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Article:

Facilitation promotes invasions in plant-associated microbial communities

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

2 **communities**

3 Running title**:** Facilitation increases invasibility

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34

Abstract

36 While several studies have established a positive correlation between

37 community diversity and invasion resistance, it is less clear how species

38 interactions within resident communities shape this process. Here we

39 experimentally tested how antagonistic and facilitative pairwise interactions

40 within resident model microbial communities predict invasion by the

41 plant-pathogenic bacterium *Ralstonia solanacearum.* We found that facilitative

42 resident community interactions promoted and antagonistic interactions

43 suppressed invasions both in the lab and in the tomato plant rhizosphere.

44 Crucially, pairwise interactions could reliably explain observed invasions

45 outcomes also in multispecies communities, and mechanistically, this was

46 linked to direct inhibition of the invader by antagonistic communities

47 (antibiosis), and to a lesser degree by resource competition between the

48 members of the resident community and the invader. Together our findings

49 suggest that the type and strength of pairwise interactions can reliably predict

50 the outcome of invasions in more complex multispecies communities.

51

Introduction

53 The characteristics of both resident communities and the invading species are

54 important for determining the outcomes of biological invasions (Williamson &

55 Fitter 1996; Catford *et al.* 2009). From the resident community perspective,

56 species diversity may be considered as a shield to invasions and this effect is

57 often attributed to competition for existing resources (Fridley *et al.* 2007;

58 Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015) where

59 highly diverse communities are thought to efficiently use all the available

60 resource niches leaving no free space for invaders (Case 1990; Tilman 2004).

61 In reality, diversity-invasion resistance relationships are more varied ranging

62 from having neutral to even negative effects (Shea & Chesson 2002; Mallon *et*

63 *al.* 2015a; Mehrabi *et al.* 2016) and are sensitive to environmental conditions

64 (Davis *et al.* 2000; Roscher *et al.* 2009; Jousset *et al.* 2011; Mallon *et al.*

65 2015b). Furthermore, it has been shown that trophic network architecture (Wei

66 *et al.* 2015), species identity effects (Yang *et al.* 2017) and food web

67 connectance (Smith-Ramesh *et al.* 2017) are important predictors of invasions

68 and are often linked with community diversity. For example, how species

69 interact might be more important than the number of interacting species within

70 the community (Wei *et al.* 2015), while invasion resistance could be sometimes

71 mediated by certain keystone taxa (Yang *et al.* 2017). However, the type and

72 strength of resident species interactions have often been overlooked in the

73 context of diversity-invasion resistance studies.

3

74 Resident species communities form complex ecological webs where

75 multiple species may interact positively or negatively with each other (Kéfi *et al.*

76 2012). Positive interactions between species at the same trophic level can

77 result from facilitation or metabolic cross-feeding, where species benefit from

78 the presence of each other (Mulder *et al.* 2001). Negative interactions may

79 result from resource competition (Wei *et al.* 2015) or direct interference

80 competition, where species directly suppress each other via antagonism (Bais

81 *et al.* 2003; Hierro & Callaway 2003; Thorpe *et al.* 2009; Hu *et al.* 2016). These

82 interactions may affect the outcomes of invasions in various ways. First,

83 facilitation and competition are likely to affect the resource availability, and

84 hence the availability of free resource niche space, and the likelihood of

85 invasions (Shea & Chesson 2002; Tilman 2004; Stachowicz & Byrnes 2006;

86 Gioria & Osborne 2014; Mallon *et al.* 2015c). It is predicted that highly

87 competitive resident communities are less prone to invasions if they can

88 efficiently utilize and consume resources that would otherwise be available for

89 invaders (Tilman 2004; Jousset *et al.* 2011; Mallon *et al.* 2015c). This effect is

90 expected to be especially strong in the resident communities that show a high

91 degree of complementarity and hence compete less strongly with each other

92 compared with the invader. In contrast, facilitative interactions between

93 resident community members could potentially increase the number of

94 resource niches via production of secondary metabolites or public goods that

95 can also be utilized by the invader (Stachowicz 2001; Mallon *et al.* 2015b;

96 Bulleri *et al.* 2016). Furthermore, competing species can inhibit each other

97 directly by producing toxic metabolites, such as antibiotics. Depending on the

98 spectrum of their activity, antibiotic compounds could have negative effects on

4

99   both resident community species and the invader (Bais *et al.* 2003; Hierro &

100   Callaway 2003; Thorpe *et al.* 2009; Becker *et al.* 2012; Hu *et al.* 2016; Wang *et*

101   *al.* 2017b). If the invader is particularly sensitive to toxins produced by the

102   resident community, it is expected that antibiotics-mediated interference

103   competition will constrain invasions. In contrast, if produced toxins have a

104   disproportionally larger negative effect on the members of the resident

105   community, such interference competition is expected to promote invasions

106   (Thorpe *et al.* 2009; Stubbendieck *et al.* 2016). Resident community species

107   interactions could further affect certain community-level properties such as

108   ecological stability (Allesina & Levine 2011), which could have indirect effects

109   on invasions (Ghoul & Mitri 2016).

