



Deposited via The University of York.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/136892/>

Version: Accepted Version

Article:

Wei, Zhong, Li, Mei, Wang, Jianing et al. (2019) Facilitation promotes invasions in plant-associated microbial communities. *Ecology Letters*. pp. 149-158. ISSN: 1461-023X

<https://doi.org/10.1111/ele.13177>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.


 The logo for Ecology Letters, featuring the words "ECOLOGY LETTERS" in a serif font, with "ECOLOGY" in a larger size than "LETTERS". The text is white and set against a green rectangular background.

Facilitation promotes invasions in plant-associated microbial communities

Journal:	<i>Ecology Letters</i>
Manuscript ID	ELE-00331-2018.R2
Manuscript Type:	Letters
Date Submitted by the Author:	27-Sep-2018
Complete List of Authors:	Zhong, Wei; Nanjing Agricultural University, Resources and Environmental Sciences Li, Mei; Nanjing Agricultural University, Resources and Environmental Sciences Wang, Jianing; Nanjing Agricultural University, Resources and Environmental Sciences Jousset, Alexandre; Utrecht University, Ecology and Biodiversity; Friman, Ville-Petri; University of York, Biology Xu, Yangchun; Nanjing Agricultural University, Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers Shen, Qirong; Nanjing Agricultural University, Resources and Environmental Sciences Pommier, Thomas; Université Claude Bernard, Laboratoire d'Ecologie Microbienne
Key Words:	community ecology, facilitation, invasion resistance, rhizosphere, microbe-microbe-plant interactions, antagonism

1
2
3 **1 Facilitation promotes invasions in plant-associated microbial**
4
5 **2 communities**

6
7
8 Running title: Facilitation increases invasibility
9

10 **4 Authors and Affiliations**

11
12 Mei Li^{1#}, Zhong Wei^{1*}, Jianing Wang¹, Alexandre Jousset^{1,2}, Ville-Petri Friman^{1,3}, Yangchun
13
14 Xu¹, Qirong Shen^{1*} and Thomas Pommier⁴
15

16
17 ¹Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative
18
19 Innovation Center for Solid Organic Waste Resource Utilization, National Engineering
20
21 Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095,
22
23 Nanjing, PR China.

24
25 ²Institute for Environmental Biology, Ecology & Biodiversity, Utrecht University, Padualaan 8,
26
27 3584 CH Utrecht, The Netherlands.

28
29 ³Department of Biology, Wentworth Way, YO10 5DD, University of York, York, UK.

30
31 ⁴Ecologie Microbienne, UMR1418, French National Institute for Agricultural Research (INRA),
32
33 University Lyon I. F-69622 Villeurbanne, France
34

35
36 **# These authors contributed equally to this work.**
37

38 **E-mail address(es) of all author(s):** 2015203041@njau.edu.cn, weizhong@njau.edu.cn,
39
40 2016103133@njau.edu.cn, A.L.C.Jousset@uu.nl, ville.friman@york.ac.uk, ycxu@njau.edu.cn,
41
42 shenqirong@njau.edu.cn, thomas.pommier@inra.fr
43

44 **20 Authorship**

45
46 ML, ZW, YX and QS designed research; ML and ZW performed research and analyzed data;
47
48 ML, ZW, AJ, VF and TP wrote the manuscript; all authors contributed to the final draft.
49

50
51 **Key words:** community ecology, facilitation, antagonism, invasion resistance, rhizosphere,
52
53 microbe-microbe-plant interactions
54

55 **25 The type of article:** Letters

1
2
3 26 The number of words in the abstract (143/150), in the main text (4930/5000)
4

5 27 The number of references (59); The number of figures (3), tables (1)
6
7

8 28 * **Corresponding author**
9

10 29 Email: weizhong@njau.edu.cn or shengqirong@njau.edu.cn; Telephone number:
11
12 30 025-84396291
13

14 31 **Data accessibility statement:** if the manuscript be accepted, the data supporting the results
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

20
21 34
22

23 35 **Abstract**
24

25
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
29
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
35
36 41 plant-pathogenic bacterium *Ralstonia solanacearum*. We found that facilitative
37
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
43
44 45 outcomes also in multispecies communities, and mechanistically, this was
45
46 46 linked to direct inhibition of the invader by antagonistic communities
47
48 47 (antibiosis), and to a lesser degree by resource competition between the
49
50 48 members of the resident community and the invader. Together our findings
51
52 49 suggest that the type and strength of pairwise interactions can reliably predict
53
54
55
56
57
58
59
60

1
2
3 50 the outcome of invasions in more complex multispecies communities.
4
5
6 51

7
8 **52 Introduction**
9

10
11 53 The characteristics of both resident communities and the invading species are
12
13 54 important for determining the outcomes of biological invasions (Williamson &
14
15 55 Fitter 1996; Catford *et al.* 2009). From the resident community perspective,
16
17 56 species diversity may be considered as a shield to invasions and this effect is
18
19 57 often attributed to competition for existing resources (Fridley *et al.* 2007;
20
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
24
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
48
49 72 strength of resident species interactions have often been overlooked in the
50
51 73 context of diversity-invasion resistance studies.
52
53
54
55
56
57
58
59
60

1
2
3 74 Resident species communities form complex ecological webs where
4
5 75 multiple species may interact positively or negatively with each other (*Kéfi et al.*
6
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
48
49 97 directly by producing toxic metabolites, such as antibiotics. Depending on the
50
51 98 spectrum of their activity, antibiotic compounds could have negative effects on
52
53
54
55
56
57
58
59
60

1
2
3 99 both resident community species and the invader (Bais *et al.* 2003; Hierro &
4
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
22
23 109 on invasions (Ghoul & Mitri 2016).
24
25
26
27

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
31
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
47
48 120 used to predict the competitive outcomes of up to 8-species communities
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 121 (Friedman *et al.* 2017). Here we extend this approach beyond competition to
5
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
27
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
31
32 135 the invader by antagonistic communities (antibiosis), and to a lesser degree by
33
34 136 resource competition between the members of the resident community and the
35
36 137 invader. Our results suggest that antagonism is an important determinant of
37
38 138 community invasion resistance (Case 1990; Tilman 2004), while facilitation
39
40 139 might promote invasions by alleviating antagonistic interactions or by releasing
41
42 140 vacant niche space for the invader.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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**

1
2
3 165 To quantify the type (facilitative, neutral or antagonistic), strength and
4
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 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)
18
19 173 in 48-well microtiter plates (ending volume of 700 μl per well) at 30°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).

28
29
30
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 $CP_{i+j} = MP_i + MP_j$.

