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**Facilitation promotes invasions in plant-associated microbial communities**

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3 **1 Facilitation promotes invasions in plant-associated microbial**  
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7  
8 Running title: Facilitation increases invasibility  
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49

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53 microbe-microbe-plant interactions  
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15  
16 32 will be archived in an appropriate public repository such as Dryad or Figshare and the data  
17  
18 33 DOI will be included at the end of the article.  
19

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21 34  
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23 35 **Abstract**  
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26 36 While several studies have established a positive correlation between  
27  
28 37 community diversity and invasion resistance, it is less clear how species  
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30 38 interactions within resident communities shape this process. Here we  
31  
32 39 experimentally tested how antagonistic and facilitative pairwise interactions  
33  
34 40 within resident model microbial communities predict invasion by the  
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36 41 plant-pathogenic bacterium *Ralstonia solanacearum*. We found that facilitative  
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38 42 resident community interactions promoted and antagonistic interactions  
39  
40 43 suppressed invasions both in the lab and in the tomato plant rhizosphere.  
41  
42 44 Crucially, pairwise interactions could reliably explain observed invasions  
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44 45 outcomes also in multispecies communities, and mechanistically, this was  
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46 46 linked to direct inhibition of the invader by antagonistic communities  
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48 47 (antibiosis), and to a lesser degree by resource competition between the  
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50 48 members of the resident community and the invader. Together our findings  
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52 49 suggest that the type and strength of pairwise interactions can reliably predict  
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3 50 the outcome of invasions in more complex multispecies communities.  
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8 **52 Introduction**  
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11 53 The characteristics of both resident communities and the invading species are  
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13 54 important for determining the outcomes of biological invasions (Williamson &  
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15 55 Fitter 1996; Catford *et al.* 2009). From the resident community perspective,  
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17 56 species diversity may be considered as a shield to invasions and this effect is  
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19 57 often attributed to competition for existing resources (Fridley *et al.* 2007;  
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21 58 Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015) where  
22  
23 59 highly diverse communities are thought to efficiently use all the available  
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25 60 resource niches leaving no free space for invaders (Case 1990; Tilman 2004).  
26  
27 61 In reality, diversity-invasion resistance relationships are more varied ranging  
28  
29 62 from having neutral to even negative effects (Shea & Chesson 2002; Mallon *et*  
30  
31 63 *al.* 2015a; Mehrabi *et al.* 2016) and are sensitive to environmental conditions  
32  
33 64 (Davis *et al.* 2000; Roscher *et al.* 2009; Jousset *et al.* 2011; Mallon *et al.*  
34  
35 65 2015b). Furthermore, it has been shown that trophic network architecture (Wei  
36  
37 66 *et al.* 2015), species identity effects (Yang *et al.* 2017) and food web  
38  
39 67 connectance (Smith-Ramesh *et al.* 2017) are important predictors of invasions  
40  
41 68 and are often linked with community diversity. For example, how species  
42  
43 69 interact might be more important than the number of interacting species within  
44  
45 70 the community (Wei *et al.* 2015), while invasion resistance could be sometimes  
46  
47 71 mediated by certain keystone taxa (Yang *et al.* 2017). However, the type and  
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49 72 strength of resident species interactions have often been overlooked in the  
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51 73 context of diversity-invasion resistance studies.  
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3 74 Resident species communities form complex ecological webs where  
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5 75 multiple species may interact positively or negatively with each other (*Kéfi et al.*  
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7 76 2012). Positive interactions between species at the same trophic level can  
8  
9 77 result from facilitation or metabolic cross-feeding, where species benefit from  
10  
11 78 the presence of each other (*Mulder et al.* 2001). Negative interactions may  
12  
13 79 result from resource competition (*Wei et al.* 2015) or direct interference  
14  
15 80 competition, where species directly suppress each other via antagonism (*Bais*  
16  
17 81 *et al.* 2003; *Hierro & Callaway* 2003; *Thorpe et al.* 2009; *Hu et al.* 2016). These  
18  
19 82 interactions may affect the outcomes of invasions in various ways. First,  
20  
21 83 facilitation and competition are likely to affect the resource availability, and  
22  
23 84 hence the availability of free resource niche space, and the likelihood of  
24  
25 85 invasions (*Shea & Chesson* 2002; *Tilman* 2004; *Stachowicz & Byrnes* 2006;  
26  
27 86 *Gioria & Osborne* 2014; *Mallon et al.* 2015c). It is predicted that highly  
28  
29 87 competitive resident communities are less prone to invasions if they can  
30  
31 88 efficiently utilize and consume resources that would otherwise be available for  
32  
33 89 invaders (*Tilman* 2004; *Jousset et al.* 2011; *Mallon et al.* 2015c). This effect is  
34  
35 90 expected to be especially strong in the resident communities that show a high  
36  
37 91 degree of complementarity and hence compete less strongly with each other  
38  
39 92 compared with the invader. In contrast, facilitative interactions between  
40  
41 93 resident community members could potentially increase the number of  
42  
43 94 resource niches via production of secondary metabolites or public goods that  
44  
45 95 can also be utilized by the invader (*Stachowicz* 2001; *Mallon et al.* 2015b;  
46  
47 96 *Bulleri et al.* 2016). Furthermore, competing species can inhibit each other  
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49 97 directly by producing toxic metabolites, such as antibiotics. Depending on the  
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51 98 spectrum of their activity, antibiotic compounds could have negative effects on  
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3 99 both resident community species and the invader (Bais *et al.* 2003; Hierro &  
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5 100 Callaway 2003; Thorpe *et al.* 2009; Becker *et al.* 2012; Hu *et al.* 2016; Wang *et*  
6  
7 101 *al.* 2017b). If the invader is particularly sensitive to toxins produced by the  
8  
9 102 resident community, it is expected that antibiotics-mediated interference  
10  
11 103 competition will constrain invasions. In contrast, if produced toxins have a  
12  
13 104 disproportionately larger negative effect on the members of the resident  
14  
15 105 community, such interference competition is expected to promote invasions  
16  
17 106 (Thorpe *et al.* 2009; Stubbendieck *et al.* 2016). Resident community species  
18  
19 107 interactions could further affect certain community-level properties such as  
20  
21 108 ecological stability (Allesina & Levine 2011), which could have indirect effects  
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23 109 on invasions (Ghoul & Mitri 2016).  
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28 110 In the present study, we explored to what extent the type (facilitative vs  
29  
30 111 antagonistic) and strength of two-species resident community species  
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32 112 interactions can predict invasions in complex multispecies bacterial  
33  
34 113 communities. Experiments conducted within one trophic level suggest that  
35  
36 114 pairwise bacterial competitions can predict three-species bacterial  
37  
38 115 competitions with as high as 90% accuracy (Friedman *et al.* 2017). While  
39  
40 116 predicting competitions in species-rich communities might require additional  
41  
42 117 information about potentially emerging higher-order interactions (Friman *et al.*  
43  
44 118 2016; Grilli *et al.* 2017; Levine *et al.* 2017), these findings suggest that  
45  
46 119 qualitative information regarding species growth in pairwise co-cultures can be  
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48 120 used to predict the competitive outcomes of up to 8-species communities  
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4 121 (Friedman *et al.* 2017). Here we extend this approach beyond competition to  
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6 122 concurrently explore the role of antagonistic and facilitative resident  
7  
8 123 community interactions for biological invasions (Bruno *et al.* 2003; Altieri *et al.*  
9  
10 124 2010; Traveset & Richardson 2014). Our study system consisted of six  
11  
12 125 non-pathogenic bacterial species (resident community), which were isolated  
13  
14 126 from the tomato plant rhizosphere, and the invader, the plant-pathogenic  
15  
16 127 *Ralstonia solanacearum* bacterium. Specifically, we first characterized  
17  
18 128 antagonistic and facilitative pairwise interactions within model resident  
19  
20 129 bacterial communities and then directly tested how these interactions predict  
21  
22 130 invasions in more complex multispecies communities both *in vitro* and *in vivo*  
23  
24 131 in the tomato rhizosphere. We found that facilitative and antagonistic pairwise  
25  
26 132 interactions reliably predicted invasions: facilitative resident communities were  
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28 133 more prone to invasions, while antagonistic resident communities were  
29  
30 134 invaded much less often. Mechanistically, this was linked to direct inhibition of  
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32 135 the invader by antagonistic communities (antibiosis), and to a lesser degree by  
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34 136 resource competition between the members of the resident community and the  
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36 137 invader. Our results suggest that antagonism is an important determinant of  
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38 138 community invasion resistance (Case 1990; Tilman 2004), while facilitation  
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40 139 might promote invasions by alleviating antagonistic interactions or by releasing  
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42 140 vacant niche space for the invader.  
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## 142 **Materials and methods**

