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Facilitation promotes invasions in plant-associated microbial communities

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Facilitation promotes invasions in plant-associated microbial communities

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Abstract

While several studies have established a positive correlation between community diversity and invasion resistance, it is less clear how species interactions within resident communities shape this process. Here we experimentally tested how antagonistic and facilitative pairwise interactions within resident model microbial communities predict invasion by the plant-pathogenic bacterium *Ralstonia solanacearum.* We found that facilitative resident community interactions promoted and antagonistic interactions suppressed invasions both in the lab and in the tomato plant rhizosphere. Crucially, pairwise interactions could reliably explain observed invasions outcomes also in multispecies communities, and mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between the members of the resident community and the invader. Together our findings suggest that the type and strength of pairwise interactions can reliably predict

the outcome of invasions in more complex multispecies communities.

Introduction

The characteristics of both resident communities and the invading species are important for determining the outcomes of biological invasions (Williamson & Fitter 1996; Catford *et al.* 2009). From the resident community perspective, species diversity may be considered as a shield to invasions and this effect is often attributed to competition for existing resources (Fridley *et al.* 2007; Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015) where highly diverse communities are thought to efficiently use all the available resource niches leaving no free space for invaders (Case 1990; Tilman 2004). In reality, diversity-invasion resistance relationships are more varied ranging from having neutral to even negative effects (Shea & Chesson 2002; Mallon *et al.* 2015a; Mehrabi *et al.* 2016) and are sensitive to environmental conditions (Davis *et al.* 2000; Roscher *et al.* 2009; Jousset *et al.* 2011; Mallon *et al.* 2015b). Furthermore, it has been shown that trophic network architecture (Wei *et al.* 2015), species identity effects (Yang *et al.* 2017) and food web connectance (Smith-Ramesh *et al.* 2017) are important predictors of invasions and are often linked with community diversity. For example, how species interact might be more important than the number of interacting species within the community (Wei *et al.* 2015), while invasion resistance could be sometimes mediated by certain keystone taxa (Yang *et al.* 2017). However, the type and strength of resident species interactions have often been overlooked in the context of diversity-invasion resistance studies.

Resident species communities form complex ecological webs where multiple species may interact positively or negatively with each other (Kéfi *et al.* 2012). Positive interactions between species at the same trophic level can result from facilitation or metabolic cross-feeding, where species benefit from the presence of each other (Mulder *et al.* 2001). Negative interactions may result from resource competition (Wei *et al.* 2015) or direct interference competition, where species directly suppress each other via antagonism (Bais *et al.* 2003; Hierro & Callaway 2003; Thorpe *et al.* 2009; Hu *et al.* 2016). These interactions may affect the outcomes of invasions in various ways. First, facilitation and competition are likely to affect the resource availability, and hence the availability of free resource niche space, and the likelihood of invasions (Shea & Chesson 2002; Tilman 2004; Stachowicz & Byrnes 2006; Gioria & Osborne 2014; Mallon *et al.* 2015c). It is predicted that highly competitive resident communities are less prone to invasions if they can efficiently utilize and consume resources that would otherwise be available for invaders (Tilman 2004; Jousset *et al.* 2011; Mallon *et al.* 2015c). This effect is expected to be especially strong in the resident communities that show a high degree of complementarity and hence compete less strongly with each other compared with the invader. In contrast, facilitative interactions between resident community members could potentially increase the number of resource niches via production of secondary metabolites or public goods that can also be utilized by the invader (Stachowicz 2001; Mallon *et al.* 2015b; Bulleri *et al.* 2016). Furthermore, competing species can inhibit each other directly by producing toxic metabolites, such as antibiotics. Depending on the spectrum of their activity, antibiotic compounds could have negative effects on both resident community species and the invader (Bais *et al.* 2003; Hierro & Callaway 2003; Thorpe *et al.* 2009; Becker *et al.* 2012; Hu *et al.* 2016; Wang *et al.* 2017b). If the invader is particularly sensitive to toxins produced by the resident community, it is expected that antibiotics-mediated interference competition will constrain invasions. In contrast, if produced toxins have a disproportionally larger negative effect on the members of the resident community, such interference competition is expected to promote invasions (Thorpe *et al.* 2009; Stubbendieck *et al.* 2016). Resident community species interactions could further affect certain community-level properties such as ecological stability (Allesina & Levine 2011), which could have indirect effects on invasions (Ghoul & Mitri 2016).