48
49
50
51 187 In order to characterize directionality of pairwise interactions, we
52
53 188 compared the ending productivity of each species (CP_i and CP_j) in two-species
54
55

1
2
3 189 co-cultures with their ending productivities in monocultures. We then
4
5 190 determined the directionality of interaction facilitative if species j had a positive
6
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
15
16
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:

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

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 - μl volumes

1
2
3 233 with 10 µl of SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), 2 µl of template,
4
5 234 0.4 µl Dye I, 0.8 µl of both forward and reverse primers (10 mM each) and 6 µl
6
7 235 sterile water. The PCR was performed by initially denaturizing at 95°C for 30 s,
8
9 236 cycling 40 times with a 5-s denaturizing 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
14
15
16 239 community sample was replicated three times.

17
18
19 240 The observed mean intensity of facilitation (OIF) was calculated using the
20
21 241 observed species proportions in the communities based on qPCR data. Similar
22
23 242 to PIF, we first determined to what extent the growth of each species was
24
25 243 affected by the presence of other species in a given community (growth in the
26
27 244 community vs. growth alone). OIF was then calculated according to the
28
29
30 245 following formula: $OIF = \frac{1}{n} \sum \log(CP_i / MP_i)$ Communities were defined as
31
32
33 246 facilitative when $OIF > 0$, antagonistic when $OIF < 0$ and neutral if $OIF = 0$. OIF
34
35 247 was calculated only based on *in vitro* data and in the case of MIF, PIF and OIF,
36
37 248 antagonism included the effects arising from both resource competition and
38
39 249 direct inhibition via toxins.

40
41
42
43 250

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

48
49
50 253 All bacteria were first grown to high densities ($OD_{600} \approx 1.0$) in liquid NA media
51
52 254 overnight at 30°C with shaking (170 rpm), washed three times in 0.85% NaCl,
53
54
55 255 and adjusted to an optical density of 0.5 at 600 nm (OD_{600}) with SpectraMax

1
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
6
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
20
21 265 assuming that interacting species would be competing for the same resources
22
23 266 even when presented with multiple different resources.
24
25
26

27 Direct antagonism between the invader and resident community species
28
29 268 was measured using supernatant assays (Hu *et al.* 2016). Briefly, after 24h of
30
31 269 growth in NA media, all bacterial monocultures were filtered to remove living
32
33 270 cells (0.22 μm filter) after 20 μl of sterile supernatant from each resident
34
35 271 species culture was mixed with 180 μl 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 μl 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
48
49 278 invader-resident species two-species combinations.
50
51
52

53
54 279

1
2
3 280 **Measuring invasion success in multispecies communities**
4

5
6 281 **a) Invasion success measured *in vitro***
7

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
15
16 286 exposed to invasion by mCherry-tagged *R. solanacearum* (10^4 cells per ml) in
17
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:
25
26 291 610 nm) with SpectraMax M5 spectrophotometer.
27
28
29
30
31
32

33 293 **b) Invasion success measured *in vivo***
34
35

36 294 We used a 50-day-long greenhouse experiment with tomato plants to measure
37
38 295 invasion success *in vivo*. The soil was collected from a rice field in Wuxi
39
40 296 (Jiangsu Province, China), sieved at 5 mm and homogenized and sterilized
41
42 297 with gamma radiation. Surface-sterilized tomato seeds (*Lycopersicon*
43
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
47
48 300 seedling substrate (Huainong, Huaian Soil and Fertilizer Institute, Huaian,
49
50 301 China). *Ralstonia solanacearum* invasion was tested in all possible
51
52 302 two-species resident communities, and due to practical reasons, in 18
53
54
55
56
57
58
59
60

1
2
3 303 multispecies resident communities that varied in their predicted mean
4
5 304 intensities of facilitation (Table S5).
6

7
8 305 Three replicates were used for each resident community, and one replicate
9
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
17
18 310 inoculated with assembled resident communities using root drenching method
19
20 311 at a final concentration of 10^8 CFU of bacteria 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
25
26 314 bacteria 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
33
34 318 after inoculation of *R. solanacearum*, rhizosphere soil was collected from one
35
36 319 plant per replicate seedling tray and the abundance of the invader determined
37
38 320 with quantitative PCR as the abundance of *R. solanacearum*-specific *fliC* gene
39
40 321 copy numbers (Hu *et al.* 2016).
41
42
43
44
45 322

46 47 48 323 **Statistical analyses**

49
50 324 To meet assumptions of normality and homogeneity of variance, invader
51
52 325 densities measured *in vitro* and *in vivo* were log₁₀-transformed. We first
53
54 326 assessed the independent effects of the proportion of facilitative interactions
55

1
2
3 327 and the mean intensity of facilitation based on pairwise resident community
4
5 328 interaction on invasions (pathogen density and disease incidence). The type of
6
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
10
11 331 multispecies communities, invasions were explained by three quantitative
12
13 332 indexes, the proportion of facilitative interactions within a community, the
14
15 333 predicted mean intensity of facilitation (PIF) and the and observed mean
16
17 334 intensity of facilitation (OIF). All indexes were fitted as continuous variables
18
19 335 and one separate model was used for each index that explained invader
20
21 336 densities *in vitro* and *in vivo* and bacterial wilt disease incidence. Additional
22
23 337 linear mixed models were used to test invasions as a function of a) niche
24
25 338 overlap between resident community and the pathogen (niche preemption by
26
27 339 the resident community), b) mean pathogen inhibition by the resident
28
29 340 community and c) resident community species identity effects. All analyses
30
31 341 were conducted with SPSS (V. 22) and R (Computing 1991; Team 2013).
32
33
34
35
36
37
38
39
40

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

1
2
3 351 interactions with each other (Figure 1B, Table S6). On average, facilitative
4
5 352 two-species communities reached higher population densities ($R^2=0.79$,
6
7 353 $P<0.001$, Figure S4), while antagonistic two-species communities were more
8
9 354 inhibitory towards each other ($R^2=0.32$, $P=0.029$, Figure S5A). No relationship
10
11 355 was found between resident species' resource niche overlap and observed
12
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
16
17 358 strength of direct inhibition was more important in explaining the type of
18
19 359 pairwise interactions between resident community members compared to
20
21 360 resource competition.
22
23
24

25 361 To link the type of pairwise interaction with the likelihood of invasions, we
26
27 362 compared *R. solanacearum* invasion success in facilitative and antagonistic
28
29 363 two-species resident communities. Compared to positive controls (*R.*
30
31 364 *solanacearum*-only: red dashed line in Figure 2A-F), pathogen densities were
32
33 365 significantly lower in the presence of resident species both *in vitro* and *in vivo*.
34
35 366 The intensity of pathogen suppression could be predicted by the type of
36
37 367 pairwise interactions between the resident species: pathogen density was
38
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
44
45 371 these results, the bacterial wilt disease incidence was also higher in facilitative
46
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
50
51 374 loss of pathogen inhibition as suggested by a negative correlation between the
52
53
54
55
56
57
58
59
60

1
2
3 375 mean intensity of facilitation and direct invader suppression ($R^2=0.45$,
4
5 376 $P<0.0001$, Figure S6). Together these results suggest that antagonistic
6
7 377 two-species resident communities were more inhibitory not only towards
8
9 378 themselves but also against the invader.