### 143 **Bacterial strains and the assembly of resident communities**

144 We used *Ralstonia solanacearum* strain QL-Rs1115 tagged with the  
145 pYC12-mCherry plasmid (Tan *et al.* 2016) as an invading pathogen in our  
146 experiments. *Ralstonia solanacearum* is a causal driver of bacterial wilt and  
147 capable of infecting various economically important crop species (Jiang *et al.*  
148 2017). We set up model resident communities using six bacterial strains  
149 isolated from the tomato rhizosphere at the same location as the pathogen  
150 (Qilin [118° 57' E, 32° 03' N], Nanjing, China). Resident community species  
151 listed in Table S1 (*Flavobacterium johnsoniae* WR4, *Chryseobacterium*  
152 *daecheongense* WR21, *Delftia acidovorans* WR42, *Bacillus amyloliquefaciens*  
153 T-5, *Lysinibacillus sphaericus* HR92 and *Ralstonia pickettii* QL-A6) have  
154 previously been shown to provide protection for associated host plants by  
155 inhibiting *R. solanacearum* pathogen growth via resource competition or direct  
156 toxin production (Figure S1). The resident community composition (Table S2)  
157 was manipulated using biodiversity-invasion resistance framework where we  
158 modulated both resident community diversity (species richness) and  
159 composition and then directly tested how this affected community invasion  
160 resistance (Wei *et al.* 2015). Invasion outcomes were then explained by  
161 interactions 1) within resident communities and 2) between resident  
162 community and the invader.

163

### 164 **Determining pairwise interactions between resident community species**

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3 165 To quantify the type (facilitative, neutral or antagonistic), strength and  
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5 166 directionality of each pairwise interaction between resident community species,  
6  
7 167 we compared the growth of each species alone and in the presence of each of  
8  
9 168 the other species in two-species co-cultures (Foster & Bell 2012). All  
10  
11 169 mono-cultures were inoculated with a starting density of  $10^5$  cells per ml and  
12  
13 170 the co-cultures were inoculated with half of this starting cell density of each  
14  
15 171 species. Resident species were grown for 48h in liquid NA medium (glucose  
16  
17 172  $10.0 \text{ g l}^{-1}$ , tryptone  $5.0 \text{ g l}^{-1}$ , yeast extract  $0.5 \text{ g l}^{-1}$ , beef extract  $3.0 \text{ g l}^{-1}$ , pH 7.0)  
18  
19 173 in 48-well microtiter plates (ending volume of 700  $\mu\text{l}$  per well) at  $30^\circ\text{C}$  with  
20  
21 174 shaking (170 rpm). Bacterial growth was measured as colony number units  
22  
23 175 (CFU) per ml by serial dilution and plating on NA agar plates after 48h growth.  
24  
25 176 All strains formed distinct colonies on agar plates and could be identified  
26  
27 177 based on colony morphology (Figure S2).

31 178 The type of pairwise interaction between two species (here  $i$  and  $j$ ) was  
32  
33 179 determined by comparing the sum of endpoint of monoculture productivity  
34  
35 180 (population densities) of  $i$  ( $MP_i$ ) and monoculture productivity of  $j$  ( $MP_j$ ) with the  
36  
37 181 ending productivity of the two-species co-culture ( $CP_{i+j}$ ). As suggested  
38  
39 182 previously, the density of a species mixture is expected to be exactly the sum  
40  
41 183 of their growth in the monocultures if species do not interact (Foster & Bell  
42  
43 184 2012). Thus, we expected that the interaction between  $i$  and  $j$  would be  
44  
45 185 facilitative if  $CP_{i+j} > MP_i + MP_j$ , antagonistic if  $CP_{i+j} < MP_i + MP_j$  and neutral if  
46  
47 186  $CP_{i+j} = MP_i + MP_j$ .

51 187 In order to characterize directionality of pairwise interactions, we  
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53 188 compared the ending productivity of each species ( $CP_i$  and  $CP_j$ ) in two-species

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3 189 co-cultures with their ending productivities in monocultures. We then  
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5 190 determined the directionality of interaction facilitative if species  $j$  had a positive  
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7 191 effect on  $i$  ( $\log_{10}(CP_i / MP_i) > 0$ ), antagonistic if  $\log_{10}(CP_i / MP_i) < 0$  and neutral if  
8  
9 192  $\log_{10}(CP_i / MP_i) = 0$ . We also calculated the mean intensity of facilitation (MIF)  
10  
11 193 of co-cultures as an average of  $\log_{10}$ -transformed pairwise interactions using  
12  
13  
14 194 the following formula:  $MIF_{ij} = \frac{1}{2}[\log(CP_i / MP_i) + \log(CP_j / MP_j)]$  The two-species  
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17 195 community was defined as facilitative when  $MIF > 0$ , antagonistic when  $MIF <$   
18  
19 196  $0$  and neutral if  $MIF = 0$ .