In the present study, we explored to what extent the type (facilitative vs antagonistic) and strength of two-species resident community species interactions can predict invasions in complex multispecies bacterial communities. Experiments conducted within one trophic level suggest that pairwise bacterial competitions can predict three-species bacterial competitions with as high as 90% accuracy (Friedman *et al.* 2017). While predicting competitions in species-rich communities might require additional information about potentially emerging higher-order interactions (Friman *et al.* 2016; Grilli *et al.* 2017; Levine *et al.* 2017), these findings suggest that qualitative information regarding species growth in pairwise co-cultures can be used to predict the competitive outcomes of up to 8-species communities

(Friedman *et al.* 2017). Here we extend this approach beyond competition to concurrently explore the role of antagonistic and facilitative resident community interactions for biological invasions (Bruno *et al.* 2003; Altieri *et al.* 2010; Traveset & Richardson 2014). Our study system consisted of six non-pathogenic bacterial species (resident community), which were isolated from the tomato plant rhizosphere, and the invader, the plant-pathogenic *Ralstonia solanacearum* bacterium. Specifically, we first characterized antagonistic and facilitative pairwise interactions within model resident bacterial communities and then directly tested how these interactions predict invasions in more complex multispecies communities both *in vitro* and *in vivo* in the tomato rhizosphere. We found that facilitative and antagonistic pairwise interactions reliably predicted invasions: facilitative resident communities were more prone to invasions, while antagonistic resident communities were invaded much less often. Mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between the members of the resident community and the invader. Our results suggest that antagonism is an important determinant of community invasion resistance (Case 1990; Tilman 2004), while facilitation might promote invasions by alleviating antagonistic interactions or by releasing vacant niche space for the invader.

Materials and methods

Bacterial strains and the assembly of resident communities

We used *Ralstonia solanacearum* strain QL-Rs1115 tagged with the pYC12-mCherry plasmid (Tan *et al.* 2016) as an invading pathogen in our experiments. *Ralstonia solanacearum* is a causal driver of bacterial wilt and capable of infecting various economically important crop species (Jiang *et al.* 2017). We set up model resident communities using six bacterial strains isolated from the tomato rhizosphere at the same location as the pathogen (Qilin [118° 57′ E, 32° 03′ N], Nanjing, China). Resident community species listed in Table S1 (*Flavobacterium johnsoniae* WR4, *Chryseobacterium daecheongense* WR21, *Delftia acidovorans* WR42, *Bacillus amyloliquefaciens* T-5, *Lysinibacillus sphaericus* HR92 and *Ralstonia pickettii* QL-A6) have previously been shown to provide protection for associated host plants by inhibiting *R. solanacearum* pathogen growth via resource competition or direct toxin production (Figure S1). The resident community composition (Table S2) was manipulated using biodiversity-invasion resistance framework where we modulated both resident community diversity (species richness) and composition and then directly tested how this affected community invasion resistance (Wei *et al.* 2015). Invasion outcomes were then explained by interactions 1) within resident communities and 2) between resident community and the invader.

Determining pairwise interactions between resident community species

To quantify the type (facilitative, neutral or antagonistic), strength and directionality of each pairwise interaction between resident community species, we compared the growth of each species alone and in the presence of each of the other species in two-species co-cultures (Foster & Bell 2012). All mono-cultures were inoculated with a starting density of 10^5 cells per ml and the co-cultures were inoculated with half of this starting cell density of each species. Resident species were grown for 48h in liquid NA medium (glucose 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) in 48-well microtiter plates (ending volume of 700 ml per well) at 30°C with shaking (170 rpm). Bacterial growth was measured as colony number units (CFU) per ml by serial dilution and plating on NA agar plates after 48h growth. All strains formed distinct colonies on agar plates and could be identified based on colony morphology (Figure S2).