10
11
12 379

13
14
15 380 **(b) Predicting and validating invasions in multispecies communities**
16
17 381 **based on pairwise interactions**

18
19
20 382 Interactions within the resident communities could well explain the invader
21
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
35
36 390 facilitative interactions within the resident communities. The predicted mean
37
38 391 intensity of facilitation explained well the increase in invader density *in vitro*
39
40 392 ($R^2=0.45$, $P<0.0001$, Figure 3D) and *in vivo* ($R^2=0.21$, $P=0.0005$, Figure 3E)
41
42 393 and correlated positively with bacterial wilt disease incidence ($R^2=0.19$,
43
44 394 $P=0.0193$, Figure 3F). The predicted and observed mean intensities of
45
46 395 facilitation correlated positively with each other ($R^2=0.44$, $P<0.0001$, Figure
47
48 396 S7), demonstrating that pairwise interactions can be used to predict
49
50 397 interactions in multispecies communities. As expected, invader densities also
51
52 398 increased with increasing observed mean intensity of facilitation both *in vitro*
53
54
55

1
2
3 399 ($R^2=0.26$, $P<0.0001$, Figure 3G) and *in vivo* ($R^2=0.17$, $P=0.0019$, Figure 3H).
4
5 400 However, the observed mean intensity of facilitation did not correlate
6
7 401 significantly with bacterial wilt disease incidence (Figure 3I).
8
9

10 402 The low invasion success observed in antagonistic resident communities
11
12 403 could be attributed to high levels of direct inhibition of the invader and/or high
13
14 404 resource niche overlap between the invader and resident community members.
15
16 405 We found that both direct pathogen inhibition and high resource niche overlap
17
18 406 reduced invader densities *in vitro* and *in vivo*, while only direct pathogen
19
20 407 inhibition significantly reduced the disease incidence (Table 1). Direct
21
22 408 pathogen suppression correlated negatively with both predicted and observed
23
24 409 mean intensities of facilitation suggesting that antagonistic multispecies
25
26 410 communities were more inhibitory to the invader (Figure S8). The species *B.*
27
28 411 *amyloliquefaciens* and *F. johnsoniae* had strong negative effects on pathogen
29
30 412 densities *in vitro* and *in vivo* (Table S7). However, only *B. amyloliquefaciens*
31
32 413 had a significant negative effect on disease incidence, while species *C.*
33
34 414 *daecheongense* had a slightly positive effect on disease incidence (Table S7).
35
36 415 Together these results suggest that pairwise resident community interactions
37
38 416 can predict invasions in multispecies communities *in vitro* and *in vivo* and that
39
40 417 these effects were primarily linked with direct pathogen suppression.
41
42
43
44

45 418

48 419 **Discussion**

49
50 420 Here we studied how resident community interactions are linked with invasions
51
52 421 in bacterial plant rhizosphere communities. We found that facilitative two-
53
54 422 species communities were invaded more easily both in the laboratory and
55
56

1
2
3 423 rhizosphere compared to antagonistic resident communities. Furthermore, we
4
5 424 could use the pairwise interactions to predict invasion outcomes in
6
7 425 multispecies communities containing up to 6 resident species. Specifically,
8
9 426 communities characterized by a high proportion of facilitative pairwise
10
11 427 interactions, and high predicted and observed mean intensities of facilitation,
12
13 428 were more susceptible to invasions. Mechanistically, this was linked to direct
14
15 429 inhibition of the invader by antagonistic communities (antibiosis), and to a
16
17 430 lesser degree by resource competition between the members of the resident
18
19 431 community and the invader. Together these findings suggest that outcomes of
20
21 432 relatively simple pairwise interactions can be used to predict invasions in
22
23 433 multispecies microbial communities especially when antagonism and
24
25 434 facilitation are strongly linked with the resistance to invasion.
26
27
28

29 435 Invasion resistance has been thus far mainly considered from the
30
31 436 perspective of resource competition and niche preemption (Case 1990; Tilman
32
33 437 2004; Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015). Our
34
35 438 results suggest that facilitative interactions should also be considered in the
36
37 439 context of invasions. While it is difficult to pinpoint the exact mechanism
38
39 440 between facilitation and invasions, most likely explanation is the loss of
40
41 441 pathogen inhibition along with the increase in the mean intensity of facilitation
42
43 442 (Figure S6). This is in line with a previous finding where the increase in the
44
45 443 antagonistic activity was found to increase the invasion resistance of
46
47 444 *Pseudomonas* resident communities (Hu *et al.* 2016). Another explanation
48
49 445 could be that facilitative resident communities were less efficient at competing
50
51 446 for resources with the invader compared to antagonistic resident communities.
52
53
54
55
56
57
58
59
60

1
2
3 447 However, this likely played a relatively small role as resource niche overlap
4
5 448 had the only significant negative effect on the invader density when measured
6
7 449 *in vitro* and *in vivo* but not on disease incidence (Table 1). It is also possible
8
9 450 that our resource competition indexes measured *in vitro* overestimated the
10
11 451 strength of resource competition or underestimated the size of the niche space
12
13 452 in the rhizosphere leading to weak correlation with invasions. Furthermore,
14
15 453 facilitative interactions could have increased the niche space in the resident
16
17 454 communities in favor of the invader, which could have promoted invasions as a
18
19 455 side effect (Bulleri *et al.* 2016). For example, previous studies have
20
21 456 demonstrated that bacteria can show diet preference between different dietary
22
23 457 glycans, which can prolong the species coexistence in co-cultures (Tuncil *et al.*
24
25 458 2017). Such dietary preference might leave some resources less utilized
26
27 459 opening opportunity for invasions (Tilman 1999). It has also been shown that
28
29 460 the breakdown of polysaccharides can allow coexistence of species that
30
31 461 liberate polysaccharide breakdown products (PBPs), which are consumed by
32
33 462 other species that are unable to grow on the polysaccharides alone (recipients)
34
35 463 (Rakoff-Nahoum *et al.* 2014). Facilitative interactions could thus potentially
36
37 464 favor the invader if it is unable to grow on the primary substrates on its own
38
39 465 (Bruno *et al.* 2003). While it is difficult to validate these hypotheses based on
40
41 466 our data, we found that facilitative communities were more productive in
42
43 467 general and reached higher total cell densities when cultured together
44
45 468 compared to alone (Figure S4). This supports the idea that facilitative resident
46
47 469 species were benefitting from the presence of each other (for example via
48
49 470 cross-feeding), which could also have benefitted the invader by creating
50
51 471 vacant niche space. The carrying capacity of resident communities could thus
52
53
54
55
56
57
58
59
60

1
2
3 472 be an important predictor of biological invasions (Gosso *et al.* 2012).
4