197

### 198 **Predicting resident species interactions in multispecies communities**

199 We simply assumed that pairwise interactions would not change in the  
200 presence of additional species and then predicted resident species  
201 interactions in multispecies communities using two different indexes: by  
202 calculating *i*) the proportion of facilitative pairwise interactions of all possible  
203 pairwise interactions and *ii*) predicted mean intensity of facilitation (PIF) in a  
204 multispecies community. For example, among the total number of all possible  
205 pairwise interactions of strains  $i$ ,  $j$  and  $k$ , if one of these interactions was  
206 facilitative ( $CP_{i+j} > MP_i + MP_j$ ), the proportion of facilitative interactions in this  
207 resident community was defined as 1/3. Analogous to MIF, we calculated the  
208 predicted intensity of facilitation (PIF) in multispecies co-cultures as the sum of  
209  $\log_{10}$ -transformed interactions divided by the number of all possible pairwise  
210 interactions within the given community using the following formula:

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$$PIF = \frac{1}{C_n^2} \sum_{i=1}^{C_n} MIF_{ij},$$

211 where  $MIF_{ij}$  refers the net intensity of one pairwise interaction between species  
212  $i$  and  $j$  in a multispecies community, which has a total of  $C_n^2$  number pairwise  
213 interactions. The communities were defined as facilitative when  $PIF > 0$ ,  
214 antagonistic when  $PIF < 0$  and neutral when  $PIF = 0$ .  $PIF$  thus accounted for  
215 both the strength and directionality of all potential pairwise interactions in a  
216 multispecies community.  
217  
218

### 219 **Validating resident species interactions in multispecies communities**

220 To verify resident species interactions in multispecies bacterial communities,  
221 we used qPCR to determine the ending densities of each resident species in  
222 monocultures and in all possible co-cultures (3, 4, 5 and 6 resident species  
223 communities). All communities were assembled in triplicate in liquid NA  
224 medium with a starting density of  $10^5$  cells per ml in monocultures and 33%,  
225 25%, 20% and 16.7% of monoculture densities in 3, 4, 5 and 6 resident  
226 species communities, respectively. After 48h in 48-well microtiter plates at  
227 30°C with shaking (170 rpm), bacterial DNA was extracted using e.Z.N.A.  
228 Bacterial DNA kit (OMEGA bio-tek) following manufacturer's protocol and  
229 extracted DNA was stored at -80°C. Species-specific primers were designed  
230 for each resident community member (Table S3, Figure S3) and qPCR  
231 analyses were carried out with an Applied Biosystems Step One Plus real-time  
232 PCR system using SYBR green I fluorescent dye detection in 20 -  $\mu$ l volumes

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3 233 with 10 µl of SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), 2 µl of template,  
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5 234 0.4 µl Dye I, 0.8 µl of both forward and reverse primers (10 mM each) and 6 µl  
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7 235 sterile water. The PCR was performed by initially denaturing at 95°C for 30 s,  
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9 236 cycling 40 times with a 5-s denaturing step at 95°C, using a 34-s  
10  
11 237 elongation/extension step at 60°C, and ending with melt curve analysis at  
12  
13 238 95°C for 15 s, at 60°C for 1 min, and at 95°C for 15 s. Each resident species  
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15 239 community sample was replicated three times.

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17  
18 240 The observed mean intensity of facilitation (OIF) was calculated using the  
19  
20 241 observed species proportions in the communities based on qPCR data. Similar  
21  
22 242 to PIF, we first determined to what extent the growth of each species was  
23  
24 243 affected by the presence of other species in a given community (growth in the  
25  
26 244 community vs. growth alone). OIF was then calculated according to the  
27  
28 245 following formula:  $OIF = \frac{1}{n} \sum \log(CP_i / MP_i)$ . Communities were defined as  
29  
30 246 facilitative when  $OIF > 0$ , antagonistic when  $OIF < 0$  and neutral if  $OIF = 0$ . OIF  
31  
32 247 was calculated only based on *in vitro* data and in the case of MIF, PIF and OIF,  
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34 248 antagonism included the effects arising from both resource competition and  
35  
36 249 direct inhibition via toxins.

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### 43 44 45 251 **Measuring resource competition and direct antagonism between the** 46 47 252 **invader and resident community species**

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50 253 All bacteria were first grown to high densities ( $OD_{600} \approx 1.0$ ) in liquid NA media  
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52 254 overnight at 30°C with shaking (170 rpm), washed three times in 0.85% NaCl,  
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54 255 and adjusted to an optical density of 0.5 at 600 nm ( $OD_{600}$ ) with SpectraMax

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2  
3 256 M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). We then  
4  
5 257 measured the growth of the invader and all six resident community species  
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7 258 individually on 48 different single-carbon resources (see Table S4)  
8  
9 259 representative of tomato root exudates (Hu *et al.* (2016)). When the invader  
10  
11 260 and resident community species were able to grow on the same resource  
12  
13 261 ( $OD_{600} > 0.05$ ), their niches were considered to overlap regarding that given  
14  
15 262 resource. In contrast, when only one strain was able to grow on a specific  
16  
17 263 resource, the niches were considered not to overlap (Wei *et al.* 2015). This  
18  
19 264 resource competition index estimated the 'apparent' resource competition  
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21 265 assuming that interacting species would be competing for the same resources  
22  
23 266 even when presented with multiple different resources.  
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27        Direct antagonism between the invader and resident community species  
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29 268 was measured using supernatant assays (Hu *et al.* 2016). Briefly, after 24h of  
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31 269 growth in NA media, all bacterial monocultures were filtered to remove living  
32  
33 270 cells (0.22  $\mu\text{m}$  filter) after 20  $\mu\text{l}$  of sterile supernatant from each resident  
34  
35 271 species culture was mixed with 180  $\mu\text{l}$  of an overnight-grown *R. solanacearum*  
36  
37 272 culture ( $OD_{600} = 0.05$ , five-fold dilution in liquid NA). The control treatments  
38  
39 273 were inoculated with 20  $\mu\text{l}$  of sterile-filtered NA media instead of bacterial  
40  
41 274 supernatant. All bacterial cultures were grown for 24h at 30°C with shaking  
42  
43 275 (170 rpm) before measuring pathogen inhibition as optical density ( $OD_{600}$   
44  
45 276 nm). Antagonism was defined as the percentage of reduction in pathogen  
46  
47 277 growth by the supernatant compared to the control treatment for all possible  
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49 278 invader-resident species two-species combinations.  
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3 280 **Measuring invasion success in multispecies communities**  
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6 281 **a) Invasion success measured *in vitro***  
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8 282 All possible multispecies resident communities were assembled in triplicate in  
9  
10 283 liquid NA medium with a starting density of  $10^5$  cells per ml (100%, 50%, 33%,  
11  
12 284 25%, 20% and 16.7% of monoculture densities in 1, 2, 3, 4, 5 and 6 resident  
13  
14 285 species communities, respectively). Communities were then subsequently  
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16 286 exposed to invasion by mCherry-tagged *R. solanacearum* ( $10^4$  cells per ml) in  
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18 287 96-well plates at 30°C with shaking (170 rpm). After 48h, total bacterial  
19  
20 288 densities were measured as optical density (OD 600 nm) and invasion success  
21  
22 289 measured as the relative invader density to total bacterial densities using red  
23  
24 290 mCherry protein fluorescence intensity (RFP; excitation: 587 nm, emission:  
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26 291 610 nm) with SpectraMax M5 spectrophotometer.  
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33 293 **b) Invasion success measured *in vivo***  
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36 294 We used a 50-day-long greenhouse experiment with tomato plants to measure  
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38 295 invasion success *in vivo*. The soil was collected from a rice field in Wuxi  
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40 296 (Jiangsu Province, China), sieved at 5 mm and homogenized and sterilized  
41  
42 297 with gamma radiation. Surface-sterilized tomato seeds (*Lycopersicon*  
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44 298 *esculentum*, cultivar "Micro-Tom") were germinated on water-agar plates for 3  
45  
46 299 days before sowing into seedling plates containing cobalt-60-sterilized  
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48 300 seedling substrate (Huainong, Huaian Soil and Fertilizer Institute, Huaian,  
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50 301 China). *Ralstonia solanacearum* invasion was tested in all possible  
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52 302 two-species resident communities, and due to practical reasons, in 18  
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3 303 multispecies resident communities that varied in their predicted mean  
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5 304 intensities of facilitation (Table S5).  
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8 305 Three replicates were used for each resident community, and one replicate  
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10 306 consisted of a seedling plate that contained six germinated tomato plants (at  
11  
12 307 the three-leaf stage of growth when grown on 700 g sterilized soil). Similar  
13  
14 308 replication was also used for positive (only the invader) and negative (no  
15  
16 309 bacteria) controls. After 3 days of growth on seedling plates, plants were  
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18 310 inoculated with assembled resident communities using root drenching method  
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20 311 at a final concentration of  $10^8$  CFU of bacteria  $\text{g}^{-1}$  soil (Wei *et al.* 2013). Seven  
21  
22 312 days after inoculation of resident communities, *R. solanacearum* was  
23  
24 313 introduced to the roots of all plants at a final concentration of  $10^7$  CFU of  
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26 314 bacteria  $\text{g}^{-1}$  soil. Tomato plants were then grown for 40 days in a greenhouse  
27  
28 315 (with natural temperature variation ranging from 25°C to 35°C) and watered  
29  
30 316 regularly with sterile water. Seedling plates were rearranged randomly every  
31  
32 317 two days and disease progression monitored at every seven days. Forty days  
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34 318 after inoculation of *R. solanacearum*, rhizosphere soil was collected from one  
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36 319 plant per replicate seedling tray and the abundance of the invader determined  
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38 320 with quantitative PCR as the abundance of *R. solanacearum*-specific *fliC* gene  
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40 321 copy numbers (Hu *et al.* 2016).  
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### 46 47 48 323 **Statistical analyses**