The type of pairwise interaction between two species (here *i* and *j*) was determined by comparing the sum of endpoint of monoculture productivity (population densities) of *i* (MP_i) and monoculture productivity of *j* (MP_j) with the ending productivity of the two-species co-culture (CP_{i+j}). As suggested previously, the density of a species mixture is expected to be exactly the sum of their growth in the monocultures if species do not interact (Foster & Bell 2012). Thus, we expected that the interaction between *i* and *j* would be facilitative if $CP_{i+j} > MP_i + MP_j$, antagonistic if $CP_{i+j} < MP_i + MP_j$ and neutral if $CP_{i+j} = MP_i + MP_j$.

In order to characterize directionality of pairwise interactions, we compared the ending productivity of each species (CP_i and CP_j) in two-species co-cultures with their ending productivities in monocultures. We then determined the directionality of interaction facilitative if species *j* had a positive effect on *i* ($\log_{10}(CP_i / MP_i) > 0$), antagonistic if $\log_{10}(CP_i / MP_i) < 0$ and neutral if $\log_{10}(CP_i / MP_i) = 0$. We also calculated the mean intensity of facilitation (MIF) of co-cultures as an average of $\log_{10}$-transformed pairwise interactions using the following formula: $MIF_{ij} = \frac{1}{2}[\log(CP_i / MP_i) + \log(CP_j / MP_j)]$. The two-species community was defined as facilitative when MIF > 0, antagonistic when MIF < 0 and neutral if MIF = 0.

Predicting resident species interactions in multispecies communities

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

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

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

Validating resident species interactions in multispecies communities

To verify resident species interactions in multispecies bacterial communities, we used qPCR to determine the ending densities of each resident species in monocultures and in all possible co-cultures (3, 4, 5 and 6 resident species communities). All communities were assembled in triplicate in liquid NA medium with a starting density of 10^5 cells per ml in monocultures and 33%, 25%, 20% and 16.7% of monoculture densities in 3, 4, 5 and 6 resident species communities, respectively. After 48h in 48-well microtiter plates at 30°C with shaking (170 rpm), bacterial DNA was extracted using e.Z.N.A. Bacterial DNA kit (OMEGA bio-tek) following manufacturer's protocol and extracted DNA was stored at -80°C. Species-specific primers were designed for each resident community member (Table S3, Figure S3) and qPCR analyses were carried out with an Applied Biosystems Step One Plus real-time PCR system using SYBR green I fluorescent dye detection in 20 - μl volumes with 10 μl of SYBR Premix Ex Taq (TaKaRa Bio Inc., Japan), 2 μl of template, 0.4 μl Dye I, 0.8 μl of both forward and reverse primers (10 mM each) and 6 μl sterile water. The PCR was performed by initially denaturizing at 95°C for 30 s, cycling 40 times with a 5-s denaturizing step at 95°C, using a 34-s elongation/extension step at 60°C, and ending with melt curve analysis at 95°C for 15 s, at 60°C for 1 min, and at 95°C for 15 s. Each resident species community sample was replicated three times.

The observed mean intensity of facilitation (OIF) was calculated using the observed species proportions in the communities based on qPCR data. Similar to PIF, we first determined to what extent the growth of each species was affected by the presence of other species in a given community (growth in the community vs. growth alone). OIF was then calculated according to the following formula: $OIF = \frac{1}{n}\sum \log(CP_i / MP_i)$. Communities were defined as facilitative when OIF > 0, antagonistic when OIF < 0 and neutral if OIF = 0. OIF was calculated only based on *in vitro* data and in the case of MIF, PIF and OIF, antagonism included the effects arising from both resource competition and direct inhibition via toxins.

Measuring resource competition and direct antagonism between the invader and resident community species

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

M5 spectrophotometer (Molecular Devices, Sunnyvale, CA). We then measured the growth of the invader and all six resident community species individually on 48 different single-carbon resources (see Table S4) representative of tomato root exudates (Hu *et al.* (2016)). When the invader and resident community species were able to grow on the same resource (OD_{600}>0.05), their niches were considered to overlap regarding that given resource. In contrast, when only one strain was able to grow on a specific resource, the niches were considered not to overlap (Wei *et al.* 2015). This resource competition index estimated the 'apparent' resource competition assuming that interacting species would be competing for the same resources even when presented with multiple different resources.

