities. Indeed, our cultivation method allowed for the isolation of well-known cultured representatives  
354 from Actinobacteria, Firmicutes and Proteobacteria phyla, consistent with previous studies [35].



355 However, we could obtain enough isolates from only two grains out of six and a low bacterial diversity  
356 was obtained compared to what could be expected based on DNA metabarcoding of other grains of soil  
357 taken near by (data not shown). Several thousands OTUs were detected by metabarcoding but the  
358 most abundant one's belong to uncultivable taxa such as DA 101 group of Acidobacteria, limiting our  
359 chances to isolate them. Nevertheless, the type of media used and short incubation time (10 days  
360 extended to 18 days) favoured fast aerobic growers, spreading phenotype, with a wide pH tolerance  
361 range [36], that may have not been active but resting as spores when grains were sampled. However,  
362 the same media have been used extensively in the past to isolate bacterial strains from soil and root  
363 habitats and allowed to isolate a much larger taxonomic diversity of strains [22, 25, 37]. Large  
364 composite samples were used in those studies while we focused here on single grains of soils,  
365 suggesting that the low diversity obtained is not a bias of the media used. Instead, it may reflect the  
366 strong heterogeneity of colonisation of soil at the micro-scale. Indeed, bacteria only occupy a small part  
367 of the total surface area of soil and individual microbial neighbourhoods are expected to contain very  
368 few cells [38]. As an example, Nunan estimated that bacteria interact with around 11 ( $\pm 4$ ) other species  
369 in low bacterial density samples and 97 ( $\pm 24$ ) species in high bacterial density [38].

370

371 The average prevalence of interactions was of 30 % in our experiments but this value strongly varied  
372 depending on the grain of soil considered, ranging from 19% to 36% (Table 1). These values are  
373 consistent with literature data in which interaction rates range from 3.4% [22] to 32% [17]. The variability  
374 among studies likely reflects the heterogeneity of the communities studied, each of the studies being  
375 performed on different sets of microorganisms with little taxonomic overlap. In addition, prevalence is  
376 influenced by the conditions of the assays (i.e. media used, temperature etc). Here, the use of delayed  
377 inoculation permitted to uncover 42 interactions (out 160 total) that were not detected in co-inoculation  
378 conditions. Deng & Wang (2017) also showed that the experimental set up modifies the number of  
379 interactions detected. In their case, the replacement of glucose by complex source of carbohydrates led

380 to a reduction of antagonistic interactions by half [20]. As many studies of interactions between bacteria  
381 are performed on a single medium and are most of time done by co-inoculating microorganisms, it is  
382 thus likely that the prevalence of potential interactions is generally under estimated. We would not have  
383 been able to observe positive interactions if we had only used co-inoculation method in the present  
384 work.

385 Despite a “low” prevalence of interactions, all strains were involved in at least one interaction, either as  
386 a sender or a receiver. All isolates except two (R25 and R28) were sender in at least one condition,  
387 highlighting the potential high level of interactions within the two bacterial communities. However, these  
388 interactions were not random as highlighted by the hierarchical organization of the networks. The overall  
389 properties of the networks were similar to those described by Vetsigian et al. (2011): there was an  
390 overall asymmetry in the sender-receiver relationship, sender degree distribution being bimodal and  
391 receiver degree unimodal and centred (Fig. 5). By contrast, we did not observe a prevalence of mutual  
392 inhibitions in our data compared to Vetsigian et al. work in which they dominated. This is likely because  
393 Vetsigian et al. focused their work on *Streptomyces* strains. Mutual inhibitions were also very frequent  
394 among *Streptomyces* isolates in our experiments, likely because *Streptomyces* tend to be more  
395 inhibitory towards coexisting populations using similar resources [39]. In addition to mutual inhibitions,  
396 cases of self-inhibition were observed in *S. olivochromogenes* and *B. subtilis*. Such phenomenon has  
397 previously been reported for *Streptomyces* and it has been interpreted as a mechanism of control of  
398 population growth [16, 40, 41].

399

400 Not surprisingly, *Streptomyces* spp., *Pseudomonas* sp. and *B. subtilis* figured in the higher part of the  
401 network as having broad inhibitory activities. These strains also tended to inhibit each-others. Many  
402 strains belonging to these genera have been described for their abilities to produce antibiotics with  
403 broad spectra activities [19, 24, 42]. Such behaviour is often associated with generalist abilities to use  
404 nutrients [43]. Russel et al. (2017) proposed that specialists and generalists have developed two

405 opposing strategies, the former having strong capacities to exploit a few resources while the generalists  
406 would produce antibiotics to counter the specialists and other generalists. In accordance with this, the  
407 only *Streptomyces* strain (B9) that had no inhibitory activity and was strongly inhibited by others isolates  
408 had very limited potential functional abilities suggesting a higher level of specialization than the other  
409 *Streptomyces* isolates (Fig. 1 & 2). In addition, all strains having little functional abilities had little to no  
410 inhibitory activities in the conditions of our study but they were highly susceptible to antagonism. Such  
411 behaviours led to a negative relationship between the ability to inhibit other strains and the probability to  
412 be inhibited by other strains, as previously described in other studies [17, 20, 22, 44]. However, the  
413 negative relationship obtained here was not as pronounced as previously described because most  
414 inhibitory strains were also inhibited by some other strains in the present work. The strength of this  
415 negative correlation may reflect the balance between generalists and specialists in the community: a  
416 dominance of specialists would lead to strong negative correlation while a larger abundance of  
417 “generalists” that competes together would lead to a weaker correlation. Two different situations were  
418 observed among isolates of the two grains of soil: the number of putative “specialists” vs “generalists”  
419 was almost balanced in grain 1 (3:4) while it was highly unbalanced in grain 2 (10:4).

420

421 More surprising was the low inhibitory activities of *Bacillus* sp. isolates. *Bacilli* are generally among the  
422 most inhibitory strains in interactions studies [10, 14, 20]. However, pairwise analyses of *Bacilli*  
423 interactions from aquatic and soil environments previously showed differences of behaviours between  
424 clades [13, 19]. Clades made of *B. subtilis* and *B. pumilus* were highly antagonists towards isolates from  
425 the 5 other clades identified by Perez-Gutierrez et al. [13], while the others had little antagonistic  
426 activities. A phylogeny analysis of our sensitive isolates indicated that they were more closely related to  
427 the clades E and G described by Perez-Gutierrez et al. [13](Suppl. Fig. 2). These two clades were  
428 characterized by a high sensitivity to *B. subtilis* strains and by a low inhibitory activity towards other  
429 *Bacilli* strains. This suggests that the ability to inhibit other strains is phylogenetically clustered in *Bacilli*

430 from both soil and aquatic environments. In addition, this would also concern sensitivities towards other  
431 taxa since our isolates were also inhibited by *Streptomyces* sp. and *Pseudomonas* sp.