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50 324 To meet assumptions of normality and homogeneity of variance, invader  
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52 325 densities measured *in vitro* and *in vivo* were log<sub>10</sub>-transformed. We first  
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54 326 assessed the independent effects of the proportion of facilitative interactions  
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3 327 and the mean intensity of facilitation based on pairwise resident community  
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5 328 interaction on invasions (pathogen density and disease incidence). The type of  
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7 329 interaction between resident community species pairs was included into  
8  
9 330 models as a categorical variable (1= facilitation; 0= antagonism). In the case of  
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11 331 multispecies communities, invasions were explained by three quantitative  
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13 332 indexes, the proportion of facilitative interactions within a community, the  
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15 333 predicted mean intensity of facilitation (PIF) and the and observed mean  
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17 334 intensity of facilitation (OIF). All indexes were fitted as continuous variables  
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19 335 and one separate model was used for each index that explained invader  
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21 336 densities *in vitro* and *in vivo* and bacterial wilt disease incidence. Additional  
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23 337 linear mixed models were used to test invasions as a function of a) niche  
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25 338 overlap between resident community and the pathogen (niche preemption by  
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27 339 the resident community), b) mean pathogen inhibition by the resident  
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29 340 community and c) resident community species identity effects. All analyses  
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31 341 were conducted with SPSS (V. 22) and R (Computing 1991; Team 2013).  
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## 343 **Results**

### 344 **(a) Two-species resident species interactions predict invasions *in vitro*** 345 **and *in vivo***

346 All species had both negative and positive effects on each other while the  
347 magnitude and directionality of these effects varied depending on specific  
348 species (Figure 1A). In particular, *B. amyloliquefaciens* was very antagonistic  
349 to the other resident community species. (Figure 1A). Furthermore, we found  
350 that 9 of the communities showed antagonistic, and 6 facilitative pairwise

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3 351 interactions with each other (Figure 1B, Table S6). On average, facilitative  
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5 352 two-species communities reached higher population densities ( $R^2=0.79$ ,  
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7 353  $P<0.001$ , Figure S4), while antagonistic two-species communities were more  
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9 354 inhibitory towards each other ( $R^2=0.32$ ,  $P=0.029$ , Figure S5A). No relationship  
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11 355 was found between resident species' resource niche overlap and observed  
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13 356 mean intensity of facilitation (Figure S5B), which suggests that facilitation did  
14  
15 357 not arise due to niche complementarity. Together these results suggest that the  
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17 358 strength of direct inhibition was more important in explaining the type of  
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19 359 pairwise interactions between resident community members compared to  
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21 360 resource competition.  
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25 361 To link the type of pairwise interaction with the likelihood of invasions, we  
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27 362 compared *R. solanacearum* invasion success in facilitative and antagonistic  
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29 363 two-species resident communities. Compared to positive controls (*R.*  
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31 364 *solanacearum*-only: red dashed line in Figure 2A-F), pathogen densities were  
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33 365 significantly lower in the presence of resident species both *in vitro* and *in vivo*.  
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35 366 The intensity of pathogen suppression could be predicted by the type of  
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37 367 pairwise interactions between the resident species: pathogen density was  
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39 368 significantly higher in facilitative compared to antagonistic communities *in vitro*  
40  
41 369 ( $F_{1,43}=16.02$ ,  $P<0.001$ , Figure 2A;  $R^2=0.49$ ,  $P<0.0001$ , Figure 2B) and *in vivo*  
42  
43 370 ( $F_{1,43}=24.40$ ,  $P<0.001$ , Figure 2C;  $R^2=0.26$ ,  $P=0.0021$ , Figure 2D). In line with  
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45 371 these results, the bacterial wilt disease incidence was also higher in facilitative  
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47 372 compared to antagonistic resident communities ( $F_{1,43}=9.03$ ,  $P=0.004$ , Figure  
48  
49 373 2E;  $R^2=0.14$ ,  $P=0.013$ , Figure 2F). Mechanistically, this could be explained by  
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51 374 loss of pathogen inhibition as suggested by a negative correlation between the  
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3 375 mean intensity of facilitation and direct invader suppression ( $R^2=0.45$ ,  
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5 376  $P<0.0001$ , Figure S6). Together these results suggest that antagonistic  
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7 377 two-species resident communities were more inhibitory not only towards  
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9 378 themselves but also against the invader.  
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15 380 **(b) Predicting and validating invasions in multispecies communities**  
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17 381 **based on pairwise interactions**