110   In the present study, we explored to what extent the type (facilitative vs

111   antagonistic) and strength of two-species resident community species

112   interactions can predict invasions in complex multispecies bacterial

113   communities. Experiments conducted within one trophic level suggest that

114   pairwise bacterial competitions can predict three-species bacterial

115   competitions with as high as 90% accuracy (Friedman *et al.* 2017). While

116   predicting competitions in species-rich communities might require additional

117   information about potentially emerging higher-order interactions (Friman *et al.*

118   2016; Grilli *et al.* 2017; Levine *et al.* 2017), these findings suggest that

119   qualitative information regarding species growth in pairwise co-cultures can be

120   used to predict the competitive outcomes of up to 8-species communities

121 (Friedman *et al.* 2017). Here we extend this approach beyond competition to

122 concurrently explore the role of antagonistic and facilitative resident

123 community interactions for biological invasions (Bruno *et al.* 2003; Altieri *et al.*

124 2010; Traveset & Richardson 2014). Our study system consisted of six

125 non-pathogenic bacterial species (resident community), which were isolated

126 from the tomato plant rhizosphere, and the invader, the plant-pathogenic

127 *Ralstonia solanacearum* bacterium. Specifically, we first characterized

128 antagonistic and facilitative pairwise interactions within model resident

129 bacterial communities and then directly tested how these interactions predict

130 invasions in more complex multispecies communities both *in vitro* and *in vivo*

131 in the tomato rhizosphere. We found that facilitative and antagonistic pairwise

132 interactions reliably predicted invasions: facilitative resident communities were

133 more prone to invasions, while antagonistic resident communities were

134 invaded much less often. Mechanistically, this was linked to direct inhibition of

135 the invader by antagonistic communities (antibiosis), and to a lesser degree by

136 resource competition between the members of the resident community and the

137 invader. Our results suggest that antagonism is an important determinant of

138 community invasion resistance (Case 1990; Tilman 2004), while facilitation

139 might promote invasions by alleviating antagonistic interactions or by releasing

140 vacant niche space for the invader.

141

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**

165   To quantify the type (facilitative, neutral or antagonistic), strength and

166   directionality of each pairwise interaction between resident community species,

167   we compared the growth of each species alone and in the presence of each of

168   the other species in two-species co-cultures (Foster & Bell 2012). All

169   mono-cultures were inoculated with a starting density of 10^5 cells per ml and

170   the co-cultures were inoculated with half of this starting cell density of each

171   species. Resident species were grown for 48h in liquid NA medium (glucose

172   10.0 g l^{-1}, tryptone 5.0 g l^{-1}, yeast extract 0.5 g l^{-1}, beef extract 3.0 g l^{-1}, pH 7.0)

173   in 48-well microtiter plates (ending volume of 700 ml per well) at 30°C with

174   shaking (170 rpm). Bacterial growth was measured as colony number units

175   (CFU) per ml by serial dilution and plating on NA agar plates after 48h growth.

176   All strains formed distinct colonies on agar plates and could be identified

177   based on colony morphology (Figure S2).

178   The type of pairwise interaction between two species (here i and j) was

179   determined by comparing the sum of endpoint of monoculture productivity

180   (population densities) of i (MP_i) and monoculture productivity of j (MP_j) with the

181   ending productivity of the two-species co-culture (CP_{i+j}). As suggested

182   previously, the density of a species mixture is expected to be exactly the sum

183   of their growth in the monocultures if species do not interact (Foster & Bell

184   2012). Thus, we expected that the interaction between i and j would be

185   facilitative if $CP_{i+j} > MP_i + MP_j$, antagonistic if $CP_{i+j} < MP_i + MP_j$ and neutral if

186    $CP_{i+j} = MP_i + MP_j$.

187   In order to characterize directionality of pairwise interactions, we

188   compared the ending productivity of each species (CP_i and CP_j) in two-species

189 co-cultures with their ending productivities in monocultures. We then

190 determined the directionality of interaction facilitative if species j had a positive

191 effect on i ($\log_{10}(CP_i / MP_i) > 0$), antagonistic if $\log_{10}(CP_i / MP_i) < 0$ and neutral if

192 $\log_{10}(CP_i / MP_i) = 0$. We also calculated the mean intensity of facilitation (MIF)

193 of co-cultures as an average of $\log_{10}$-transformed pairwise interactions using

194 the following formula: $MIF_{ij} = \frac{1}{2}[\log(CP_i / MP_i) + \log(CP_j / MP_j)]$ The two-species

195 community was defined as facilitative when MIF > 0, antagonistic when MIF <

196 0 and neutral if MIF = 0.

197

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:

211 $$PIF = \frac{1}{C_n^2} \sum_1^{C_n^2} MIF_{ij}$$,

212 where MIF_{ij} refers the net intensity of one pairwise interaction between species

213 i and j in a multispecies community, which has a total of C_n^2 number pairwise

214 interactions. The communities were defined as facilitative when PIF > 0,

215 antagonistic when PIF < 0 and neutral when PIF = 0. PIF thus accounted for

216 both the strength and directionality of all potential pairwise interactions in a

217 multispecies community.