432 Instead of being inhibitors, these strains tended to be growth promoters. Few studies have assessed  
433 potential beneficial interactions within bacterial communities [14, 17, 45], with the exception of mixed  
434 biofilms [46]. The proportion of beneficial interactions was lower than antagonistic one's, as measured in  
435 other studies. However, it is noteworthy that an equal number of strains isolated from grain 2 had  
436 beneficial and antagonistic activities, suggesting that both patterns can be widespread. All pairwise  
437 interactions studies have been performed so far on strains that are naturally able to grow alone by  
438 themselves. But, these represent a little portion of the full diversity and it is has been suggested that  
439 part of our incapacity to grow microorganisms is due to their interdependency [9]. In the present case,  
440 we were able to rescue the growth of two isolates thanks to co-cultivation with delay. Thus, it is very  
441 possible that beneficial interactions are much more prevalent than what we can measure and further  
442 studies are needed to better explore positive interactions within microbial communities.

443 Surprisingly, there was almost no overlap between stimulatory and inhibitory isolates; only one of our  
444 isolate could both inhibit and stimulate growth depending of the receiver. One can wonder whether this  
445 is a bias due to the reduced number of isolates tested and their low taxonomic diversity or whether this  
446 is a generalist property of bacterial behaviours. One could argue that it is unlikely because of the high  
447 versatility of microbial behaviours [47]. Yet, a similar pattern was found among *Streptomyces*: most  
448 isolates with stimulatory activities had little antagonistic activities [17]. Further investigations on larger  
449 libraries of strains will be necessary to test this hypothesis.

450 In contrast to antagonistic interactions, the sender-receiver relationship was balanced: stimulatory  
451 isolates tended to promote the growth of several strains and conversely. This suggests that growth  
452 promotion occurred by generalist mechanisms, in line with the public good theory [48]. However, it is  
453 also possible that a single isolate stimulate the growth of other through different mechanisms. By  
454 contrast, it is more likely that the two cases of mutual stimulations relied on specific mechanisms of

455 interaction. Growth promotion occurred between strains belonging to the *Bacilliales* order but between  
456 isolates from different species and having different potential functional abilities. While kin inhibition has  
457 been deeply studied in *Bacillus* [19, 49], little is known on positive interactions among non-kin *Bacilli* to  
458 the best of our knowledge [50].

459

#### 460 *Concluding remarks*

461 In conclusion, as hypothesised, delayed inoculation led to an increase of the number and the intensity of  
462 antagonistic activities and it permitted to uncover positive interactions. However a non-negligible  
463 number of interactions were not modified by the interaction or were specific to the co-inoculation.  
464 Despite its focus on well-known taxa, *Streptomyces* and *Bacilli*, this work has uncovered new properties  
465 that require further investigations. Firstly, it showed that clades of *Bacilli* with low inhibitory potential can  
466 stimulate and/or rescue the growth of other *Bacilliales* through mechanisms that have yet to be  
467 discovered. Secondly, it suggests that the number of isolates with possible stimulatory activities can be  
468 higher than previously suspected. Indeed the number of isolates with stimulatory abilities are equal to  
469 the number of inhibitory strains in grain 2. Overall, our results call for more research on the positive  
470 interactions between microorganisms to determine their prevalence, whether they are carried by  
471 "specific" organisms, and the mechanisms behind these stimulations. Such information would be very  
472 useful for example for people who create synthetic communities.

473

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482

#### 483 **Conflict of Interest**

484 The authors declare that they have no conflict of interest.

485

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603

604 **Figure legends**

605

606 **Figure 1. Taxonomic affiliation and potential functional abilities of isolates from grain 1 and**  
607 **grain 2 further analysed. A.** Phylogram of the 25 isolates based on 16S partial rRNA sequences. **B.**  
608 Heat map representing potential abilities of the bacterial isolates, organized based on similarities of  
609 activities. Double clustering was performed : bacterial isolates were first clustered based on similarity of  
610 activity profile (horizontal clustering) and functional activities were clustered based on similarities  
611 among isolates (vertical clustering). Strains labelled “B” are from grain 1, strains labelled “R” are from  
612 grain 2.

613 **Figure 2. Hierarchical network representation of interactions between isolates of grain 1 or grain**  
614 **2 when co-inoculated or inoculated with delay.** Color and shape of the nodes refer to taxonomic  
615 affiliation of the isolates: pink diamonds-Actinomycetes, blue circles – Firmicutes, green squares –  
616 Proteobacteria. Color of the edges indicate the type of interaction: yellow – one way inhibition, red –  
617 mutual inhibition, blue – one way growth promotion, green – mutual growth promotion. The size of the  
618 nodes is proportional to the number of interactions as a sender. Nodes in the top of the graphs have  
619 antagonist higher sender degree than nodes in the bottom part.

620

621 **Figure 3. Relationships between bacterial behaviours: antagonisms vs susceptibility (red),**  
622 **stimulation vs being stimulated (blue).** Each point represents the sender degree vs receiver degree of  
623 a bacterial isolate. Sender degree is the number of bacterial isolates inhibited or stimulated by the  
624 isolate. Receiver degree is the number of bacterial isolates that inhibit or stimulate the growth of the  
625 isolate. Circles and triangle depict isolate from grain 1 and 2, respectively.

626

627 **Figure 4. Effect of inoculation time on the outputs of the interactions. A.** Number of antagonist  
628 interactions and their intensities when strains were co-inoculated (black bars) or inoculated with a 48  
629 hours delay (grey bars). Distribution of intensities of inhibition significantly differs between inoculation  
630 time according to  $X^2$  test. Comparison of behaviours between co- (**B**) and delayed inoculation (**C**).  
631 Colors of each square indicates whether the output of the interaction between two isolates was identical  
632 or different between co- and delayed-inoculation. NA – non applicable because the isolate did not grow  
633 alone and its effect on other strains could not be assessed.

634

635 **Figure 5.** Sender and receiver degree distributions for inhibition (A, B) and stimulation (C) interactions  
636 in co-inoculation (A) or delayed conditions (B,C). Black bars = receiver, white bars = sender. Differences  
637 of distribution between sender and receiver degrees were tested with  $X^2$  tests.