Direct antagonism between the invader and resident community species was measured using supernatant assays (Hu *et al.* 2016). Briefly, after 24h of growth in NA media, all bacterial monocultures were filtered to remove living cells (0.22 μm filter) after 20 μl of sterile supernatant from each resident species culture was mixed with 180 μl of an overnight-grown *R. solanacearum* culture (OD_{600} = 0.05, five-fold dilution in liquid NA). The control treatments were inoculated with 20 μl of sterile-filtered NA media instead of bacterial supernatant. All bacterial cultures were grown for 24h at 30°C with shaking (170 rpm) before measuring pathogen inhibition as optical density (OD 600 nm). Antagonism was defined as the percentage of reduction in pathogen growth by the supernatant compared to the control treatment for all possible invader-resident species two-species combinations.

Measuring invasion success in multispecies communities

a) Invasion success measured *in vitro*

All possible multispecies resident communities were assembled in triplicate in liquid NA medium with a starting density of 10^5 cells per ml (100%, 50%, 33%, 25%, 20% and 16.7% of monoculture densities in 1, 2, 3, 4, 5 and 6 resident species communities, respectively). Communities were then subsequently exposed to invasion by mCherry-tagged *R. solanacearum* (10^4 cells per ml) in 96-well plates at 30°C with shaking (170 rpm). After 48h, total bacterial densities were measured as optical density (OD 600 nm) and invasion success measured as the relative invader density to total bacterial densities using red mCherry protein fluorescence intensity (RFP; excitation: 587 nm, emission: 610 nm) with SpectraMax M5 spectrophotometer.

b) Invasion success measured *in vivo*

We used a 50-day-long greenhouse experiment with tomato plants to measure invasion success *in vivo*. The soil was collected from a rice field in Wuxi (Jiangsu Province, China), sieved at 5 mm and homogenized and sterilized with gamma radiation. Surface-sterilized tomato seeds (*Lycopersicon esculentum*, cultivar “*Micro-Tom*”) were germinated on water-agar plates for 3 days before sowing into seedling plates containing cobalt-60-sterilized seedling substrate (Huainong, Huaian Soil and Fertilizer Institute, Huaian, China). *Ralstonia solanacearum* invasion was tested in all possible two-species resident communities, and due to practical reasons, in 18 multispecies resident communities that varied in their predicted mean intensities of facilitation (Table S5).

Three replicates were used for each resident community, and one replicate consisted of a seedling plate that contained six germinated tomato plants (at the three-leaf stage of growth when grown on 700 g sterilized soil). Similar replication was also used for positive (only the invader) and negative (no bacteria) controls. After 3 days of growth on seedling plates, plants were inoculated with assembled resident communities using root drenching method at a final concentration of 10^8 CFU of bacteria g^{-1} soil (Wei *et al.* 2013). Seven days after inoculation of resident communities, *R. solanacearum* was introduced to the roots of all plants at a final concentration of 10^7 CFU of bacteria g^{-1} soil. Tomato plants were then grown for 40 days in a greenhouse (with natural temperature variation ranging from 25°C to 35°C) and watered regularly with sterile water. Seedling plates were rearranged randomly every two days and disease progression monitored at every seven days. Forty days after inoculation of *R. solanacearum*, rhizosphere soil was collected from one plant per replicate seedling tray and the abundance of the invader determined with quantitative PCR as the abundance of *R. solanacearum*-specific *fliC* gene copy numbers (Hu *et al.* 2016).

Statistical analyses

To meet assumptions of normality and homogeneity of variance, invader densities measured *in vitro* and *in vivo* were log10-transformed. We first assessed the independent effects of the proportion of facilitative interactions and the mean intensity of facilitation based on pairwise resident community interaction on invasions (pathogen density and disease incidence). The type of interaction between resident community species pairs was included into models as a categorical variable (1= facilitation; 0= antagonism). In the case of multispecies communities, invasions were explained by three quantitative indexes, the proportion of facilitative interactions within a community, the predicted mean intensity of facilitation (PIF) and the and observed mean intensity of facilitation (OIF). All indexes were fitted as continuous variables and one separate model was used for each index that explained invader densities *in vitro* and *in vivo* and bacterial wilt disease incidence. Additional linear mixed models were used to test invasions as a function of a) niche overlap between resident community and the pathogen (niche preemption by the resident community), b) mean pathogen inhibition by the resident community and c) resident community species identity effects. All analyses were conducted with SPSS (V. 22) and R (Computing 1991; Team 2013).