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 - μl volumes

233 with 10 µl of SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), 2 µl of template,

234 0.4 µl Dye I, 0.8 µl of both forward and reverse primers (10 mM each) and 6 µl

235 sterile water. The PCR was performed by initially denaturizing at 95°C for 30 s,

236 cycling 40 times with a 5-s denaturizing step at 95°C, using a 34-s

237 elongation/extension step at 60°C, and ending with melt curve analysis at

238 95°C for 15 s, at 60°C for 1 min, and at 95°C for 15 s. Each resident species

239 community sample was replicated three times.

240 The observed mean intensity of facilitation (OIF) was calculated using the

241 observed species proportions in the communities based on qPCR data. Similar

242 to PIF, we first determined to what extent the growth of each species was

243 affected by the presence of other species in a given community (growth in the

244 community vs. growth alone). OIF was then calculated according to the

245 following formula: $OIF = \frac{1}{n}\sum \log(CP_i / MP_i)$. Communities were defined as

246 facilitative when OIF > 0, antagonistic when OIF < 0 and neutral if OIF = 0. OIF

247 was calculated only based on *in vitro* data and in the case of MIF, PIF and OIF,

248 antagonism included the effects arising from both resource competition and

249 direct inhibition via toxins.

250

251 **Measuring resource competition and direct antagonism between the**

252 **invader and resident community species**

253 All bacteria were first grown to high densities (OD$_{600}$≈1.0) in liquid NA media

254 overnight at 30°C with shaking (170 rpm), washed three times in 0.85% NaCl,

255 and adjusted to an optical density of 0.5 at 600 nm (OD$_{600}$) with SpectraMax

256 M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). We then

257 measured the growth of the invader and all six resident community species

258 individually on 48 different single-carbon resources (see Table S4)

259 representative of tomato root exudates (Hu *et al.* (2016)). When the invader

260 and resident community species were able to grow on the same resource

261 ($OD_{600} > 0.05$), their niches were considered to overlap regarding that given

262 resource. In contrast, when only one strain was able to grow on a specific

263 resource, the niches were considered not to overlap (Wei *et al.* 2015). This

264 resource competition index estimated the 'apparent' resource competition

265 assuming that interacting species would be competing for the same resources

266 even when presented with multiple different resources.

267   Direct antagonism between the invader and resident community species

268 was measured using supernatant assays (Hu *et al.* 2016). Briefly, after 24h of

269 growth in NA media, all bacterial monocultures were filtered to remove living

270 cells (0.22 µm filter) after 20 µl of sterile supernatant from each resident

271 species culture was mixed with 180 µl of an overnight-grown *R. solanacearum*

272 culture (OD_{600} = 0.05, five-fold dilution in liquid NA). The control treatments

273 were inoculated with 20 µl of sterile-filtered NA media instead of bacterial

274 supernatant. All bacterial cultures were grown for 24h at 30°C with shaking

275 (170 rpm) before measuring pathogen inhibition as optical density (OD 600

276 nm). Antagonism was defined as the percentage of reduction in pathogen

277 growth by the supernatant compared to the control treatment for all possible

278 invader-resident species two-species combinations.

279

280 **Measuring invasion success in multispecies communities**

281 **a) Invasion success measured *in vitro***

282 All possible multispecies resident communities were assembled in triplicate in

283 liquid NA medium with a starting density of 10^5 cells per ml (100%, 50%, 33%,

284 25%, 20% and 16.7% of monoculture densities in 1, 2, 3, 4, 5 and 6 resident

285 species communities, respectively). Communities were then subsequently

286 exposed to invasion by mCherry-tagged *R. solanacearum* (10^4 cells per ml) in

287 96-well plates at 30°C with shaking (170 rpm). After 48h, total bacterial

288 densities were measured as optical density (OD 600 nm) and invasion success

289 measured as the relative invader density to total bacterial densities using red

290 mCherry protein fluorescence intensity (RFP; excitation: 587 nm, emission:

291 610 nm) with SpectraMax M5 spectrophotometer.

292

293 **b) Invasion success measured *in vivo***

294 We used a 50-day-long greenhouse experiment with tomato plants to measure

295 invasion success *in vivo*. The soil was collected from a rice field in Wuxi

296 (Jiangsu Province, China), sieved at 5 mm and homogenized and sterilized

297 with gamma radiation. Surface-sterilized tomato seeds (*Lycopersicon*

298 *esculentum*, cultivar "*Micro-Tom*") were germinated on water-agar plates for 3

299 days before sowing into seedling plates containing cobalt-60-sterilized

300 seedling substrate (Huainong, Huaian Soil and Fertilizer Institute, Huaian,

301 China). *Ralstonia solanacearum* invasion was tested in all possible

302 two-species resident communities, and due to practical reasons, in 18

303 multispecies resident communities that varied in their predicted mean

304 intensities of facilitation (Table S5).