638

639

640 **Supplementary Figure 1. Scheme of the set up used to analyse pairwise bacterial interactions**  
641 **and examples of the outputs obtained.**

642 **A.** Simultaneous co-inoculation is based on bacterial cross-streaks (Centre and sides) inoculated at the  
643 same time. **B.** Delayed co-inoculation is based on bacteria streaked first in the centre, then 48 hours  
644 later, additional bacterial were cross-streaked on the sides. Interactions (effect of the central streak on  
645 the side streaks) were determined by measuring the size of the bacterial streaks on the sides (denoted  
646 with a dotted square). **C.** Example of inhibitory activity. **D.** Example of beneficial activity. **E.** Example of  
647 rescue of growth.

648

649 **Supplemental Figure 2.** Phylogram of *Bacillus* isolates based on 16S partial rRNA sequences.

650 Reference sequences from Perez-Gutierrez et al. (2013) were introduced as markers of *Bacillus* groups  
651 with high antagonistic potential : A. *B. pumilus*, B : *B. subtilis*, and low antagonistic potential : C. *B.*  
652 *alqualophilus*, D. *B. aquamiris*, E. *Bacillus*. sp. NRRL14911, F. *B. cereus*, G. *B. horikoshii*. Tree was  
653 built after Muscle alignment of sequences, Gblock curation and PhyML inference of phylogeny.

654 **Supplemental Figure 3. Comparison of inhibition behaviours of isolates from grain 1 and grain 2.**

655 **A.** Distribution of intensities of inhibitions of isolates from grain 1 (black bars) and from grain 2 (white  
656 bars). **B.** Distribution of the receiver degree for inhibition of isolates from grain 1 (black bars) and from  
657 grain 2 (white bars). Differences among distributions were tested with  $X^2$  tests.

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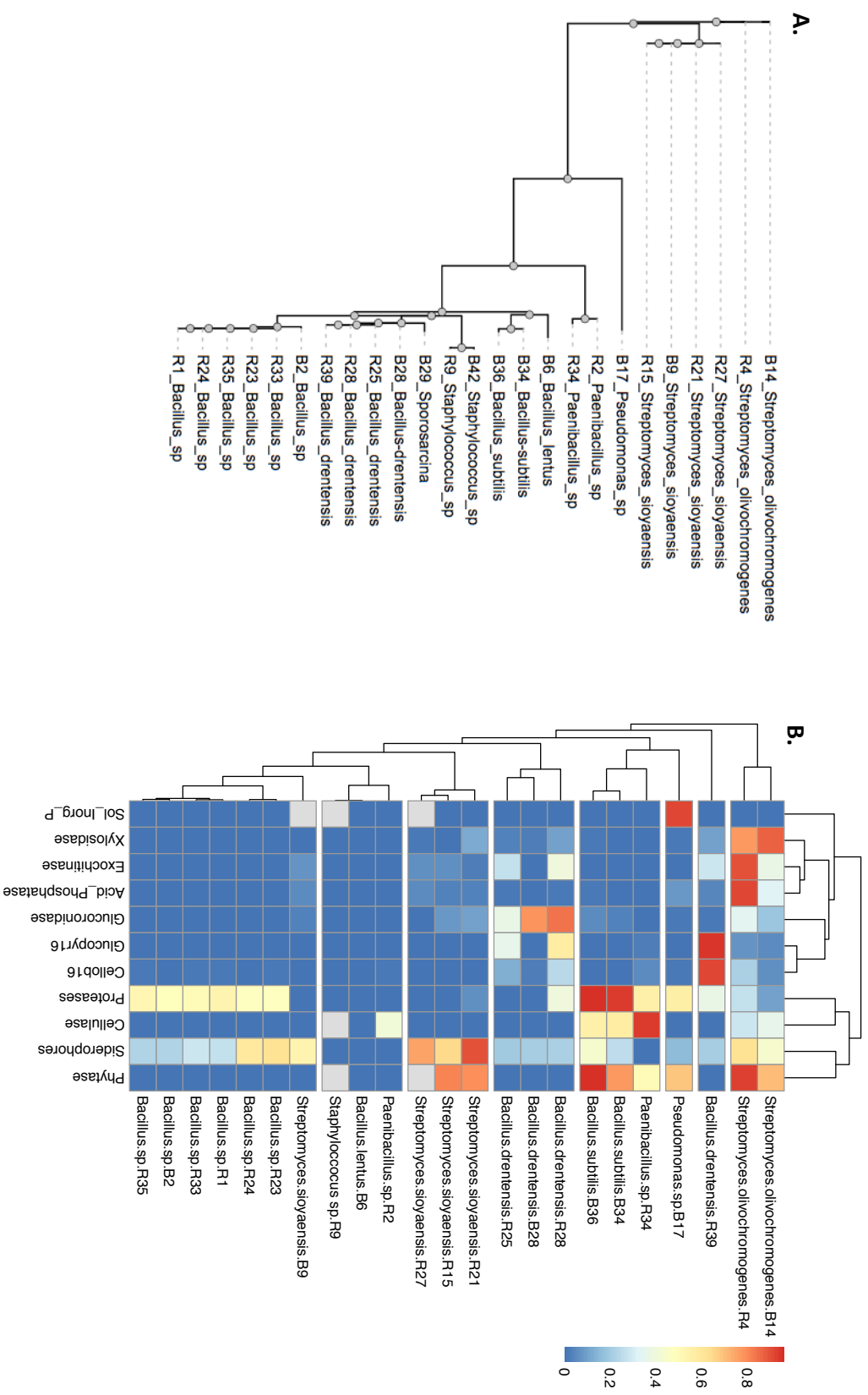