5
6 473 In addition to within-resident community interactions, the interactions
7
8 474 between resident communities and the invader were also good predictors of
9
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,
13
14 477 previous studies have shown that soil bacteria are capable of producing a wide
15
16 478 variety of antimicrobials that often suppress *R. solanacearum* (Hu *et al.* 2016;
17
18 479 Wang *et al.* 2017b). For example, the *B. amyloliquefaciens* T-5 strain used in
19
20 480 this study has been shown to efficiently suppress *R. solanacearum* both in the
21
22 481 lab and plant rhizosphere (Wang *et al.* 2017b) and this strain also had the
23
24 482 greatest negative effect on the pathogen densities and disease incidence in
25
26 483 this study (Figure S1). In addition, the strain *F. johnsoniae* had a negative
27
28 484 effect on pathogen densities both *in vitro* and *in vivo*. Together these results
29
30 485 suggest that pathogen suppression via toxins was likely mediated by the
31
32 486 presence of these species.
33
34
35

36 487 In general, pairwise resident community interactions predicted well the
37
38 488 observed invasion outcomes in multispecies communities. (Figure 3, Table 1).
39
40 489 However, no correlation was found between the observed mean intensity of
41
42 490 facilitation and bacterial wilt disease incidence (Figure 3I). This suggests that
43
44 491 while *in vitro* mechanisms (resource competition and antibiosis) can robustly
45
46 492 predict invasions in more complex *in vivo* environments (Wei *et al.* 2015; Hu *et*
47
48 493 *al.* 2016), they do not account for all aspects of more complex natural
49
50 494 environments. There are many potential explanations for these discrepancies
51
52 495 that should be validated in future studies. First, investigating the role of
53
54
55

1
2
3 496 microbe-mediated plant immunity is important as both pathogenic and
4
5 497 non-pathogenic bacteria can trigger or suppress plant immunity (Chen *et al.*
6
7 498 2017; Rautenbach *et al.* 2017). Furthermore, several bacterial secondary
8
9 499 metabolites involved in pathogen suppression also impact plant immunity: for
10
11 500 example, 2, 4-diacetylphloroglucinol (DAPG) produced by fluorescent
12
13 501 *Pseudomonas* spp. (Bulai & Venturino 2017) or lipopeptide surfactins
14
15 502 produced by *Bacillus subtilis* (Wang *et al.* 2017a) have a such dual-function.
16
17 503 Second, the rhizosphere bacterial communities we used were rather simple,
18
19 504 and hence, predictions based on pairwise species interactions should be
20
21 505 tested in more complex multi-trophic communities in the future. Lastly, our
22
23 506 predictive indexes only estimated the mean net effects and did not distinguish
24
25 507 if both or only one of the species benefitted and vice versa (Foster & Bell 2012).
26
27 508 While this approach seems to be a good predictor of invasion outcomes,
28
29 509 accounting for the directionality of interactions and potential emerging
30
31 510 higher-order interactions (Friman *et al.* 2016; Grilli *et al.* 2017; Levine *et al.*
32
33 511 2017) is likely to improve these predictions.
34
35
36
37

38 512 In conclusion, our results suggest that qualitative information regarding
39
40 513 species growth in pairwise co-cultures can be used to predict the outcomes of
41
42 514 invasions in multispecies communities. Even though our results can be broadly
43
44 515 applied across different biological problems, they could offer direct solutions in
45
46 516 the context of crop protection. Bacterial pathogens impose an ever-increasing
47
48 517 threat for agriculture (Olson & Stenlid 2001; Choudhary & Johri 2009; Nicol *et*
49
50 518 *al.* 2011) and recent evidence suggests that the rhizosphere microbiome plays
51
52 519 an essential role in controlling the onset of diseases (Berendsen *et al.* 2012;
53
54
55

1
2
3 520 Lozupone *et al.* 2012). Understanding the characteristics that make certain
4
5 521 microbiomes more resistant to invasions could potentially allow one to harness
6
7 522 beneficial bacterial communities for crop protection. While recent studies have
8
9 523 shown that microbial diversity alone may be such important characteristic (Wei
10
11 524 *et al.* 2015; Hu *et al.* 2016) we here suggest that highly antagonistic microbial
12
13 525 communities might also be efficient at constraining pathogen invasions.
14
15
16
17 526

19 527 **Acknowledgements**

22 528 This research was financially supported by the National Key Basic Research
23
24 529 Program of China (2015CB150503, Qirong Shen), National Natural Science
25
26 530 Foundation of China (41471213 to Yangchun Xu; 41671248 to Zhong Wei), the
27
28 531 Natural Science Foundation of Jiangsu Province (BK20170085, Zhong Wei),
29
30 532 the 111 project (B12009, Qirong Shen), Young Elite Scientist Sponsorship
31
32 533 Program by CAST (2015QNRC001 to Zhong Wei), and the Qing Lan Project
33
34 534 (funding to Yangchun Xu and Zhong Wei). Ville-Petri Friman is supported by
35
36 535 the Wellcome Trust [reference no. 105624] through the Centre for Chronic
37
38 536 Diseases and Disorders (C2D2) and Royal Society Research Grant
39
40 537 (RSG\R1\180213) at the University of York. Alexandre Jousset is supported by
41
42 538 the Nederlandse Organisatie voor Wetenschappelijk Onderzoek
43
44 539 (ALW.870.15.050) and the Koninklijke Nederlandse Akademie van
45
46 540 Wetenschappen (530-5CDP18). We thank Jack Law for insightful comments
47
48 541 and suggestions for the manuscript.
49
50
51
52
53
54 542

1
2
3 543 **References**
4
5

- 6 544 Allesina, S. & Levine, J.M. (2011). A competitive network theory of species diversity. *Proc Natl Acad Sci*
7
8
9 545 *U S A*, 108, 5638-5642.
- 10
11 546 Altieri, A.H., van Wesenbeeck, B.K., Bertness, M.D. & Silliman, B.R. (2010). Facilitation cascade drives
12
13 547 positive relationship between native biodiversity and invasion success. *Ecology*, 91,
14
15 548 1269-1275.
- 16
17
18 549 Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. & Vivanco, J.M. (2003). Allelopathy and exotic plant
19
20
21 550 invasion: From molecules and genes to species interactions. *Science*, 301, 1377-1380.
- 22
23 551 Becker, J., Eisenhauer, N., Scheu, S. & Jousset, A. (2012). Increasing antagonistic interactions cause
24
25 552 bacterial communities to collapse at high diversity. *Ecol Lett*, 15, 468-474.
- 26
27
28 553 Berendsen, R.L., Pieterse, C.M.J. & Bakker, P.A.H.M. (2012). The rhizosphere microbiome and plant
29
30 554 health. *Trends Plant Sci*, 17, 478-486.
- 31
32
33 555 Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003). Inclusion of facilitation into ecological theory.
34
35 556 *Trends Ecol. Evol.*, 18, 119-125.
- 36
37
38 557 Bulai, I.M. & Venturino, E. (2017). Two mathematical models for dissolved oxygen in a lake-CMMSE-16.
39
40 558 *J Math Chem*, 55, 1481-1504.
- 41
42
43 559 Bulleri, F., Bruno, J.F., Silliman, B.R. & Stachowicz, J.J. (2016). Facilitation and the niche: implications
44
45 560 for coexistence, range shifts and ecosystem functioning. *Funct Ecol*, 30, 70-78.
- 46
47
48 561 Case, T.J. (1990). Invasion Resistance Arises In Strongly Interacting Species-Rich Model Competition
49
50 562 Communities. *P Natl Acad Sci USA*, 87, 9610-9614.
- 51
52
53 563 Catford, J.A., Jansson, R. & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating
54
55