Results

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

All species had both negative and positive effects on each other while the magnitude and directionality of these effects varied depending on specific species (Figure 1A). In particular, *B. amyloliquefaciens* was very antagonistic to the other resident community species. (Figure 1A). Furthermore, we found that 9 of the communities showed antagonistic, and 6 facilitative pairwise interactions with each other (Figure 1B, Table S6). On average, facilitative two-species communities reached higher population densities (R^2=0.79, P<0.001, Figure S4), while antagonistic two-species communities were more inhibitory towards each other (R^2=0.32, P=0.029, Figure S5A). No relationship was found between resident species' resource niche overlap and observed mean intensity of facilitation (Figure S5B), which suggests that facilitation did not arise due to niche complementarity. Together these results suggest that the strength of direct inhibition was more important in explaining the type of pairwise interactions between resident community members compared to resource competition.

To link the type of pairwise interaction with the likelihood of invasions, we compared *R. solanacearum* invasion success in facilitative and antagonistic two-species resident communities. Compared to positive controls (*R. solanacearum*-only: red dashed line in Figure 2A-F), pathogen densities were significantly lower in the presence of resident species both *in vitro* and *in vivo*. The intensity of pathogen suppression could be predicted by the type of pairwise interactions between the resident species: pathogen density was significantly higher in facilitative compared to antagonistic communities *in vitro* ($F_{1,43}$=16.02, P<0.001, Figure 2A; R^2=0.49, P<0.0001, Figure 2B) and *in vivo* ($F_{1,43}$=24.40, P<0.001, Figure 2C; R^2=0.26, P=0.0021, Figure 2D). In line with these results, the bacterial wilt disease incidence was also higher in facilitative compared to antagonistic resident communities ($F_{1,43}$=9.03, P=0.004, Figure 2E; R^2=0.14, P=0.013, Figure 2F). Mechanistically, this could be explained by loss of pathogen inhibition as suggested by a negative correlation between the mean intensity of facilitation and direct invader suppression (R^2=0.45, P<0.0001, Figure S6). Together these results suggest that antagonistic two-species resident communities were more inhibitory not only towards themselves but also against the invader.

(b) Predicting and validating invasions in multispecies communities based on pairwise interactions

Interactions within the resident communities could well explain the invader abundance *in vitro* (R^2: 0.45, P<0.0001) and *in vivo* (R^2: 0.28, P<0.0001), and bacterial wilt disease incidence (R^2: 0.18, P=0.0002) *in vivo* (Table 1). The proportion of facilitative interactions were well explained by the increase in invader density in all tested resident communities *in vitro* (R^2=0.35, P<0.0001, Figure 3A). Similarly, both the density of the invader in the tomato rhizosphere (R^2=0.22, P=0.0004, Figure 3B) and bacterial wilt disease incidence (R^2=0.21, P=0.0004, Figure 3C) increased significantly with increasing proportion of facilitative interactions within the resident communities. The predicted mean intensity of facilitation explained well the increase in invader density *in vitro* (R^2=0.45, P<0.0001, Figure 3D) and *in vivo* (R^2=0.21, P=0.0005, Figure 3E) and correlated positively with bacterial wilt disease incidence (R^2=0.19, P=0.0193, Figure 3F). The predicted and observed mean intensities of facilitation correlated positively with each other (R^2=0.44, P<0.0001, Figure S7), demonstrating that pairwise interactions can be used to predict interactions in multispecies communities. As expected, invader densities also increased with increasing observed mean intensity of facilitation both *in vitro*

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

The low invasion success observed in antagonistic resident communities could be attributed to high levels of direct inhibition of the invader and/or high resource niche overlap between the invader and resident community members. We found that both direct pathogen inhibition and high resource niche overlap reduced invader densities *in vitro* and *in vivo*, while only direct pathogen inhibition significantly reduced the disease incidence (Table 1). Direct pathogen suppression correlated negatively with both predicted and observed mean intensities of facilitation suggesting that antagonistic multispecies communities were more inhibitory to the invader (Figure S8). The species *B. amyloliquefaciens* and *F. johnsoniae* had strong negative effects on pathogen densities *in vitro* and *in vivo* (Table S7). However, only *B. amyloliquefaciens* had a significant negative effect on disease incidence, while species *C. daecheongense* had a slightly positive effect on disease incidence (Table S7). Together these results suggest that pairwise resident community interactions can predict invasions in multispecies communities *in vitro* and *in vivo* and that these effects were primarily linked with direct pathogen suppression.