Figure 1

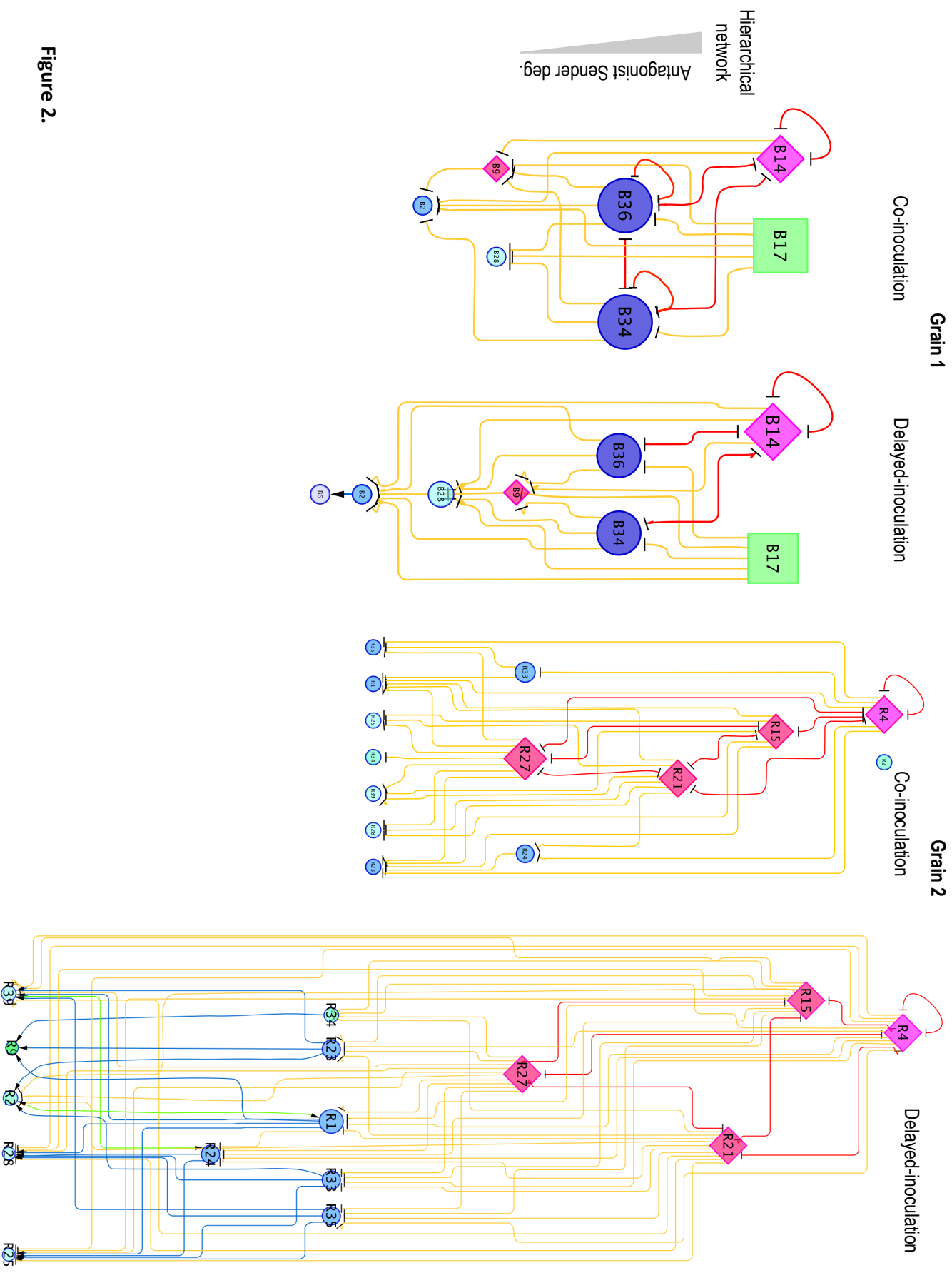


Figure 2.