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20 382 Interactions within the resident communities could well explain the invader  
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22 383 abundance *in vitro* ( $R^2: 0.45$ ,  $P<0.0001$ ) and *in vivo* ( $R^2: 0.28$ ,  $P<0.0001$ ), and  
23  
24 384 bacterial wilt disease incidence ( $R^2: 0.18$ ,  $P=0.0002$ ) *in vivo* (Table 1). The  
25  
26 385 proportion of facilitative interactions were well explained by the increase in  
27  
28 386 invader density in all tested resident communities *in vitro* ( $R^2=0.35$ ,  $P<0.0001$ ,  
29  
30 387 Figure 3A). Similarly, both the density of the invader in the tomato rhizosphere  
31  
32 388 ( $R^2=0.22$ ,  $P=0.0004$ , Figure 3B) and bacterial wilt disease incidence ( $R^2=0.21$ ,  
33  
34 389  $P=0.0004$ , Figure 3C) increased significantly with increasing proportion of  
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36 390 facilitative interactions within the resident communities. The predicted mean  
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38 391 intensity of facilitation explained well the increase in invader density *in vitro*  
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40 392 ( $R^2=0.45$ ,  $P<0.0001$ , Figure 3D) and *in vivo* ( $R^2=0.21$ ,  $P=0.0005$ , Figure 3E)  
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42 393 and correlated positively with bacterial wilt disease incidence ( $R^2=0.19$ ,  
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44 394  $P=0.0193$ , Figure 3F). The predicted and observed mean intensities of  
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46 395 facilitation correlated positively with each other ( $R^2=0.44$ ,  $P<0.0001$ , Figure  
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48 396 S7), demonstrating that pairwise interactions can be used to predict  
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50 397 interactions in multispecies communities. As expected, invader densities also  
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52 398 increased with increasing observed mean intensity of facilitation both *in vitro*  
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3 399 ( $R^2=0.26$ ,  $P<0.0001$ , Figure 3G) and *in vivo* ( $R^2=0.17$ ,  $P=0.0019$ , Figure 3H).

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5 400 However, the observed mean intensity of facilitation did not correlate  
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7 401 significantly with bacterial wilt disease incidence (Figure 3I).

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10 402 The low invasion success observed in antagonistic resident communities  
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12 403 could be attributed to high levels of direct inhibition of the invader and/or high  
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14 404 resource niche overlap between the invader and resident community members.  
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16 405 We found that both direct pathogen inhibition and high resource niche overlap  
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18 406 reduced invader densities *in vitro* and *in vivo*, while only direct pathogen  
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20 407 inhibition significantly reduced the disease incidence (Table 1). Direct  
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22 408 pathogen suppression correlated negatively with both predicted and observed  
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24 409 mean intensities of facilitation suggesting that antagonistic multispecies  
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26 410 communities were more inhibitory to the invader (Figure S8). The species *B.*  
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28 411 *amyloliquefaciens* and *F. johnsoniae* had strong negative effects on pathogen  
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30 412 densities *in vitro* and *in vivo* (Table S7). However, only *B. amyloliquefaciens*  
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32 413 had a significant negative effect on disease incidence, while species *C.*  
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34 414 *daecheongense* had a slightly positive effect on disease incidence (Table S7).  
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36 415 Together these results suggest that pairwise resident community interactions  
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38 416 can predict invasions in multispecies communities *in vitro* and *in vivo* and that  
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40 417 these effects were primarily linked with direct pathogen suppression.  
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## 48 419 **Discussion**

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50 420 Here we studied how resident community interactions are linked with invasions  
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52 421 in bacterial plant rhizosphere communities. We found that facilitative two-  
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54 422 species communities were invaded more easily both in the laboratory and