Discussion

Here we studied how resident community interactions are linked with invasions in bacterial plant rhizosphere communities. We found that facilitative two-species communities were invaded more easily both in the laboratory and rhizosphere compared to antagonistic resident communities. Furthermore, we could use the pairwise interactions to predict invasion outcomes in multispecies communities containing up to 6 resident species. Specifically, communities characterized by a high proportion of facilitative pairwise interactions, and high predicted and observed mean intensities of facilitation, were more susceptible to invasions. Mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between the members of the resident community and the invader. Together these findings suggest that outcomes of relatively simple pairwise interactions can be used to predict invasions in multispecies microbial communities especially when antagonism and facilitation are strongly linked with the resistance to invasion.

Invasion resistance has been thus far mainly considered from the perspective of resource competition and niche preemption (Case 1990; Tilman 2004; Theoharides & Dukes 2007; van Elsas *et al.* 2012; Wei *et al.* 2015). Our results suggest that facilitative interactions should also be considered in the context of invasions. While it is difficult to pinpoint the exact mechanism between facilitation and invasions, most likely explanation is the loss of pathogen inhibition along with the increase in the mean intensity of facilitation (Figure S6). This is in line with a previous finding where the increase in the antagonistic activity was found to increase the invasion resistance of *Pseudomonas* resident communities (Hu *et al.* 2016). Another explanation could be that facilitative resident communities were less efficient at competing for resources with the invader compared to antagonistic resident communities.

However, this likely played a relatively small role as resource niche overlap had the only significant negative effect on the invader density when measured *in vitro* and *in vivo* but not on disease incidence (Table 1). It is also possible that our resource competition indexes measured *in vitro* overestimated the strength of resource competition or underestimated the size of the niche space in the rhizosphere leading to weak correlation with invasions. Furthermore, facilitative interactions could have increased the niche space in the resident communities in favor of the invader, which could have promoted invasions as a side effect (Bulleri *et al.* 2016). For example, previous studies have demonstrated that bacteria can show diet preference between different dietary glycans, which can prolong the species coexistence in co-cultures (Tuncil *et al.* 2017). Such dietary preference might leave some resources less utilized opening opportunity for invasions (Tilman 1999). It has also been shown that the breakdown of polysaccharides can allow coexistence of species that liberate polysaccharide breakdown products (PBPs), which are consumed by other species that are unable to grow on the polysaccharides alone (recipients) (Rakoff-Nahoum *et al.* 2014). Facilitative interactions could thus potentially favor the invader if it is unable to grow on the primary substrates on its own (Bruno *et al.* 2003). While it is difficult to validate these hypotheses based on our data, we found that facilitative communities were more productive in general and reached higher total cell densities when cultured together compared to alone (Figure S4). This supports the idea that facilitative resident species were benefitting from the presence of each other (for example via cross-feeding), which could also have benefitted the invader by creating vacant niche space. The carrying capacity of resident communities could thus be an important predictor of biological invasions (Gosso *et al.* 2012).

In addition to within-resident community interactions, the interactions between resident communities and the invader were also good predictors of invasions, albeit to a lesser extent (Figure 3 and Table 1). While it remains unclear what exact compounds were produced by the resident communities, previous studies have shown that soil bacteria are capable of producing a wide variety of antimicrobials that often suppress *R. solanacearum* (Hu *et al.* 2016; Wang *et al.* 2017b). For example, the *B. amyloliquefaciens* T-5 strain used in this study has been shown to efficiently suppress *R. solanacearum* both in the lab and plant rhizosphere (Wang *et al.* 2017b) and this strain also had the greatest negative effect on the pathogen densities and disease incidence in this study (Figure S1). In addition, the strain *F. johnsoniae* had a negative effect on pathogen densities both *in vitro* and *in vivo*. Together these results suggest that pathogen suppression via toxins was likely mediated by the presence of these species.