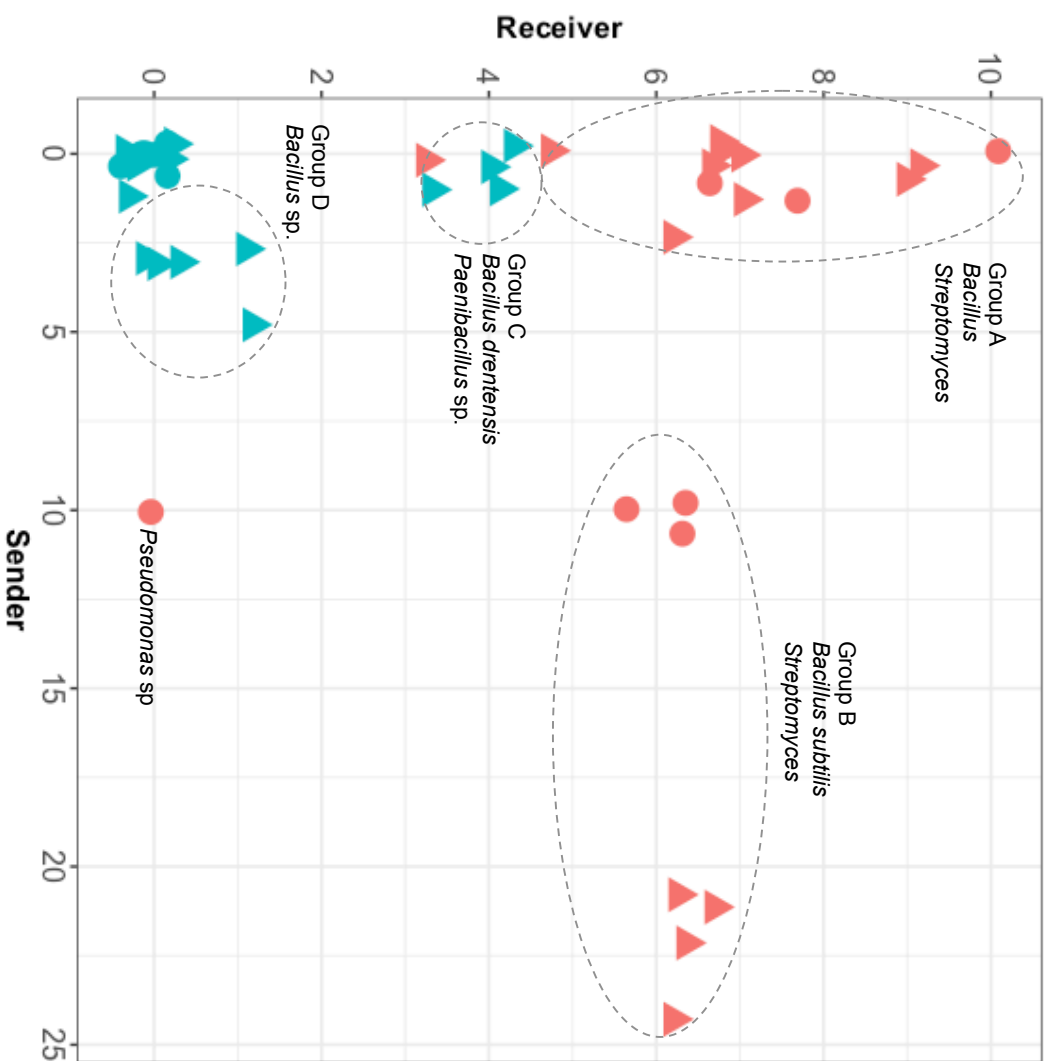


Figure 3

Figure 4

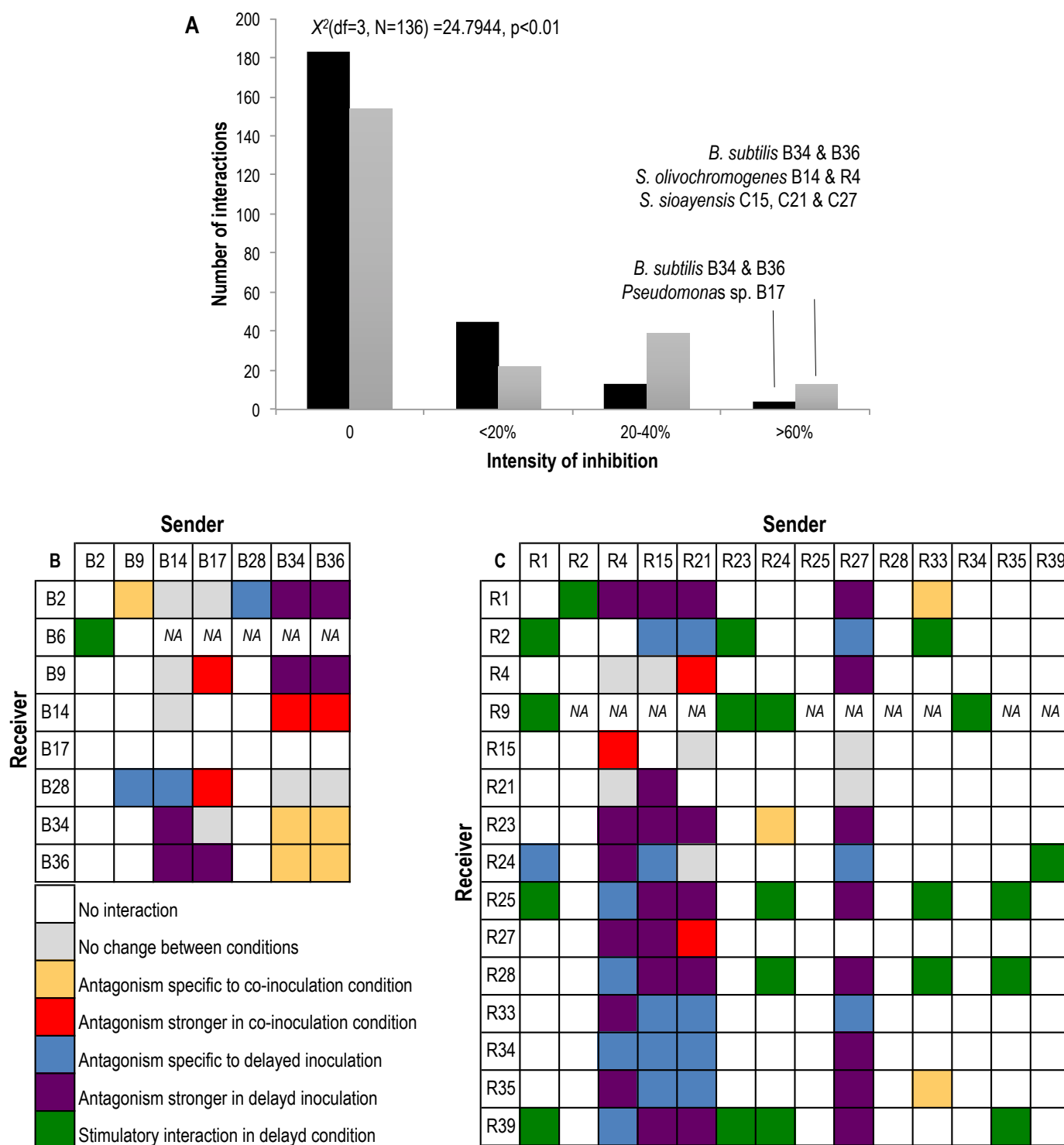


Figure 4



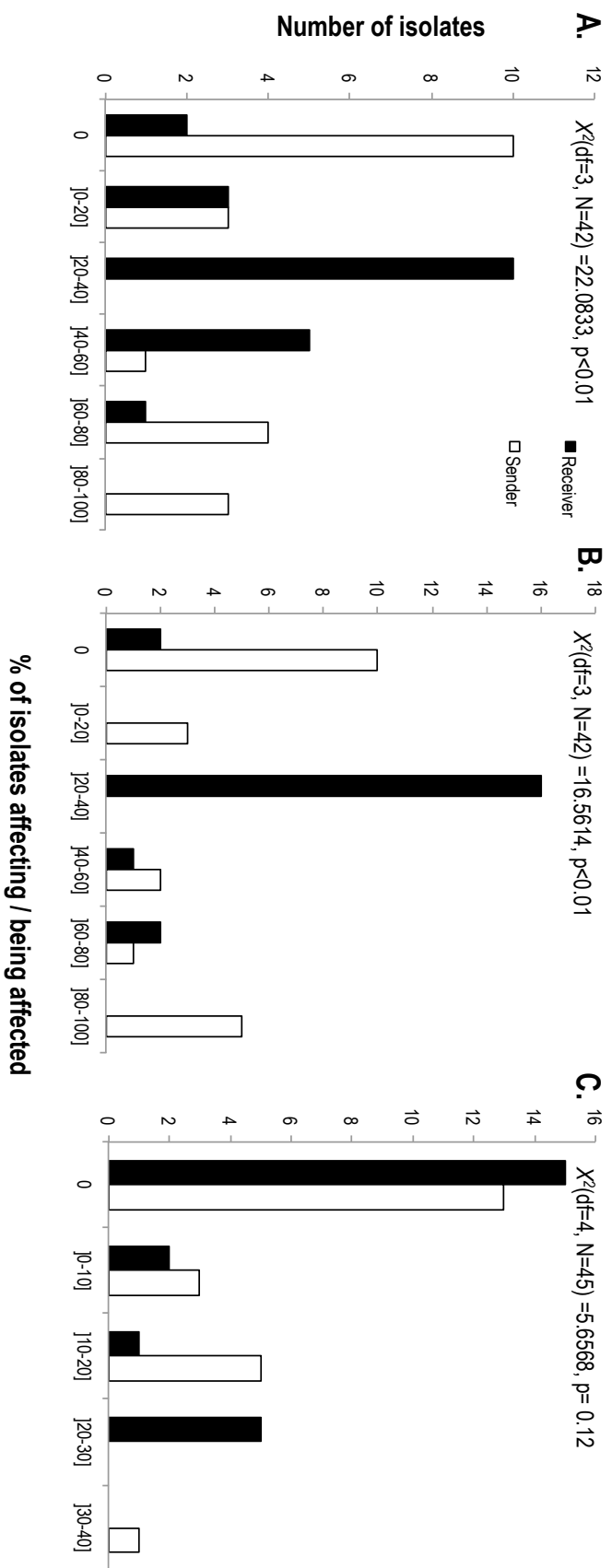


Figure 5.

Table 1

**Table 1.** Summary of the interactions occurring between isolates from grain 1 and grain 2 in co- and delayed inoculation conditions.