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3 423 rhizosphere compared to antagonistic resident communities. Furthermore, we  
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5 424 could use the pairwise interactions to predict invasion outcomes in  
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7 425 multispecies communities containing up to 6 resident species. Specifically,  
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9 426 communities characterized by a high proportion of facilitative pairwise  
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11 427 interactions, and high predicted and observed mean intensities of facilitation,  
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13 428 were more susceptible to invasions. Mechanistically, this was linked to direct  
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15 429 inhibition of the invader by antagonistic communities (antibiosis), and to a  
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17 430 lesser degree by resource competition between the members of the resident  
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19 431 community and the invader. Together these findings suggest that outcomes of  
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21 432 relatively simple pairwise interactions can be used to predict invasions in  
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23 433 multispecies microbial communities especially when antagonism and  
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25 434 facilitation are strongly linked with the resistance to invasion.  
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29 435 Invasion resistance has been thus far mainly considered from the  
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31 436 perspective of resource competition and niche preemption (Case 1990; Tilman  
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33 437 2004; Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015). Our  
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35 438 results suggest that facilitative interactions should also be considered in the  
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37 439 context of invasions. While it is difficult to pinpoint the exact mechanism  
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39 440 between facilitation and invasions, most likely explanation is the loss of  
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41 441 pathogen inhibition along with the increase in the mean intensity of facilitation  
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43 442 (Figure S6). This is in line with a previous finding where the increase in the  
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45 443 antagonistic activity was found to increase the invasion resistance of  
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47 444 *Pseudomonas* resident communities (Hu *et al.* 2016). Another explanation  
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49 445 could be that facilitative resident communities were less efficient at competing  
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51 446 for resources with the invader compared to antagonistic resident communities.  
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3 447 However, this likely played a relatively small role as resource niche overlap  
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5 448 had the only significant negative effect on the invader density when measured  
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7 449 *in vitro* and *in vivo* but not on disease incidence (Table 1). It is also possible  
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9 450 that our resource competition indexes measured *in vitro* overestimated the  
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11 451 strength of resource competition or underestimated the size of the niche space  
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13 452 in the rhizosphere leading to weak correlation with invasions. Furthermore,  
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15 453 facilitative interactions could have increased the niche space in the resident  
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17 454 communities in favor of the invader, which could have promoted invasions as a  
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19 455 side effect (Bulleri *et al.* 2016). For example, previous studies have  
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21 456 demonstrated that bacteria can show diet preference between different dietary  
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23 457 glycans, which can prolong the species coexistence in co-cultures (Tuncil *et al.*  
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25 458 2017). Such dietary preference might leave some resources less utilized  
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27 459 opening opportunity for invasions (Tilman 1999). It has also been shown that  
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29 460 the breakdown of polysaccharides can allow coexistence of species that  
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31 461 liberate polysaccharide breakdown products (PBPs), which are consumed by  
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33 462 other species that are unable to grow on the polysaccharides alone (recipients)  
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35 463 (Rakoff-Nahoum *et al.* 2014). Facilitative interactions could thus potentially  
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37 464 favor the invader if it is unable to grow on the primary substrates on its own  
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39 465 (Bruno *et al.* 2003). While it is difficult to validate these hypotheses based on  
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41 466 our data, we found that facilitative communities were more productive in  
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43 467 general and reached higher total cell densities when cultured together  
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45 468 compared to alone (Figure S4). This supports the idea that facilitative resident  
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47 469 species were benefitting from the presence of each other (for example via  
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49 470 cross-feeding), which could also have benefitted the invader by creating  
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51 471 vacant niche space. The carrying capacity of resident communities could thus  
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3 472 be an important predictor of biological invasions (Gosso *et al.* 2012).  
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6 473 In addition to within-resident community interactions, the interactions  
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8 474 between resident communities and the invader were also good predictors of  
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10 475 invasions, albeit to a lesser extent (Figure 3 and Table 1). While it remains  
11  
12 476 unclear what exact compounds were produced by the resident communities,  
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14 477 previous studies have shown that soil bacteria are capable of producing a wide  
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16 478 variety of antimicrobials that often suppress *R. solanacearum* (Hu *et al.* 2016;  
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18 479 Wang *et al.* 2017b). For example, the *B. amyloliquefaciens* T-5 strain used in  
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20 480 this study has been shown to efficiently suppress *R. solanacearum* both in the  
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22 481 lab and plant rhizosphere (Wang *et al.* 2017b) and this strain also had the  
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24 482 greatest negative effect on the pathogen densities and disease incidence in  
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26 483 this study (Figure S1). In addition, the strain *F. johnsoniae* had a negative  
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28 484 effect on pathogen densities both *in vitro* and *in vivo*. Together these results  
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30 485 suggest that pathogen suppression via toxins was likely mediated by the  
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32 486 presence of these species.  
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36 487 In general, pairwise resident community interactions predicted well the  
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38 488 observed invasion outcomes in multispecies communities. (Figure 3, Table 1).  
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40 489 However, no correlation was found between the observed mean intensity of  
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42 490 facilitation and bacterial wilt disease incidence (Figure 3I). This suggests that  
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44 491 while *in vitro* mechanisms (resource competition and antibiosis) can robustly  
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46 492 predict invasions in more complex *in vivo* environments (Wei *et al.* 2015; Hu *et*  
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48 493 *al.* 2016), they do not account for all aspects of more complex natural  
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50 494 environments. There are many potential explanations for these discrepancies  
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52 495 that should be validated in future studies. First, investigating the role of  
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3 496 microbe-mediated plant immunity is important as both pathogenic and  
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5 497 non-pathogenic bacteria can trigger or suppress plant immunity (Chen *et al.*  
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7 498 2017; Rautenbach *et al.* 2017). Furthermore, several bacterial secondary  
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9 499 metabolites involved in pathogen suppression also impact plant immunity: for  
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11 500 example, 2, 4-diacetylphloroglucinol (DAPG) produced by fluorescent  
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13 501 *Pseudomonas* spp. (Bulai & Venturino 2017) or lipopeptide surfactins  
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15 502 produced by *Bacillus subtilis* (Wang *et al.* 2017a) have a such dual-function.  
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17 503 Second, the rhizosphere bacterial communities we used were rather simple,  
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19 504 and hence, predictions based on pairwise species interactions should be  
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21 505 tested in more complex multi-trophic communities in the future. Lastly, our  
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23 506 predictive indexes only estimated the mean net effects and did not distinguish  
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25 507 if both or only one of the species benefitted and vice versa (Foster & Bell 2012).  
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27 508 While this approach seems to be a good predictor of invasion outcomes,  
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29 509 accounting for the directionality of interactions and potential emerging  
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31 510 higher-order interactions (Friman *et al.* 2016; Grilli *et al.* 2017; Levine *et al.*  
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33 511 2017) is likely to improve these predictions.  
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38 512 In conclusion, our results suggest that qualitative information regarding  
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40 513 species growth in pairwise co-cultures can be used to predict the outcomes of  
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42 514 invasions in multispecies communities. Even though our results can be broadly  
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44 515 applied across different biological problems, they could offer direct solutions in  
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46 516 the context of crop protection. Bacterial pathogens impose an ever-increasing  
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48 517 threat for agriculture (Olson & Stenlid 2001; Choudhary & Johri 2009; Nicol *et*  
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50 518 *al.* 2011) and recent evidence suggests that the rhizosphere microbiome plays  
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52 519 an essential role in controlling the onset of diseases (Berendsen *et al.* 2012;  
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3 520 Lozupone *et al.* 2012). Understanding the characteristics that make certain  
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5 521 microbiomes more resistant to invasions could potentially allow one to harness  
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7 522 beneficial bacterial communities for crop protection. While recent studies have  
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9 523 shown that microbial diversity alone may be such important characteristic (Wei  
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11 524 *et al.* 2015; Hu *et al.* 2016) we here suggest that highly antagonistic microbial  
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13 525 communities might also be efficient at constraining pathogen invasions.  
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679

## 680 **Figure legends**

681 **Figure 1. The type and relative strength of resident species pairwise interactions.** (A)

682 Network diagram showing the strength and directionality of all pairwise interactions between

683 resident community species. The thickness of lines represents the strength and green and red

684 color the facilitative or antagonistic effects between different species. (B) Nine of the fifteen

685 pairwise interactions were on average antagonistic (co-culture density < monoculture density)

686 and six facilitative (co-culture density > monoculture density). Panels show two examples: Left,

687 antagonism between species Ba and Cd; Right, facilitation between species Rp and Cd. \*\*\*

688 denotes for statistical significance at  $p < 0.001$ . All error bars denote for  $\pm 1$  s.e.m.

689

690 **Figure 2. The type of pairwise resident community interactions predicts invasions *in***

691 ***vitro* and *in vivo*.** (A) The *R. solanacearum* invader abundance in antagonistic and facilitative

692 two-species resident communities measured *in vitro*. (B) The relationship between invader

693 abundance and the mean intensity of facilitation in resident communities measured *in vitro*. (C)

694 The relative invader abundance in antagonistic and facilitative two-species resident

695 communities measured in the tomato rhizosphere 40 days after inoculation of the invader. (D)

696 The relationship between invader abundance and the mean intensity of facilitation in resident

697 communities measured *in vivo* in the tomato rhizosphere. (E) The bacterial wilt disease

698 incidence (%) in antagonistic and facilitative pairwise resident communities 40 days after

699 inoculation of the invader. (F) The relationship between disease incidence and the mean