305 Three replicates were used for each resident community, and one replicate

306 consisted of a seedling plate that contained six germinated tomato plants (at

307 the three-leaf stage of growth when grown on 700 g sterilized soil). Similar

308 replication was also used for positive (only the invader) and negative (no

309 bacteria) controls. After 3 days of growth on seedling plates, plants were

310 inoculated with assembled resident communities using root drenching method

311 at a final concentration of 10^8 CFU of bacteria g^{-1} soil (Wei *et al.* 2013). Seven

312 days after inoculation of resident communities, *R. solanacearum* was

313 introduced to the roots of all plants at a final concentration of 10^7 CFU of

314 bacteria g^{-1} soil. Tomato plants were then grown for 40 days in a greenhouse

315 (with natural temperature variation ranging from 25°C to 35°C) and watered

316 regularly with sterile water. Seedling plates were rearranged randomly every

317 two days and disease progression monitored at every seven days. Forty days

318 after inoculation of *R. solanacearum*, rhizosphere soil was collected from one

319 plant per replicate seedling tray and the abundance of the invader determined

320 with quantitative PCR as the abundance of *R. solanacearum*-specific *fliC* gene

321 copy numbers (Hu *et al.* 2016).

322

323 **Statistical analyses**

324 To meet assumptions of normality and homogeneity of variance, invader

325 densities measured *in vitro* and *in vivo* were log10-transformed. We first

326 assessed the independent effects of the proportion of facilitative interactions

14

327   and the mean intensity of facilitation based on pairwise resident community

328   interaction on invasions (pathogen density and disease incidence). The type of

329   interaction between resident community species pairs was included into

330   models as a categorical variable (1= facilitation; 0= antagonism). In the case of

331   multispecies communities, invasions were explained by three quantitative

332   indexes, the proportion of facilitative interactions within a community, the

333   predicted mean intensity of facilitation (PIF) and the and observed mean

334   intensity of facilitation (OIF). All indexes were fitted as continuous variables

335   and one separate model was used for each index that explained invader

336   densities *in vitro* and *in vivo* and bacterial wilt disease incidence. Additional

337   linear mixed models were used to test invasions as a function of a) niche

338   overlap between resident community and the pathogen (niche preemption by

339   the resident community), b) mean pathogen inhibition by the resident

340   community and c) resident community species identity effects. All analyses

341   were conducted with SPSS (V. 22) and R (Computing 1991; Team 2013).

342

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

15

351   interactions with each other (Figure 1B, Table S6). On average, facilitative

352   two-species communities reached higher population densities (R^2=0.79,

353   P<0.001, Figure S4), while antagonistic two-species communities were more

354   inhibitory towards each other (R^2=0.32, P=0.029, Figure S5A). No relationship

355   was found between resident species' resource niche overlap and observed

356   mean intensity of facilitation (Figure S5B), which suggests that facilitation did

357   not arise due to niche complementarity. Together these results suggest that the

358   strength of direct inhibition was more important in explaining the type of

359   pairwise interactions between resident community members compared to

360   resource competition.

361   To link the type of pairwise interaction with the likelihood of invasions, we

362   compared *R. solanacearum* invasion success in facilitative and antagonistic

363   two-species resident communities. Compared to positive controls (*R.*

364   *solanacearum*-only: red dashed line in Figure 2A-F), pathogen densities were

365   significantly lower in the presence of resident species both *in vitro* and *in vivo*.

366   The intensity of pathogen suppression could be predicted by the type of

367   pairwise interactions between the resident species: pathogen density was

368   significantly higher in facilitative compared to antagonistic communities *in vitro*

369   ($F_{1,43}$=16.02, P<0.001, Figure 2A; R^2=0.49, P<0.0001, Figure 2B) and *in vivo*

370   ($F_{1,43}$=24.40, P<0.001, Figure 2C; R^2=0.26, P=0.0021, Figure 2D). In line with

371   these results, the bacterial wilt disease incidence was also higher in facilitative

372   compared to antagonistic resident communities ($F_{1,43}$=9.03, P=0.004, Figure

373   2E; R^2=0.14, P=0.013, Figure 2F). Mechanistically, this could be explained by

374   loss of pathogen inhibition as suggested by a negative correlation between the

375 mean intensity of facilitation and direct invader suppression (R^2=0.45,

376 P<0.0001, Figure S6). Together these results suggest that antagonistic

377 two-species resident communities were more inhibitory not only towards

378 themselves but also against the invader.

379

380 **(b) Predicting and validating invasions in multispecies communities**

381 **based on pairwise interactions**

382 Interactions within the resident communities could well explain the invader

383 abundance *in vitro* (R^2: 0.45, P<0.0001) and *in vivo* (R^2: 0.28, P<0.0001), and

384 bacterial wilt disease incidence (R^2: 0.18, P=0.0002) *in vivo* (Table 1). The

385 proportion of facilitative interactions were well explained by the increase in

386 invader density in all tested resident communities *in vitro* (R^2=0.35, P<0.0001,

387 Figure 3A). Similarly, both the density of the invader in the tomato rhizosphere

388 (R^2=0.22, P=0.0004, Figure 3B) and bacterial wilt disease incidence (R^2=0.21,

389 P=0.0004, Figure 3C) increased significantly with increasing proportion of

390 facilitative interactions within the resident communities. The predicted mean

391 intensity of facilitation explained well the increase in invader density *in vitro*

392 (R^2=0.45, P<0.0001, Figure 3D) and *in vivo* (R^2=0.21, P=0.0005, Figure 3E)

393 and correlated positively with bacterial wilt disease incidence (R^2=0.19,

394 P=0.0193, Figure 3F). The predicted and observed mean intensities of

395 facilitation correlated positively with each other (R^2=0.44, P<0.0001, Figure

396 S7), demonstrating that pairwise interactions can be used to predict

397 interactions in multispecies communities. As expected, invader densities also

398 increased with increasing observed mean intensity of facilitation both *in vitro*

399 (R^2=0.26, P<0.0001, Figure 3G) and *in vivo* (R^2=0.17, P=0.0019, Figure 3H).