		Grain 1		Grain 2	
		Co-inoculation	Delayed-inoculation	Co-inoculation	Delayed-inoculation
Total number of isolates		10	10	15	15
Number of isolates growing on mMS		7	7	14	14
Possible number of inhibitions or stimulations		49	49	196	196
Possible number of rescue		21	21	14	14
Total number of possible interactions		560			
Total number of interactions		160			
<b>Inhibition</b>	Number of senders [%]	5 [71%]	6 [86 %]	6 [43%]	4 [29 %]
	Number of receivers [%]	6 [86%]	6 [86 %]	13 [87%]	14 [100 %]
	Visible inhibitions [%]	23 [47%]	21 [43 %]	39 [20%]	53 [27%]
	One way inhibitions	14	16	26	40
	Mutual inhibitions*	3	2	6	6
	Self-inhibitions	3	1	1	1
<b>Stimulation</b>	Number of senders [%]	0	0	0	5 [36 %]
	Number of receivers [%]	0	0	0	6 [43 %]
	Visible stimulations [%]	0	0	0	20 [10 %]
	One way stimulations	0	0	0	16
	Mutual stimulations*	0	0	0	2
<b>Rescue</b>	Number of senders [%]	0	1 [14 %]	0	3 [21 %]
	Number of receivers [%]	0	1 [33 %]	0	1 [100 %]
	Visible rescues [%]	0	1 [0.05%]	0	3 [21 %]
<b>Total</b>	Local density of interaction **	0.33	0.31	0.19	0.36
	Average density of interaction	0.29 +/- 0.04			
	Average density of inhibition	0.28 +/- 0.06			
	Average density of stimulation	0.04 +/- 0.03			
	Average density of rescue	0.06 +/- 0.05			

\* n mutual interaction = 2n visible interactions

\*\* number of interaction realized / number of possible interactions

**Table 2.** Frequencies of antagonist interactions among bacterial groups from the two grains of soils. Data are given as average of co-inoculation and delayed inoculation +/- SD. Self-inhibition were not taken into account. Senders are given in columns and receivers in rows.

<b>Grain 1</b>	<i>Bacillus</i>	<i>Streptomyces</i>	<i>Pseudomonas</i>
<i>Bacillus</i>	46 ± 6	28 ± 4	50 ± 0
<i>Streptomyces</i>	25 ± 0	50 ± 0	25 ± 0
<i>Pseudomonas</i>	0 ± 0	0 ± 0	NA

<b>Grain 2</b>	<i>Bacillus</i>	<i>Streptomyces</i>	<i>Paenibacillus</i>
<i>Bacillus</i>	4 ± 3	42 ± 11	0 ± 0
<i>Streptomyces</i>	0 ± 0	100 ± 0	0 ± 0
<i>Paenibacillus</i>	0 ± 0	16 ± 13	0 ± 0

## Supplemental methods

### ***Molecular identification of tree roots***

In order to verify that the roots belonged to the *Quercus robur* species, DNA extractions from the roots were performed using REExtract-N-Amp Plant PCR Kit using manufacturer recommendations (Sigma-Aldrich). The Internal Transcribed Spacer 2 (ITS2) was amplified by PCR with the primers ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described in White et al. (1990) and Yao et al. (2010). Band of 400 bp, corresponding to the expected size of plant ITS2 was extracted from 1% agarose electrophoresis gel, purified with QIAquick PCR purification kit according to manufacturer recommendation (Qiagen) and sequenced by Sanger sequencing using ITS3 and ITS4 primers by Eurofins (Germany). Taxonomy affiliation was determined by BLASTn on All plant EST database (Altschul, et al. 1990).

### ***Media compositions***

1/10th Tryptic soy agar (1/10th TSA) per liter: 3 g of tryptic soy broth (Difco) and 15 g of agar

SIM agar per liter: 1 g starch, 0.4 g casein, 0.1 g CaCO<sub>3</sub>, 0.25 g KNO<sub>3</sub>, 0.1 g MgSO<sub>4</sub>, 0.2 g K<sub>2</sub>HPO<sub>4</sub>, 20 g agar, pH 7.0

Soy flour medium (SFM) per liter: 20 g soy flour, 20 g mannitol and 20 g agar, pH 6.6

Modified Murashige and Skoog (mMS) per liter: 1 g glucose, 1 g MES, 100 mL of Murashige and Skoog basal salt micronutrient solution (M0529, Sigma-Aldrich), 50 mL of Murashige and Skoog basal salt macronutrient solution (M0654, Sigma-Aldrich), 1 mL of Gamborg vitamins (Sigma-Aldrich), 10 mL of 100X supplementary vitamins (20 mg mL<sup>-1</sup> L-glutamine, 0.1 mg mL<sup>-1</sup> Ca-pantothenate, 0.1 mg mL<sup>-1</sup> L-cysteine hydrochloride, 0.1 mg mL<sup>-1</sup> Biotin) and 10 g of agar; pH 5.8 adjusted with 3% HCl).

### ***Functional bioassays: agar tests and fluorogenic assays***

A combination of tests on agar media (further referred as « agar tests ») and in liquid using fluorogenic probes (« fluorescence assays ») were used to assess functional abilities to access organic and inorganic nutrients.

For all agar tests, 5 µL of normalized bacterial (non-actinomycetes) or spore (actinomycetes) suspensions were inoculated in triplicate on Petri dishes containing further described agar media. If

bacterial isolates had the ability to degrade a compound, then a halo surrounding the colony was visible. After 7 days of incubation at 25°C in the dark, diameters of halos were measured.

The fluorescence assays were done as described by Uroz et al. (2013) using 4-methylumbelliferone (MU) – linked to exo-enzyme substrates. Briefly, substrates coupled with MU were incubated with bacterial suspensions ( $A_{600nm} = 0.6$ ) or heat shocked spores ( $10^8$  cfu.mL<sup>-1</sup>) for 16 hours, shaken in the dark and reactions were stopped using 1 M Tris solution pH 11.

Results of agar tests and fluorogenic assays were normalized by dividing by the maximum unit per assay. All experiments were performed in triplicate and the average value for each activity of each isolate was calculated. Heatmap produced with the Pheatmap function in R was used for data visualisation.