In general, pairwise resident community interactions predicted well the observed invasion outcomes in multispecies communities. (Figure 3, Table 1). However, no correlation was found between the observed mean intensity of facilitation and bacterial wilt disease incidence (Figure 3I). This suggests that while *in vitro* mechanisms (resource competition and antibiosis) can robustly predict invasions in more complex *in vivo* environments (Wei *et al.* 2015; Hu *et al.* 2016), they do not account for all aspects of more complex natural environments. There are many potential explanations for these discrepancies 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;

Lozupone *et al.* 2012). Understanding the characteristics that make certain microbiomes more resistant to invasions could potentially allow one to harness beneficial bacterial communities for crop protection. While recent studies have shown that microbial diversity alone may be such important characteristic (Wei *et al.* 2015; Hu *et al.* 2016) we here suggest that highly antagonistic microbial communities might also be efficient at constraining pathogen invasions.

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References

Allesina, S. & Levine, J.M. (2011). A competitive network theory of species diversity. *Proc Natl Acad Sci U S A*, 108, 5638-5642.

Altieri, A.H., van Wesenbeeck, B.K., Bertness, M.D. & Silliman, B.R. (2010). Facilitation cascade drives positive relationship between native biodiversity and invasion success. *Ecology*, 91, 1269-1275.

Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. & Vivanco, J.M. (2003). Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science*, 301, 1377-1380.

Becker, J., Eisenhauer, N., Scheu, S. & Jousset, A. (2012). Increasing antagonistic interactions cause bacterial communities to collapse at high diversity. *Ecol Lett*, 15, 468-474.

Berendsen, R.L., Pieterse, C.M.J. & Bakker, P.A.H.M. (2012). The rhizosphere microbiome and plant health. *Trends Plant Sci*, 17, 478-486.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.*, 18, 119-125.

Bulai, I.M. & Venturino, E. (2017). Two mathematical models for dissolved oxygen in a lake-CMMSE-16. *J Math Chem*, 55, 1481-1504.

Bulleri, F., Bruno, J.F., Silliman, B.R. & Stachowicz, J.J. (2016). Facilitation and the niche: implications for coexistence, range shifts and ecosystem functioning. *Funct Ecol*, 30, 70-78.

Case, T.J. (1990). Invasion Resistance Arises In Strongly Interacting Species-Rich Model Competition Communities. *P Natl Acad Sci USA*, 87, 9610-9614.

Catford, J.A., Jansson, R. & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Divers Distrib*, 15, 22-40.

Chen, Q.Q., Lin, L., Tan, Z.Y. & Yan, Y.J. (2017). Coordination mechanisms for scheduling games with proportional deterioration. *Eur J Oper Res*, 263, 380-389.

Choudhary, D.K. & Johri, B.N. (2009). Interactions of Bacillus spp. and plants – With special reference to induced systemic resistance (ISR). *Microbiol Res*, 164, 493-513.

Computing, S. (1991). R Foundation for Statistical Computing, Vienna, Austria. *URL* http://www. *R-project. org*.

Davis, M.A., Grime, J.P. & Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *J Ecol*, 88, 528-534.

Foster, K.R. & Bell, T. (2012). 17Competition, not cooperation, dominates interactions among culturable microbial species. *Current biology : CB*, 22, 1845-1850.

Fridley, J.D., Stachowicz, J.J., Naeem, S., Sax, D.F., Seabloom, E.W., Smith, M.D. *et al.* (2007). The invasion paradox: Reconciling pattern and process in species invasions. *Ecology*, 88, 3-17.

Friedman, J., Higgins, L.M. & Gore, J. (2017). Community structure follows simple assembly rules in microbial microcosms. *Nat Ecol Evol*, 1.

Friman, V.P., Dupont, A., Bass, D., Murrell, D.J. & Bell, T. (2016). Relative importance of evolutionary dynamics depends on the composition of microbial predator-prey community. *Isme J*, 10, 1352-1362.

Ghoul, M. & Mitri, S. (2016). 6The Ecology and Evolution of Microbial Competition. *Trends Microbiol*, 24, 833-845.

Gioria, M. & Osborne, B.A. (2014). Resource competition in plant invasions: emerging patterns and research needs. *Front Plant Sci*, 5.