- 1
2
3 564 hypotheses into a single theoretical framework. *Divers Distrib*, 15, 22-40.
- 4
5
6 565 Chen, Q.Q., Lin, L., Tan, Z.Y. & Yan, Y.J. (2017). Coordination mechanisms for scheduling games with
7
8 566 proportional deterioration. *Eur J Oper Res*, 263, 380-389.
- 9
10
11 567 Choudhary, D.K. & Johri, B.N. (2009). Interactions of *Bacillus* spp. and plants – With special reference
12
13 568 to induced systemic resistance (ISR). *Microbiol Res*, 164, 493-513.
- 14
15
16 569 Computing, S. (1991). R Foundation for Statistical Computing, Vienna, Austria. URL [http://www.](http://www.R-project.org)
17
18 570 *R-project.org*.
- 19
20
21 571 Davis, M.A., Grime, J.P. & Thompson, K. (2000). Fluctuating resources in plant communities: a general
22
23 572 theory of invasibility. *J Ecol*, 88, 528-534.
- 24
25
26 573 Foster, K.R. & Bell, T. (2012). Competition, not cooperation, dominates interactions among
27
28 574 culturable microbial species. *Current biology : CB*, 22, 1845-1850.
- 29
30
31 575 Fridley, J.D., Stachowicz, J.J., Naeem, S., Sax, D.F., Seabloom, E.W., Smith, M.D. *et al.* (2007). The
32
33 576 invasion paradox: Reconciling pattern and process in species invasions. *Ecology*, 88, 3-17.
- 34
35
36 577 Friedman, J., Higgins, L.M. & Gore, J. (2017). Community structure follows simple assembly rules in
37
38 578 microbial microcosms. *Nat Ecol Evol*, 1.
- 39
40
41 579 Friman, V.P., Dupont, A., Bass, D., Murrell, D.J. & Bell, T. (2016). Relative importance of evolutionary
42
43 580 dynamics depends on the composition of microbial predator-prey community. *Isme J*, 10,
44
45 581 1352-1362.
- 46
47
48 582 Ghoul, M. & Mitri, S. (2016). The Ecology and Evolution of Microbial Competition. *Trends Microbiol*,
49
50 583 24, 833-845.
- 51
52
53 584 Gioria, M. & Osborne, B.A. (2014). Resource competition in plant invasions: emerging patterns and
54
55 585 research needs. *Front Plant Sci*, 5.

- 1
2
3
4 586 Gosso, A., LA MORGIA, V., MARCHISIO, P., TELVE, O. & VENTURINO, E. (2012). Does a larger carrying
5
6 587 capacity for an exotic species allow environment invasion?- Some considerations on the
7
8 588 competition of red and grey squirrels *Journal of Biological Systems*, 20, 221-234.
9
10
11 589 Grilli, J., Barabas, G., Michalska-Smith, M.J. & Allesina, S. (2017). Higher-order interactions stabilize
12
13 590 dynamics in competitive network models. *Nature*, 548, 210-+.
14
15
16 591 Hierro, J.L. & Callaway, R.M. (2003). Allelopathy and exotic plant invasion. *Plant Soil*, 256, 29-39.
17
18 592 Hu, J., Wei, Z., Friman, V.-P., Gu, S.-h., Wang, X.-f., Eisenhauer, N. *et al.* (2016). Probiotic Diversity
19
20 593 Enhances Rhizosphere Microbiome Function and Plant Disease Suppression. *Mbio*, 7,
21
22 594 e01790-01716.
23
24
25 595 Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X. *et al.* (2017). Bacterial Wilt in China: History,
26
27 596 Current Status, and Future Perspectives. *Front Plant Sci*, 8, 1549.
28
29
30 597 Jousset, A., Schulz, W., Scheu, S. & Eisenhauer, N. (2011). Intraspecific genotypic richness and
31
32 598 relatedness predict the invasibility of microbial communities. *Isme J*, 5, 1108-1114.
33
34
35 599 Kéfi, S., Berlow, E.L., Wieters, E.A., Navarrete, S.A. & Petchey, O.L. (2012). More than a meal...
36
37 600 integrating non-feeding interactions into food webs. *Ecol Lett.*
38
39
40 601 Levine, J.M., Bascompte, J., Adler, P.B. & Allesina, S. (2017). Beyond pairwise mechanisms of species
41
42 602 coexistence in complex communities. *Nature*, 546, 56-64.
43
44
45 603 Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. & Knight, R. (2012). Diversity, stability and
46
47 604 resilience of the human gut microbiota. *Nature*, 489, 220-230.
48
49
50 605 Mallon, C.A., Elsas, J.D. & Salles, J.F. (2015a). Microbial invasions: the process, patterns, and
51
52 606 mechanisms. *Trends Microbiol*, 23, 719-729.
53
54
55 607 Mallon, C.A., Poly, F., Le Roux, X., Marring, I., van Elsas, J.D. & Salles, J.F. (2015b). Resource pulses can