#### *Degradation of organic matter*

Agar tests were used to determine the potential ability of bacterial isolates to hydrolyze cellulose and cleave proteolytic bonds. The hydrolysis of cellulose was tested as described by Ulrich et al. (2008) using carboxy-methyl-cellulose (CMC) agar (5 g L<sup>-1</sup> carboxy-methyl-cellulose, 1 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 1 g L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.5 g L<sup>-1</sup> MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5 g L<sup>-1</sup> NaCl and 15 g L<sup>-1</sup> agar). To reveal the enzymatic activity, the agar plates were flooded with 3 mL of 1 mg/mL Congo red solution for 40 min, then poured off and washed with 1 M NaCl for 15 min. Transparent halos were measured as positive hydrolyzed cellulose. The cleavage of proteolytic bonds was tested using casein agar (10 g L<sup>-1</sup> skimmed milk, 5 g L<sup>-1</sup> tryptone, 2.5 g L<sup>-1</sup> yeast extract, 1 g L<sup>-1</sup> glucose and 15 g L<sup>-1</sup> agar). Diameters of clear halos were measured as indicators of the production of proteases.

To measure the production of exo-enzymes potentially involved in organic matter degradation, six MU-bound exo-enzyme substrates (Sigma Aldrich) were tested: MU-β-D-glucopyranoside to measure β-glucosidase (EC 3.2.1.3), MU-β-D-glucuronide hydrate for β-glucuronidase (EC 3.2.1.31), MU-β-D-xylopyranoside for β-xylosidase (EC 3.2.1.37), MU-β-D-cellobioside for cellobiohydrolase (EC 3.2.1.91) and MU-N-acetyl-β-D-glucosaminide for exochitinase (EC 3.2.1.14) following procedure described by Uroz et al. (2013).

#### *Siderophore production*

The potential ability to produce siderophores was assessed using chrome azurol S (CAS) agar by adapting the method described by Alexander et al. (1991). Due to toxicity of the medium against certain bacterial isolates, bacterial strains were first grown on R2A agar (Difco) for 24 h (Loaces et al. 2011). CAS agar was then poured on top of the colonies and incubated at 25°C for 6 additional days. Diameters of pink/orange halos were measured as a reflection of the potential ability of bacteria to mobilize iron.

#### *Phosphorous mobilization*

Potential ability to solubilize inorganic phosphorus was assessed as described by Lepleux et al., using tri-calcium phosphate agar bioassay (TCP). Potential stepwise dephosphorylation of phytate carried out by phytases was measured using phytase agar bioassay method (Kerovua, et al. 1998). In both cases, clear halos were measured as indicator of the ability of bacterial isolates to mobilize inorganic or organic phosphorous. MU-phosphate substrate was used to detect the production of acid phosphatases as described by Uroz et al. (2013).

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**A. Grain 1. Co-inoculation**

		Sender							
		B2	B6	B9	B14	B17	B28	B34	B36
Receiver	B2	0	NA	-1	-2	-2	0	-1	-1
	B6	no growth	NA	no growth	no growth	no growth	no growth	no growth	no growth
	B9	0	NA	0	-2	-4	0	-1	-1
	B14	0	NA	0	-1	0	0	-2	-2
	B17	0	NA	0	0	0	0	0	0
	B28	0	NA	0	0	-3	0	-3	-3
	B34	0	NA	0	-1	-2	0	-1	-1
	B36	0	NA	0	-1	-1	0	-1	-1

**B. Grain 1. Delayed inoculation**

		Sender							
		B2	B6	B9	B14	B17	B28	B34	B36
Receiver	B2	0	NA	0	-2	-2	-1	-3	-3
	B6	1	NA	no growth	no growth	no growth	no growth	no growth	no growth
	B9	0	NA	0	-2	-2	0	-2	-2
	B14	0	NA	0	-1	0	0	-1	-1
	B17	0	NA	0	0	0	0	0	0
	B28	0	NA	-1	-3	-2	0	-3	-3
	B34	0	NA	0	-2	-2	0	0	0
	B36	0	NA	0	-2	-2	0	0	0

**C. Grain 2 Co-inoculation**

		Sender							
		R1	R2	R4	R9	R15	R21	R23	R24
Receiver	R1	0	0	-1	NA	-1	-1	0	0
	R2	0	0	0	NA	0	0	0	0
	R4	0	0	-1	NA	-2	-2	0	0
	R9	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth
	R15	0	0	-2	NA	0	-1	0	0
	R21	0	0	-2	NA	-1	0	0	0
	R23	0	0	-1	NA	-1	-1	0	-1
	R24	0	0	-1	NA	0	-1	0	0
	R25	0	0	0	NA	-1	-1	0	0
	R27	0	0	-1	NA	-1	-2	0	0
	R28	0	0	0	NA	-1	-1	0	0
	R33	0	0	-1	NA	0	0	0	0
	R34	0	0	0	NA	0	0	0	0
	R35	0	0	-1	NA	0	0	0	0
R39	0	0	0	NA	-1	-2	0	0	

**D. Grain 2. Delayed-inoculation**

								Sender
R1	R2	R4	R9	R15	R21	R23	R24	

<b>Receiver</b>	<b>R1</b>	0	1	-2	NA	-2	-2	0	0
	<b>R2</b>	1	0	0	NA	-1	-1	1	0
	<b>R4</b>	0	0	-1	NA	-2	-1	0	0
	<b>R9</b>	1	no growth	no growth	no growth	no growth	no growth	1	no growth
	<b>R15</b>	0	0	-1	NA	0	-1	0	0
	<b>R21</b>	0	0	-2	NA	-2	0	0	0
	<b>R23</b>	0	0	-2	NA	-2	-2	0	0
	<b>R24</b>	-1	0	-2	NA	-2	-1	0	0
	<b>R25</b>	1	0	-3	NA	-3	-3	0	1
	<b>R27</b>	0	0	-2	NA	-2	-1	0	0
	<b>R28</b>	1	0	-1	NA	-2	-2	0	1
	<b>R33</b>	0	0	-2	NA	-2	-2	0	0
	<b>R34</b>	0	0	-1	NA	-1	-1	0	0
	<b>R35</b>	0	0	-2	NA	-2	-1	0	0
<b>R39</b>	1	0	-1	NA	-2	-3	1	1	