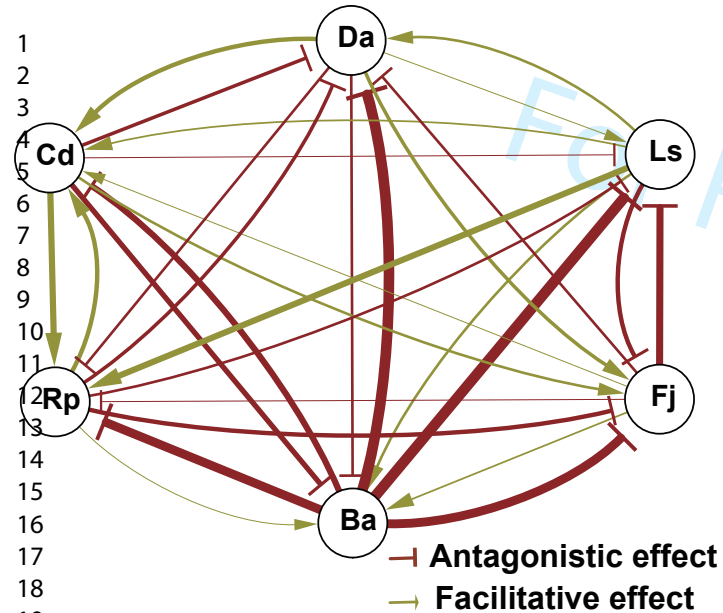


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3 700 intensity of facilitation in resident communities measured *in vivo* in the tomato rhizosphere. In  
4 701 all panels, the red dashed lines show the baseline for positive control treatments (invader-only).  
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6 702 In panels, B, D and F, values below and above zero denote for antagonistic and antagonistic  
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8 703 pairwise resident communities, respectively. Two and three stars denote for statistical  
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10 704 significance at  $p < 0.01$  and  $p < 0.001$  significance levels, respectively. All the bars denote for  $\pm$   
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16 707 **Figure 3. The relationship between invader abundance and disease incidence with**  
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18 708 **predicted and observed mean intensities of facilitation within multispecies**  
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20 709 **communities.** (A-B) The relationship between invader abundance and the proportion of  
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22 710 facilitative interactions in the resident communities measured *in vitro* and *in vivo*, respectively.  
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24 711 (C) The relationship between bacterial wilt disease incidence (%) and the proportion of  
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26 712 facilitative interactions in the resident communities. (D-E) The relationship between invader  
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28 713 abundance and the predicted mean intensity of facilitation in the resident communities  
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30 714 measured *in vitro* and *in vivo*, respectively. (F) The relationship between bacterial wilt disease  
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32 715 incidence (%) and the predicted mean intensity of facilitation in the resident communities. (G-H)  
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34 716 The relationship between invader abundance and the observed mean intensity of facilitation in  
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36 717 the resident communities measured *in vitro* and *in vivo*, respectively. (I) The relationship  
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38 718 between bacterial wilt disease incidence (%) and the observed mean intensity of facilitation in  
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40 719 the resident communities. In all panels, red dashed lines show the baseline of invader  
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42 720 densities in control treatments (invader-only). In panels D-I, values below and above zero  
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44 721 denote for competitive and antagonistic resident communities, respectively.

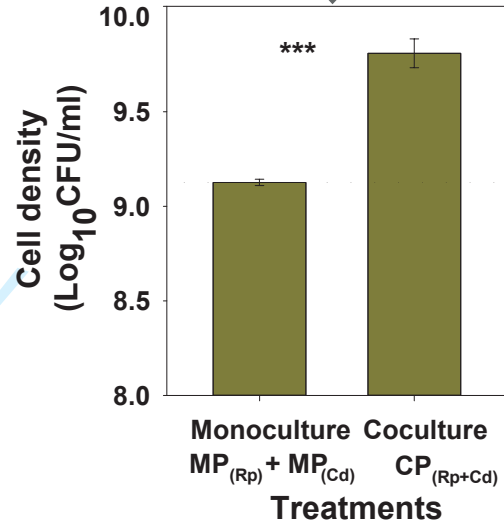
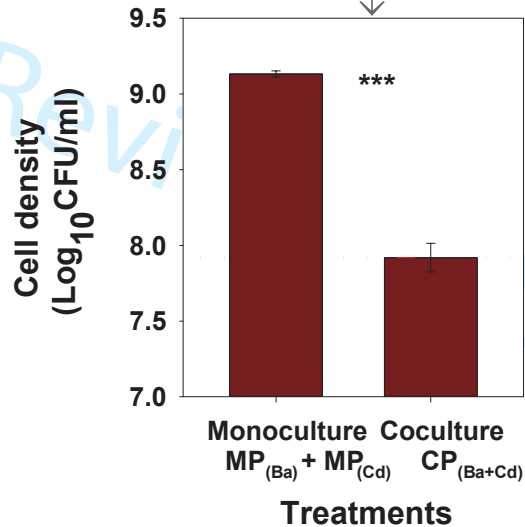
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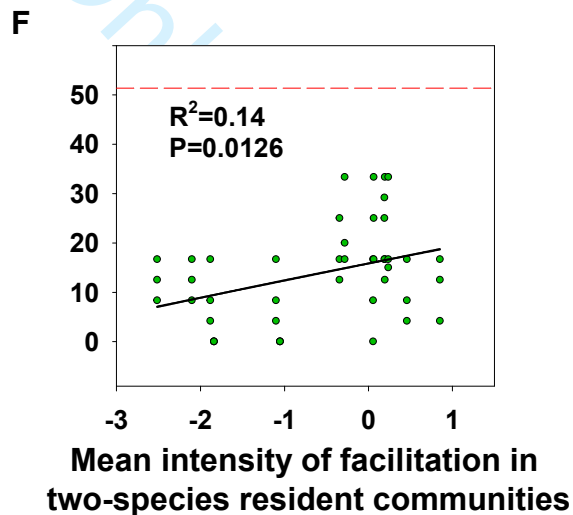
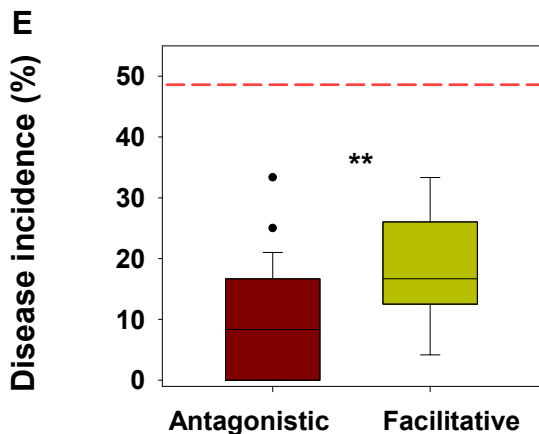
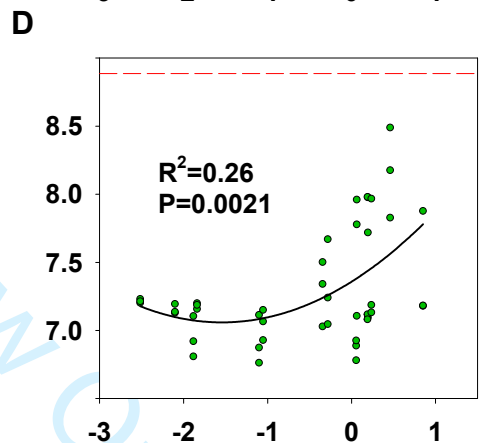
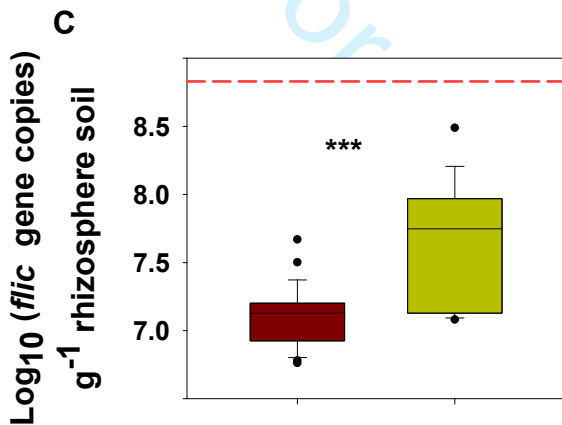
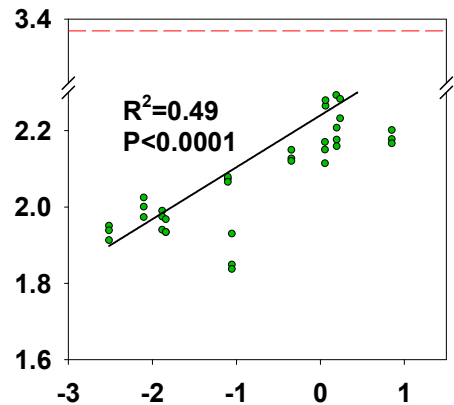
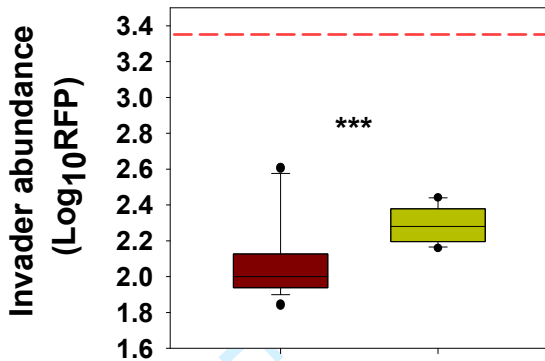