400 However, the observed mean intensity of facilitation did not correlate

401 significantly with bacterial wilt disease incidence (Figure 3I).

402   The low invasion success observed in antagonistic resident communities

403 could be attributed to high levels of direct inhibition of the invader and/or high

404 resource niche overlap between the invader and resident community members.

405 We found that both direct pathogen inhibition and high resource niche overlap

406 reduced invader densities *in vitro* and *in vivo*, while only direct pathogen

407 inhibition significantly reduced the disease incidence (Table 1). Direct

408 pathogen suppression correlated negatively with both predicted and observed

409 mean intensities of facilitation suggesting that antagonistic multispecies

410 communities were more inhibitory to the invader (Figure S8). The species *B.*

411 *amyloliquefaciens* and *F. johnsoniae* had strong negative effects on pathogen

412 densities *in vitro* and *in vivo* (Table S7). However, only *B. amyloliquefaciens*

413 had a significant negative effect on disease incidence, while species *C.*

414 *daecheongense* had a slightly positive effect on disease incidence (Table S7).

415 Together these results suggest that pairwise resident community interactions

416 can predict invasions in multispecies communities *in vitro* and *in vivo* and that

417 these effects were primarily linked with direct pathogen suppression.

418

419 **Discussion**

420 Here we studied how resident community interactions are linked with invasions

421 in bacterial plant rhizosphere communities. We found that facilitative two-

422 species communities were invaded more easily both in the laboratory and

423 rhizosphere compared to antagonistic resident communities. Furthermore, we

424 could use the pairwise interactions to predict invasion outcomes in

425 multispecies communities containing up to 6 resident species. Specifically,

426 communities characterized by a high proportion of facilitative pairwise

427 interactions, and high predicted and observed mean intensities of facilitation,

428 were more susceptible to invasions. Mechanistically, this was linked to direct

429 inhibition of the invader by antagonistic communities (antibiosis), and to a

430 lesser degree by resource competition between the members of the resident

431 community and the invader. Together these findings suggest that outcomes of

432 relatively simple pairwise interactions can be used to predict invasions in

433 multispecies microbial communities especially when antagonism and

434 facilitation are strongly linked with the resistance to invasion.

435   Invasion resistance has been thus far mainly considered from the

436 perspective of resource competition and niche preemption (Case 1990; Tilman

437 2004; Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015). Our

438 results suggest that facilitative interactions should also be considered in the

439 context of invasions. While it is difficult to pinpoint the exact mechanism

440 between facilitation and invasions, most likely explanation is the loss of

441 pathogen inhibition along with the increase in the mean intensity of facilitation

442 (Figure S6). This is in line with a previous finding where the increase in the

443 antagonistic activity was found to increase the invasion resistance of

444 *Pseudomonas* resident communities (Hu *et al.* 2016). Another explanation

445 could be that facilitative resident communities were less efficient at competing

446 for resources with the invader compared to antagonistic resident communities.

447   However, this likely played a relatively small role as resource niche overlap

448   had the only significant negative effect on the invader density when measured

449   *in vitro* and *in vivo* but not on disease incidence (Table 1). It is also possible

450   that our resource competition indexes measured *in vitro* overestimated the

451   strength of resource competition or underestimated the size of the niche space

452   in the rhizosphere leading to weak correlation with invasions. Furthermore,

453   facilitative interactions could have increased the niche space in the resident

454   communities in favor of the invader, which could have promoted invasions as a

455   side effect (Bulleri *et al.* 2016). For example, previous studies have

456   demonstrated that bacteria can show diet preference between different dietary

457   glycans, which can prolong the species coexistence in co-cultures (Tuncil *et al.*

458   2017). Such dietary preference might leave some resources less utilized

459   opening opportunity for invasions (Tilman 1999). It has also been shown that

460   the breakdown of polysaccharides can allow coexistence of species that

461   liberate polysaccharide breakdown products (PBPs), which are consumed by

462   other species that are unable to grow on the polysaccharides alone (recipients)

463   (Rakoff-Nahoum *et al.* 2014). Facilitative interactions could thus potentially

464   favor the invader if it is unable to grow on the primary substrates on its own

465   (Bruno *et al.* 2003). While it is difficult to validate these hypotheses based on

466   our data, we found that facilitative communities were more productive in

467   general and reached higher total cell densities when cultured together

468   compared to alone (Figure S4). This supports the idea that facilitative resident

469   species were benefitting from the presence of each other (for example via

470   cross-feeding), which could also have benefitted the invader by creating

471   vacant niche space. The carrying capacity of resident communities could thus

472   be an important predictor of biological invasions (Gosso *et al.* 2012).