0: no effect

1: stimulation of growth or rescue

- 1: less than 20 % of inhibition

-2: 20 to 40 % inhibition

-3: 40 to 60% inhibition

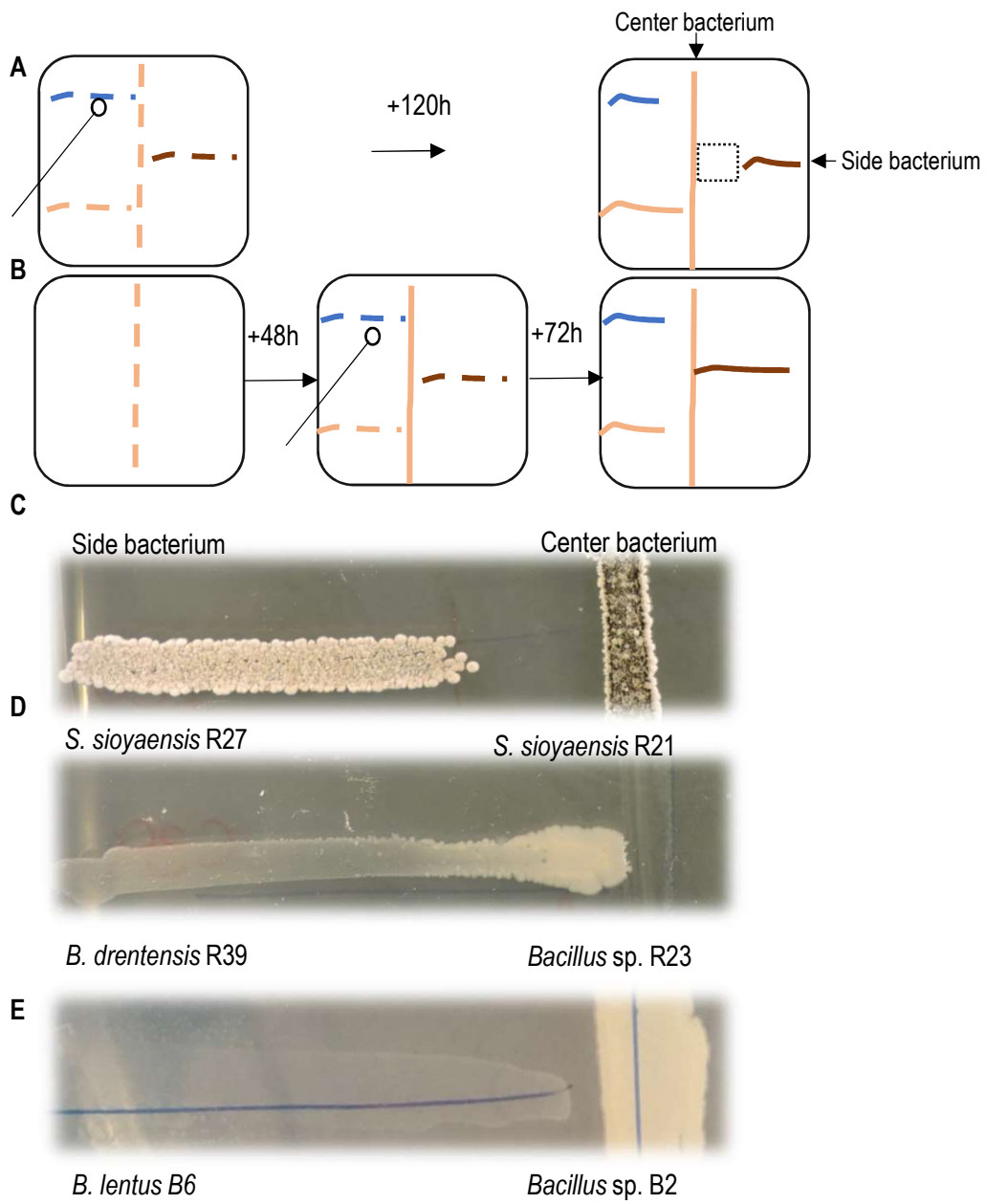
-4 more than 60% inhibition



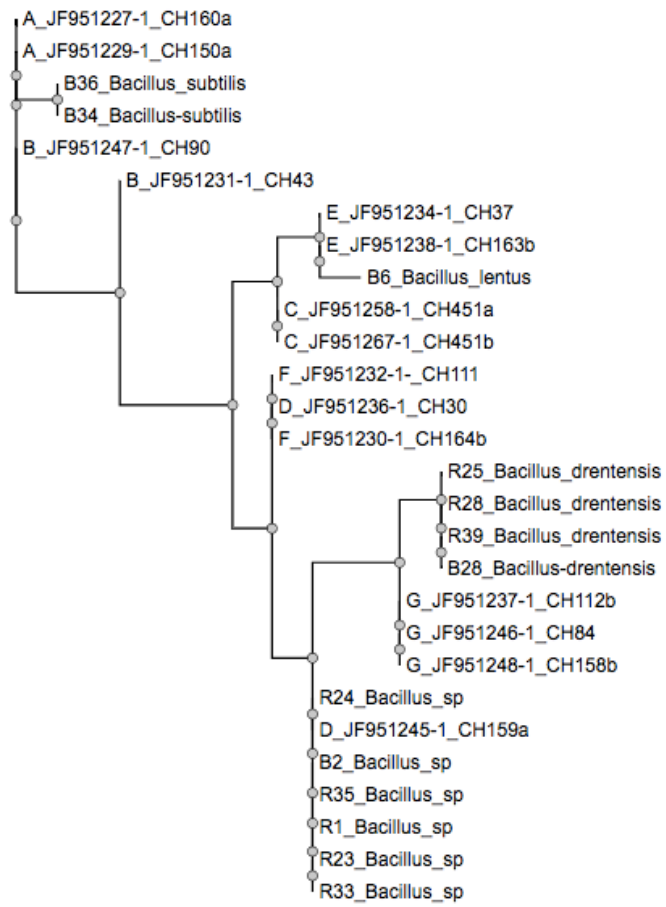
R25	R27	R28	R33	R34	R35	R39
0	-1	0	-1	0	0	0
0	0	0	0	0	0	0
0	-1	0	0	0	0	0
no growth	no growth	no growth	no growth	no growth	no growth	no growth
0	-1	0	0	0	0	0
0	-2	0	0	0	0	0
0	-1	0	0	0	0	0
0	0	0	0	0	0	0
0	-1	0	0	0	0	0
0	0	0	0	0	0	0
0	-1	0	0	0	0	0
0	0	0	0	0	0	0
0	-1	0	0	0	0	0
0	-1	0	-1	0	0	0
0	-1	0	0	0	0	0

R25	R27	R28	R33	R34	R35	R39
-----	-----	-----	-----	-----	-----	-----

0	-2	0	0	0	0	0
0	-1	0	1	0	0	0
0	-2	0	0	0	0	0
no growth	no growth	no growth	no growth	1	no growth	no growth
0	-1	0	0	0	0	0
0	-2	0	0	0	0	0
0	-2	0	0	0	0	0
0	-2	0	0	0	0	1
0	-3	0	1	0	1	0
0	0	0	0	0	0	0
0	-3	0	1	0	1	0
0	-2	0	0	0	0	0
0	-2	0	0	0	0	0
0	-3	0	0	0	0	0
0	-3	0	0	0	1	0



**Supplemental Figure 1.**



**Supplemental Figure 2**