- 1
2
3
4 608 alleviate the biodiversity-invasion relationship in soil microbial communities. *Ecology*, 96,
5
6 609 915-926.
7
8 610 Mallon, C.A., van Elsas, J.D. & Salles, J.F. (2015c). Microbial Invasions: The Process, Patterns, and
9
10 611 Mechanisms. *Trends Microbiol*, 23, 719-729.
11
12 612 Mehrabi, Z., McMillan, V.E., Clark, I.M., Canning, G., Hammond-Kosack, K.E., Preston, G. *et al.* (2016).
13
14 613 *Pseudomonas* spp. diversity is negatively associated with suppression of the wheat take-all
15
16 614 pathogen. *Sci Rep-Uk*, 6.
17
18 615 Mulder, C.P., Uliassi, D.D. & Doak, D.F. (2001). Physical stress and diversity-productivity relationships:
19
20 616 the role of positive interactions. *Proc Natl Acad Sci U S A*, 98, 6704-6708.
21
22 617 Nicol, J.M., Turner, S.J., Coyne, D.L., Nijs, L.d., Hockland, S. & Maafi, Z.T. (2011). Current Nematode
23
24 618 Threats to World Agriculture. In: *Genomics and Molecular Genetics of Plant-Nematode*
25
26 619 *Interactions* (eds. Jones, J, Gheysen, G & Fenoll, C). Springer Netherlands Dordrecht, pp.
27
28 620 21-43.
29
30 621 Olson, A. & Stenlid, J. (2001). Plant pathogens - Mitochondrial control of fungal hybrid virulence.
31
32 622 *Nature*, 411, 438-438.
33
34 623 Rakoff-Nahoum, S., Coyne, M.J. & Comstock, L.E. (2014). An Ecological Network of Polysaccharide
35
36 624 Utilization among Human Intestinal Symbionts. *Current Biology*, 24, 40-49.
37
38 625 Rautenbach, M., Vlok, N.M., Eyeghe-Bickong, H.A., van der Merwe, M.J. & Stander, M.A. (2017). An
39
40 626 Electrospray Ionization Mass Spectrometry Study on the "In Vacuo" Hetero-Oligomers
41
42 627 Formed by the Antimicrobial Peptides, Surfactin and Gramicidin S. *J Am Soc Mass Spectr*, 28,
43
44 628 1623-1637.
45
46 629 Roscher, C., Bessler, H., Oelmann, Y., Engels, C., Wilcke, W. & Schulze, E.D. (2009). Resources,
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 630 recruitment limitation and invader species identity determine pattern of spontaneous
4
5
6 631 invasion in experimental grasslands. *J Ecol*, 97, 32-47.
7
8 632 Shea, K. & Chesson, P. (2002). Community ecology theory as a framework for biological invasions.
9
10 633 *Trends Ecol Evol*, 17, 170-176.
11
12
13 634 Smith-Ramesh, L.M., Moore, A.C. & Schmitz, O.J. (2017). Global synthesis suggests that food web
14
15 635 connectance correlates to invasion resistance. *Global Change Biol*, 23, 465-473.
16
17
18 636 Stachowicz, J.J. (2001). Mutualism, Facilitation, and the Structure of Ecological Communities.
19
20 637 *Bioscience*, 51, 235-246.
21
22
23 638 Stachowicz, J.J. & Byrnes, J.E. (2006). Species diversity, invasion success, and ecosystem functioning:
24
25 639 disentangling the influence of resource competition, facilitation, and extrinsic factors. *Mar*
26
27 640 *Ecol Prog Ser*, 311, 251-262.
28
29
30 641 Stubbendieck, R.M., Vargas-Bautista, C. & Straight, P.D. (2016). Bacterial Communities: Interactions to
31
32 642 Scale. *Front Microbiol*, 7.
33
34
35 643 Tan, S.Y., Gu, Y., Yang, C.L., Dong, Y., Mei, X.L., Shen, Q.R. *et al.* (2016). *Bacillus amyloliquefaciens* T-5
36
37 644 may prevent *Ralstonia solanacearum* infection through competitive exclusion. *Biol Fert Soils*,
38
39 645 52, 341-351.
40
41
42 646 Team, R.C. (2013). R: A language and environment for statistical computing.
43
44
45 647 Theoharides, K.A. & Dukes, J.S. (2007). Plant invasion across space and time: factors affecting
46
47 648 nonindigenous species success during four stages of invasion. *New Phytologist*, 176, 256-273.
48
49
50 649 Thorpe, A.S., Thelen, G.C., Diaconu, A. & Callaway, R.M. (2009). Root exudate is allelopathic in invaded
51
52 650 community but not in native community: field evidence for the novel weapons hypothesis. *J*
53
54 651 *Ecol*, 97, 641-645.

- 1
2
3
4 652 Tilman, D. (1999). The ecological consequences of changes in biodiversity: A search for general
5
6 653 principles. *Ecology*, 80, 1455-1474.
7
8 654 Tilman, D. (2004). Niche tradeoffs, neutrality, and community structure: A stochastic theory of
9
10 655 resource competition, invasion, and community assembly. *P Natl Acad Sci USA*, 101,
11
12 656 10854-10861.
13
14
15 657 Traveset, A. & Richardson, D.M. (2014). Mutualistic Interactions and Biological Invasions. *Annu Rev*
16
17 658 *Ecol Evol S*, 45, 89-+.
18
19
20 659 Tuncil, Y.E., Xiao, Y., Porter, N.T., Reuhs, B.L., Martens, E.C. & Hamaker, B.R. (2017). Reciprocal
21
22 660 Prioritization to Dietary Glycans by Gut Bacteria in a Competitive Environment Promotes
23
24 661 Stable Coexistence. *Mbio*, 8.
25
26
27
28 662 van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V. & Salles, J.F. (2012). Microbial
29
30 663 diversity determines the invasion of soil by a bacterial pathogen. *Proc Natl Acad Sci U S A*,
31
32 664 109, 1159-1164.
33
34
35 665 Wang, L., Wu, Y.P. & Xu, Q. (2017a). Instability of spiky steady states for S-K-T biological competing
36
37 666 model with cross-diffusion. *Nonlinear Anal-Theor*, 159, 424-457.
38
39
40 667 Wang, X., Wei, Z., Li, M., Wang, X., Shan, A., Mei, X. *et al.* (2017b). Parasites and competitors suppress
41
42 668 bacterial pathogen synergistically due to evolutionary trade-offs. *Evolution*, 71, 733-746.
43
44
45 669 Wei, Z., Huang, J., Tan, S., Mei, X., Shen, Q. & Xu, Y. (2013). The congeneric strain *Ralstonia pickettii*
46
47 670 QL-A6 of *Ralstonia solanacearum* as an effective biocontrol agent for bacterial wilt of tomato.
48
49 671 *Biological Control*, 65, 278-285.
50
51
52 672 Wei, Z., Yang, T., Friman, V.P., Xu, Y., Shen, Q. & Jousset, A. (2015). Trophic network architecture of
53
54 673 root-associated bacterial communities determines pathogen invasion and plant health.

674 *Nature communications*, 6, 8413.

675 Williamson, M. & Fitter, A. (1996). The varying success of invaders. *Ecology*, 77, 1661-1666.

676 Yang, T., Wei, Z., Friman, V.P., Xu, Y., Shen, Q., Kowalchuk, G.A. *et al.* (2017). Resource availability
677 modulates biodiversity-invasion relationships by altering competitive interactions. *Environ*
678 *Microbiol*, 19, 2984-2991.