473   In addition to within-resident community interactions, the interactions

474   between resident communities and the invader were also good predictors of

475   invasions, albeit to a lesser extent (Figure 3 and Table 1). While it remains

476   unclear what exact compounds were produced by the resident communities,

477   previous studies have shown that soil bacteria are capable of producing a wide

478   variety of antimicrobials that often suppress *R. solanacearum* (Hu *et al.* 2016;

479   Wang *et al.* 2017b). For example, the *B. amyloliquefaciens* T-5 strain used in

480   this study has been shown to efficiently suppress *R. solanacearum* both in the

481   lab and plant rhizosphere (Wang *et al.* 2017b) and this strain also had the

482   greatest negative effect on the pathogen densities and disease incidence in

483   this study (Figure S1). In addition, the strain *F. johnsoniae* had a negative

484   effect on pathogen densities both *in vitro* and *in vivo*. Together these results

485   suggest that pathogen suppression via toxins was likely mediated by the

486   presence of these species.

487   In general, pairwise resident community interactions predicted well the

488   observed invasion outcomes in multispecies communities. (Figure 3, Table 1).

489   However, no correlation was found between the observed mean intensity of

490   facilitation and bacterial wilt disease incidence (Figure 3I). This suggests that

491   while *in vitro* mechanisms (resource competition and antibiosis) can robustly

492   predict invasions in more complex *in vivo* environments (Wei *et al.* 2015; Hu *et*

493   *al.* 2016), they do not account for all aspects of more complex natural

494   environments. There are many potential explanations for these discrepancies

495   that should be validated in future studies. First, investigating the role of

microbe-mediated plant immunity is important as both pathogenic and non-pathogenic bacteria can trigger or suppress plant immunity (Chen *et al.* 2017; Rautenbach *et al.* 2017). Furthermore, several bacterial secondary metabolites involved in pathogen suppression also impact plant immunity: for example, 2, 4-diacetylphloroglucinol (DAPG) produced by fluorescent *Pseudomonas* spp. (Bulai & Venturino 2017) or lipopeptide surfactins produced by *Bacillus subtilis* (Wang *et al.* 2017a) have a such dual-function. Second, the rhizosphere bacterial communities we used were rather simple, and hence, predictions based on pairwise species interactions should be tested in more complex multi-trophic communities in the future. Lastly, our predictive indexes only estimated the mean net effects and did not distinguish if both or only one of the species benefitted and vice versa (Foster & Bell 2012). While this approach seems to be a good predictor of invasion outcomes, accounting for the directionality of interactions and potential emerging higher-order interactions (Friman *et al.* 2016; Grilli *et al.* 2017; Levine *et al.* 2017) is likely to improve these predictions.

In conclusion, our results suggest that qualitative information regarding species growth in pairwise co-cultures can be used to predict the outcomes of invasions in multispecies communities. Even though our results can be broadly applied across different biological problems, they could offer direct solutions in the context of crop protection. Bacterial pathogens impose an ever-increasing threat for agriculture (Olson & Stenlid 2001; Choudhary & Johri 2009; Nicol *et al.* 2011) and recent evidence suggests that the rhizosphere microbiome plays an essential role in controlling the onset of diseases (Berendsen *et al.* 2012;

520 Lozupone *et al.* 2012). Understanding the characteristics that make certain

521 microbiomes more resistant to invasions could potentially allow one to harness

522 beneficial bacterial communities for crop protection. While recent studies have

523 shown that microbial diversity alone may be such important characteristic (Wei

524 *et al.* 2015; Hu *et al.* 2016) we here suggest that highly antagonistic microbial

525 communities might also be efficient at constraining pathogen invasions.

526

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542

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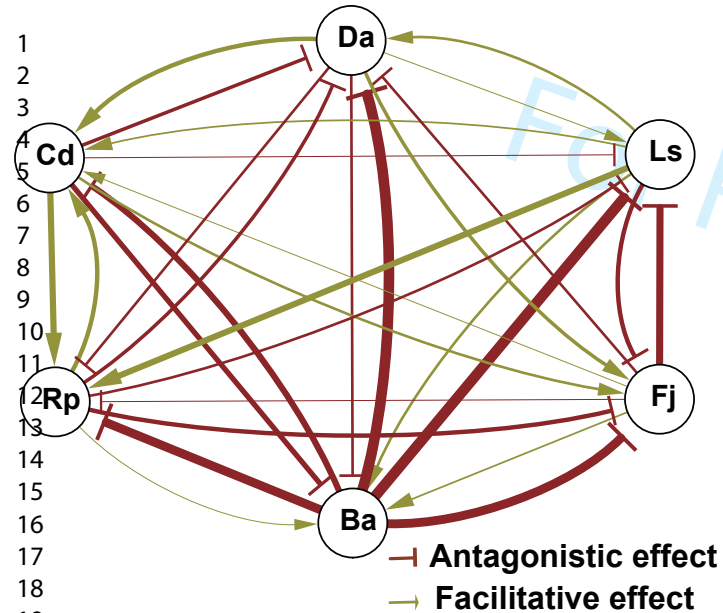
699   inoculation of the invader. (F) The relationship between disease incidence and the mean

700 intensity of facilitation in resident communities measured *in vivo* in the tomato rhizosphere. In

701 all panels, the red dashed lines show the baseline for positive control treatments (invader-only).