B

Antagonistic interaction (n=9)

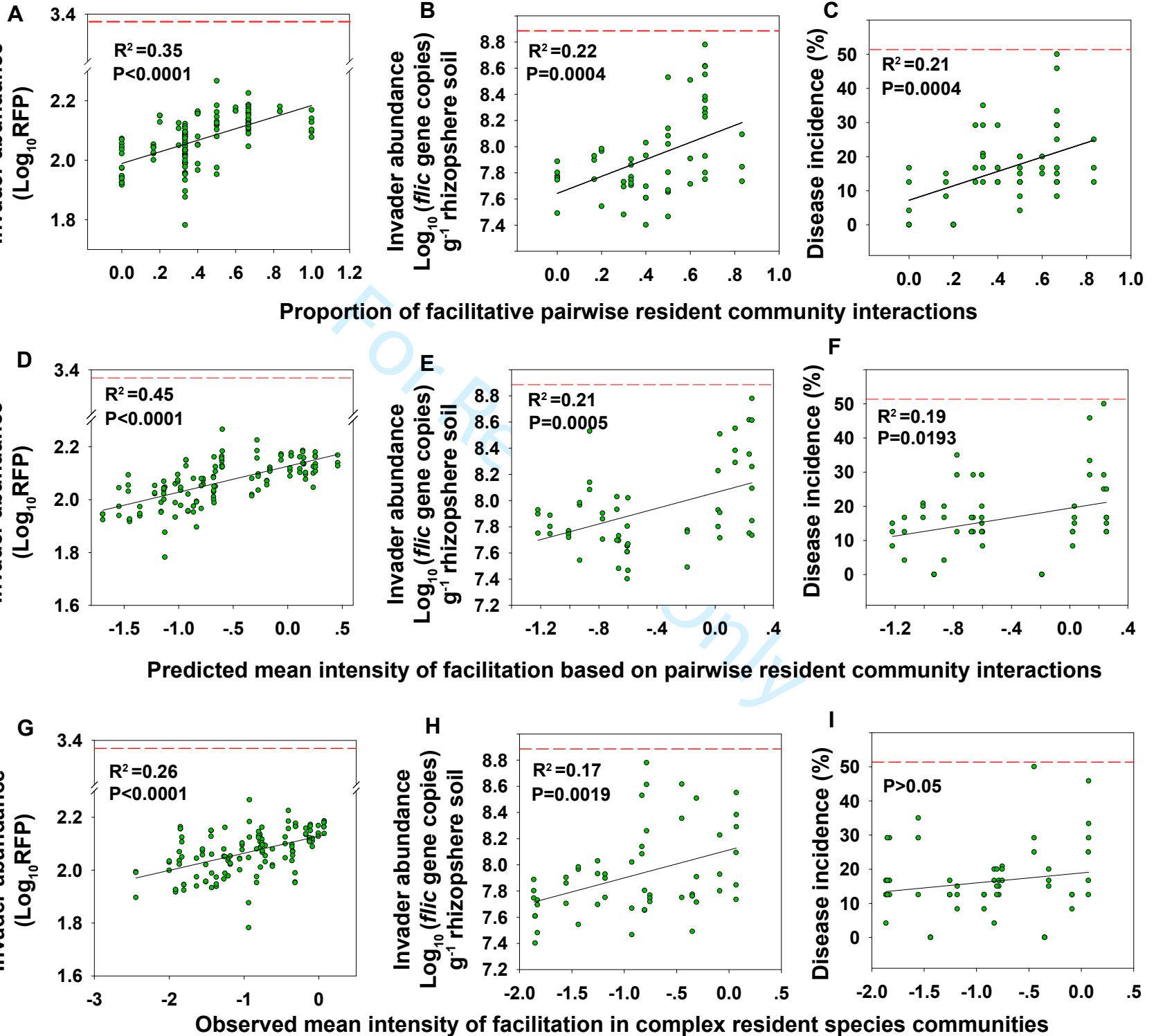
Facilitative interaction (n=6)





Interaction type in two-species resident communities

Mean intensity of facilitation in two-species resident communities



**Table 1** Two different general linear mixed models (GLM) comparing the interactions within resident communities (a), and interactions between community and invader (b) on invader abundance *in vitro* and *in vivo* and disease incidence measured *in vivo*.

	Invader relative abundance <i>in vitro</i>			Invader abundance measured <i>in vivo</i>			Disease incidence measured <i>in vivo</i>		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
<b>(a) Interactions within resident communities</b>									
Proportion of facilitative interactions	1	0.02	0.885	↑ 1	11.82	0.0009	↑ 1	7.01	0.009
Predicted Mean intensity of interactions	↑ 1	129.8	<2E-16	↑ 1	14.29	0.0003	↑ 1	12.66	0.0006
Observed Mean intensity of interactions	↑ 1	8.18	0.005	↑ 1	11.24	0.001	1	1.56	0.215
No. of Residuals	167			95			95		
Model summary	$R^2$ : 0.45 AIC: -303.69			$R^2$ : 0.28 AIC: 111.10			$R^2$ : 0.18 AIC: 731.07		
<b>(b) Interaction between community and invader</b>									
Niche breadth									
Niche overlap between the invader and resident communities	↓ 1	13.76	0.0003	↓ 1	8.62	0.004	1	1.29	0.258
Direct invader inhibition by resident communities	↓ 1	79.15	8.881E-16	↓ 1	5.24	0.024	↓ 1	12.46	0.0006
No. of Residuals	168			96			96		
Model summary	$R^2$ : 0.36 AIC: -277.97			$R^2$ : 0.13 AIC: 128.58			$R^2$ : 0.13 AIC: 735.84		

All response variables were treated as continuous variables. The table shows the most parsimonious models selected based on the AIC

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4 information. The up and downwards arrows denote for positive and negative effects on response variables, respectively.  
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