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***

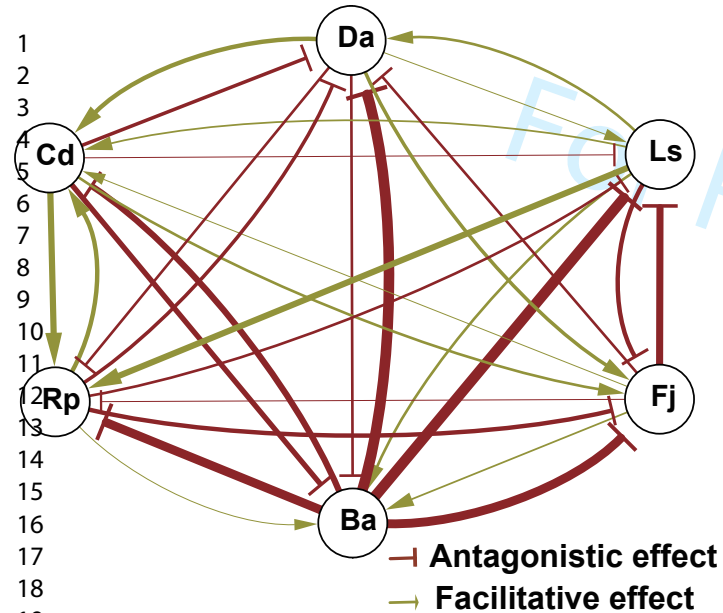
691 ***vitro* and *in vivo*.** (A) The *R. solanacearum* invader abundance in antagonistic and facilitative
692 two-species resident communities measured *in vitro*. (B) The relationship between invader
693 abundance and the mean intensity of facilitation in resident communities measured *in vitro*. (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

1
2
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).
5
6 702 In panels, B, D and F, values below and above zero denote for antagonistic and antagonistic
7
8 703 pairwise resident communities, respectively. Two and three stars denote for statistical
9
10 704 significance at $p < 0.01$ and $p < 0.001$ significance levels, respectively. All the bars denote for \pm
11
12 705 1 s.e.m.

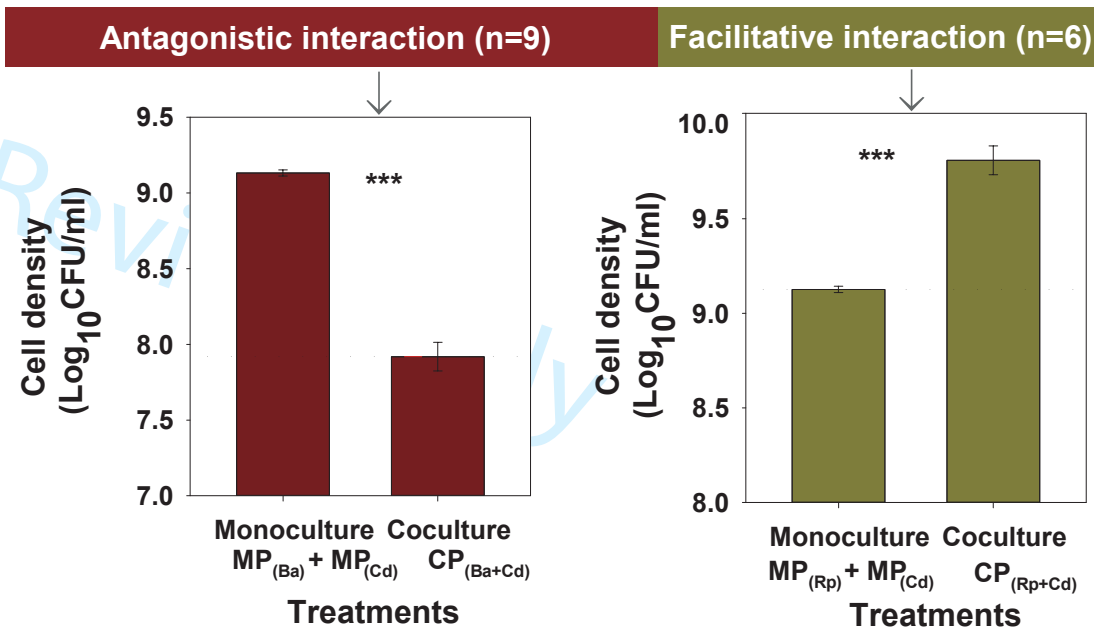
13
14 706

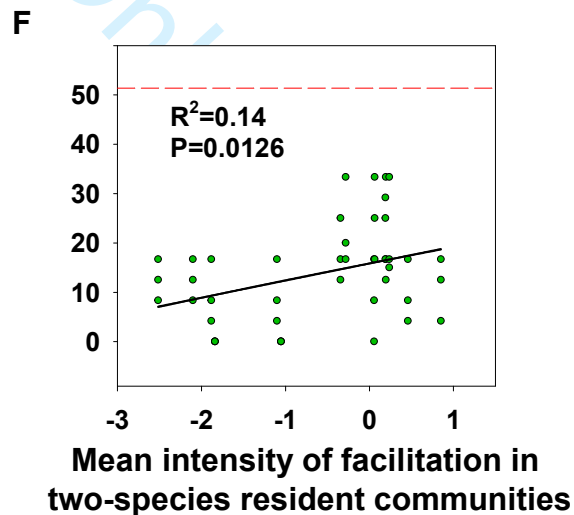
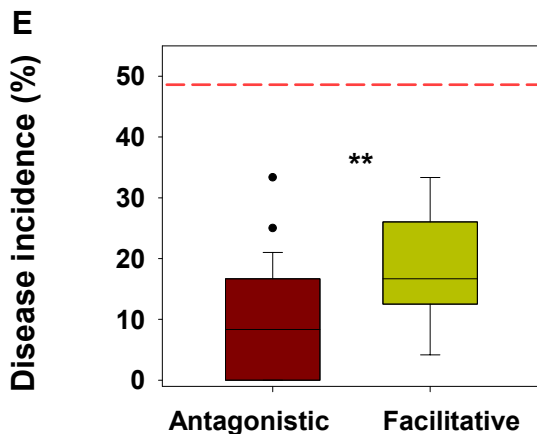
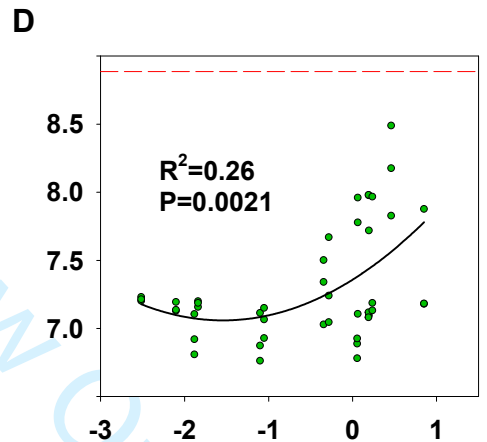
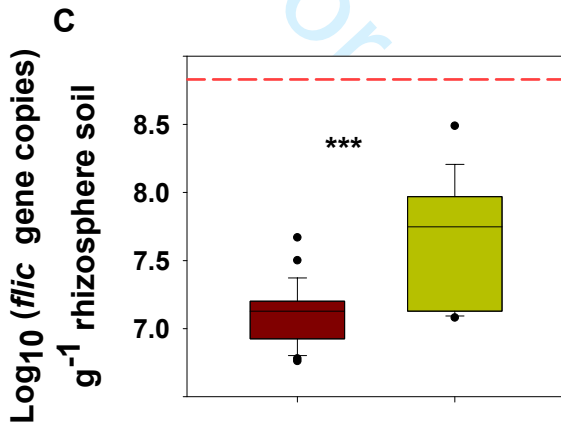
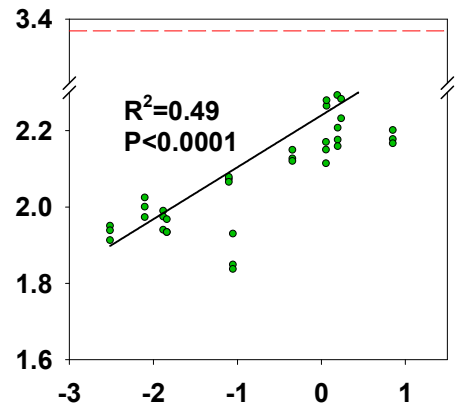
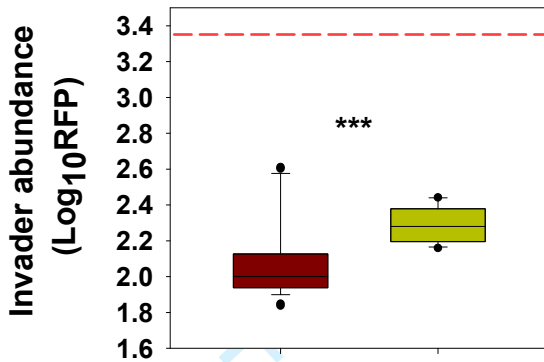
15
16 707 **Figure 3. The relationship between invader abundance and disease incidence with**
17
18 708 **predicted and observed mean intensities of facilitation within multispecies**
19
20 709 **communities.** (A-B) The relationship between invader abundance and the proportion of
21
22 710 facilitative interactions in the resident communities measured *in vitro* and *in vivo*, respectively.
23
24 711 (C) The relationship between bacterial wilt disease incidence (%) and the proportion of
25
26 712 facilitative interactions in the resident communities. (D-E) The relationship between invader
27
28 713 abundance and the predicted mean intensity of facilitation in the resident communities
29
30 714 measured *in vitro* and *in vivo*, respectively. (F) The relationship between bacterial wilt disease
31
32 715 incidence (%) and the predicted mean intensity of facilitation in the resident communities. (G-H)
33
34 716 The relationship between invader abundance and the observed mean intensity of facilitation in
35
36 717 the resident communities measured *in vitro* and *in vivo*, respectively. (I) The relationship
37
38 718 between bacterial wilt disease incidence (%) and the observed mean intensity of facilitation in
39
40 719 the resident communities. In all panels, red dashed lines show the baseline of invader
41
42 720 densities in control treatments (invader-only). In panels D-I, values below and above zero
43
44 721 denote for competitive and antagonistic resident communities, respectively.