702 In panels, B, D and F, values below and above zero denote for antagonistic and antagonistic

703 pairwise resident communities, respectively. Two and three stars denote for statistical

704 significance at p < 0.01 and p < 0.001 significance levels, respectively. All the bars denote for ±

705 1 s.e.m.

706

707 **Figure 3. The relationship between invader abundance and disease incidence with**

708 **predicted and observed mean intensities of facilitation within multispecies**

709 **communities.** (A-B) The relationship between invader abundance and the proportion of

710 facilitative interactions in the resident communities measured *in vitro* and *in vivo*, respectively.

711 (C) The relationship between bacterial wilt disease incidence (%) and the proportion of

712 facilitative interactions in the resident communities. (D-E) The relationship between invader

713 abundance and the predicted mean intensity of facilitation in the resident communities

714 measured *in vitro* and *in vivo*, respectively. (F) The relationship between bacterial wilt disease

715 incidence (%) and the predicted mean intensity of facilitation in the resident communities. (G-H)

716 The relationship between invader abundance and the observed mean intensity of facilitation in

717 the resident communities measured *in vitro* and *in vivo*, respectively. (I) The relationship

718 between bacterial wilt disease incidence (%) and the observed mean intensity of facilitation in

719 the resident communities. In all panels, red dashed lines show the baseline of invader

720 densities in control treatments (invader-only). In panels D-I, values below and above zero

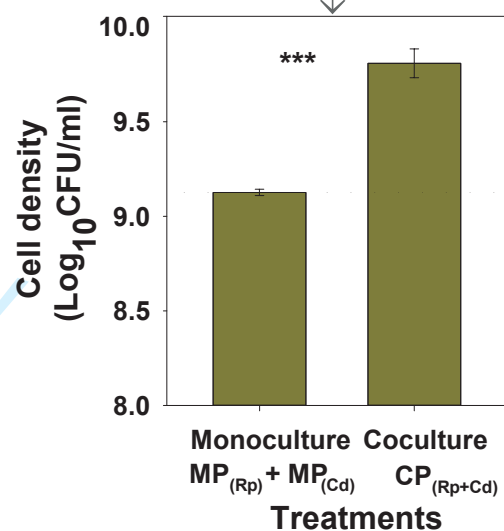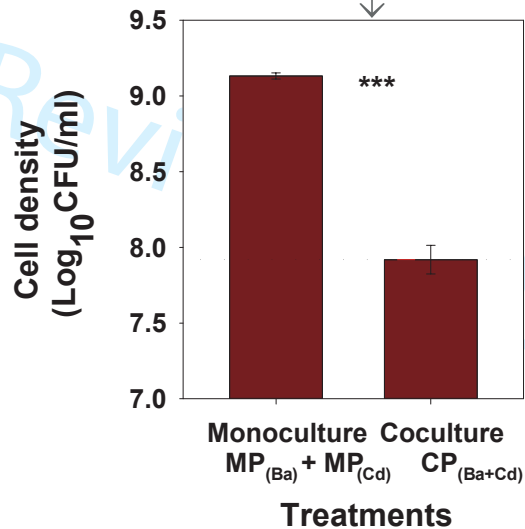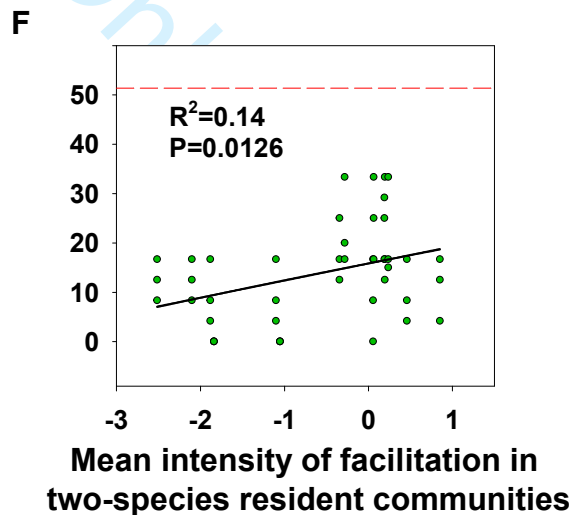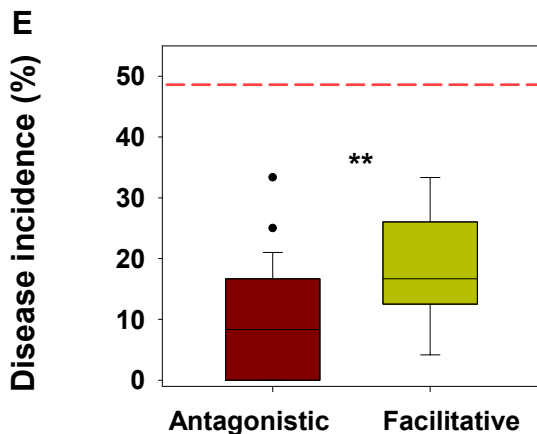721 denote for competitive and antagonistic resident communities, respectively.