A



B





Interaction type in two-species
resident communities

Mean intensity of facilitation in
two-species resident communities

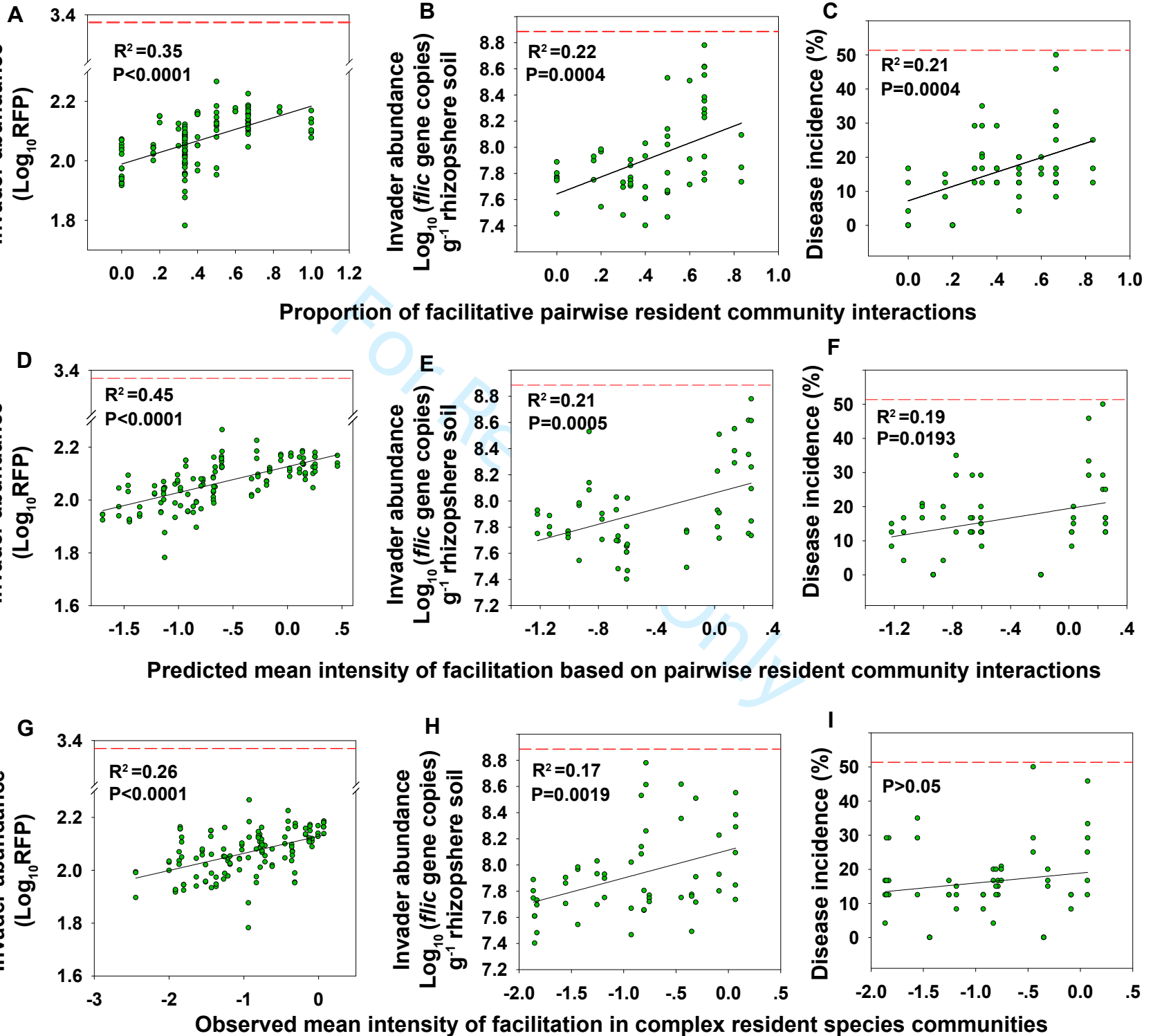


Table 1 Two different general linear mixed models (GLM) comparing the interactions within resident communities (a), and interactions between community and invader (b) on invader abundance *in vitro* and *in vivo* and disease incidence measured *in vivo*.

	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>
(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

1
2
3
4 information. The up and downwards arrows denote for positive and negative effects on response variables, respectively.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

For Review Only