A

B

A — Invader abundance (Log₁₀RFP) vs Proportion of facilitative pairwise resident community interactions. R² =0.35, P<0.0001

B — Invader abundance Log₁₀(flic gene copies) g⁻¹ rhizopshere soil. R² =0.22, P=0.0004

C — Disease incidence (%). R² =0.21, P=0.0004

Proportion of facilitative pairwise resident community interactions

D — Invader abundance (Log₁₀RFP). R² =0.45, P<0.0001

E — Invader abundance Log₁₀(flic gene copies) g⁻¹ rhizopshere soil. R² =0.21, P=0.0005

F — Disease incidence (%). R² =0.19, P=0.0193

Predicted mean intensity of facilitation based on pairwise resident community interactions

G — Invader abundance (Log₁₀RFP). R² =0.26, P<0.0001

H — Invader abundance Log₁₀(flic gene copies) g⁻¹ rhizopshere soil. R² =0.17, P=0.0019

I — Disease incidence (%). P>0.05

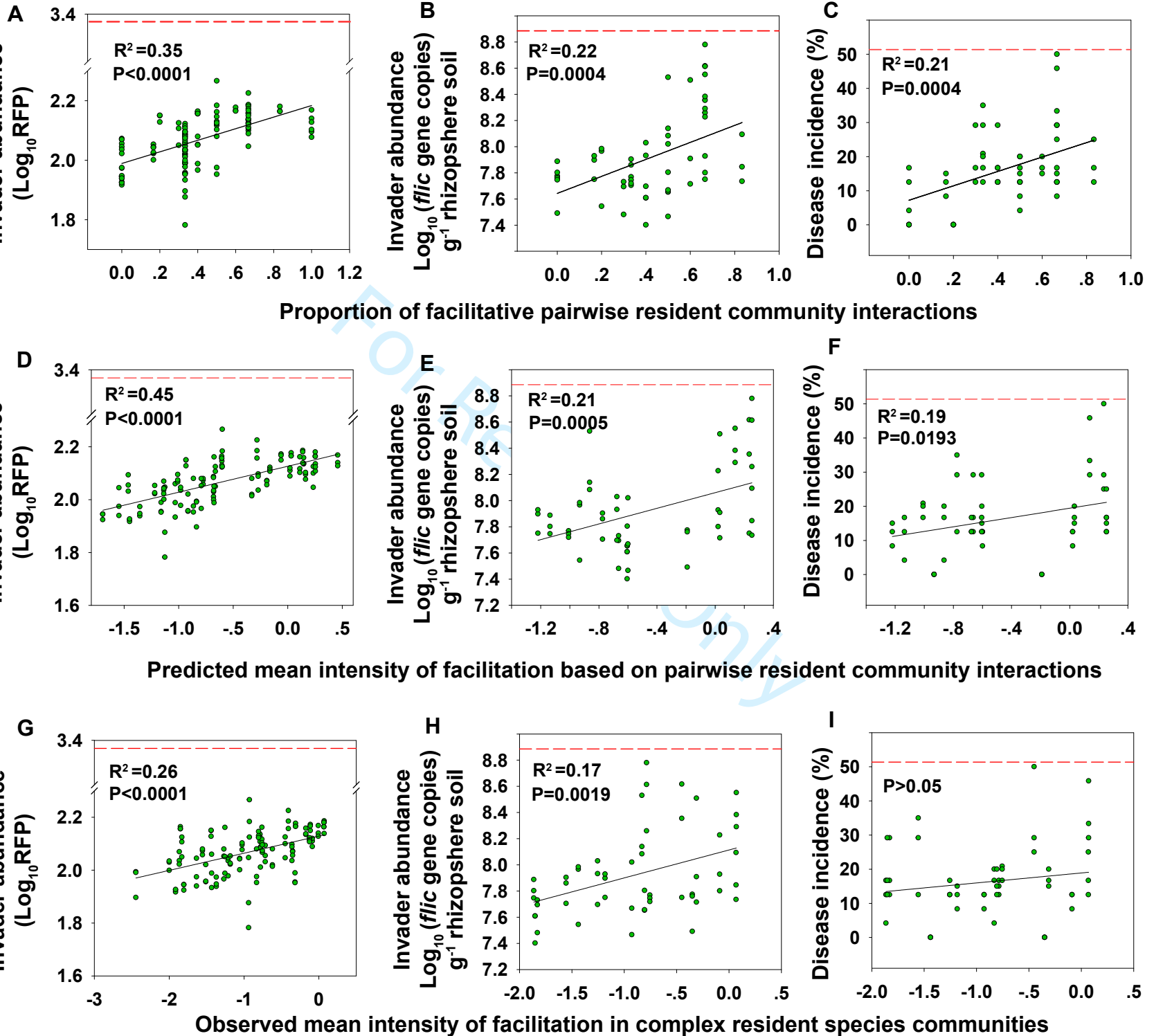Observed mean intensity of facilitation in complex resident species communities

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 *in vitro*			Invader abundance measured *in vivo*			Disease incidence measured *in vivo*		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
(a) Interactions within resident communities									
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) Interaction between community and invader									
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

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