Gosso, A., LA MORGIA, V., MARCHISIO, P., TELVE, O. & VENTURINO, E. (2012). Does a larger carrying capacity for an exotic species allow environment invasion?- Some considerations on the competition of red and grey souirrals *Journal of Biological Systems*, 20, 221-234.

Grilli, J., Barabas, G., Michalska-Smith, M.J. & Allesina, S. (2017). Higher-order interactions stabilize dynamics in competitive network models. *Nature*, 548, 210-+.

Hierro, J.L. & Callaway, R.M. (2003). Allelopathy and exotic plant invasion. *Plant Soil*, 256, 29-39.

Hu, J., Wei, Z., Friman, V.-P., Gu, S.-h., Wang, X.-f., Eisenhauer, N. *et al.* (2016). Probiotic Diversity Enhances Rhizosphere Microbiome Function and Plant Disease Suppression. *Mbio*, 7, e01790-01716.

Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X. *et al.* (2017). Bacterial Wilt in China: History, Current Status, and Future Perspectives. *Front Plant Sci*, 8, 1549.

Jousset, A., Schulz, W., Scheu, S. & Eisenhauer, N. (2011). Intraspecific genotypic richness and relatedness predict the invasibility of microbial communities. *Isme J*, 5, 1108-1114.

Kéfi, S., Berlow, E.L., Wieters, E.A., Navarrete, S.A. & Petchey, O.L. (2012). More than a meal… integrating non-feeding interactions into food webs. *Ecol Lett*.

Levine, J.M., Bascompte, J., Adler, P.B. & Allesina, S. (2017). Beyond pairwise mechanisms of species coexistence in complex communities. *Nature*, 546, 56-64.

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, 489, 220-230.

Mallon, C.A., Elsas, J.D. & Salles, J.F. (2015a). Microbial invasions: the process, patterns, and mechanisms. *Trends Microbiol*, 23, 719-729.

Mallon, C.A., Poly, F., Le Roux, X., Marring, I., van Elsas, J.D. & Salles, J.F. (2015b). Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. *Ecology*, 96, 915-926.

Mallon, C.A., van Elsas, J.D. & Salles, J.F. (2015c). Microbial Invasions: The Process, Patterns, and Mechanisms. *Trends Microbiol*, 23, 719-729.

Mehrabi, Z., McMillan, V.E., Clark, I.M., Canning, G., Hammond-Kosack, K.E., Preston, G. *et al.* (2016). Pseudomonas spp. diversity is negatively associated with suppression of the wheat take-all pathogen. *Sci Rep-Uk*, 6.

Mulder, C.P., Uliassi, D.D. & Doak, D.F. (2001). Physical stress and diversity-productivity relationships: the role of positive interactions. *Proc Natl Acad Sci U S A*, 98, 6704-6708.

Nicol, J.M., Turner, S.J., Coyne, D.L., Nijs, L.d., Hockland, S. & Maafi, Z.T. (2011). Current Nematode Threats to World Agriculture. In: *Genomics and Molecular Genetics of Plant-Nematode Interactions* (eds. Jones, J, Gheysen, G & Fenoll, C). Springer Netherlands Dordrecht, pp. 21-43.

Olson, A. & Stenlid, J. (2001). Plant pathogens - Mitochondrial control of fungal hybrid virulence. *Nature*, 411, 438-438.

Rakoff-Nahoum, S., Coyne, M.J. & Comstock, L.E. (2014). An Ecological Network of Polysaccharide Utilization among Human Intestinal Symbionts. *Current Biology*, 24, 40-49.

Rautenbach, M., Vlok, N.M., Eyeghe-Bickong, H.A., van der Merwe, M.J. & Stander, M.A. (2017). An Electrospray Ionization Mass Spectrometry Study on the "In Vacuo" Hetero-Oligomers Formed by the Antimicrobial Peptides, Surfactin and Gramicidin S. *J Am Soc Mass Spectr*, 28, 1623-1637.

Roscher, C., Bessler, H., Oelmann, Y., Engels, C., Wilcke, W. & Schulze, E.D. (2009). Resources, recruitment limitation and invader species identity determine pattern of spontaneous invasion in experimental grasslands. *J Ecol*, 97, 32-47.

Shea, K. & Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *Trends Ecol Evol*, 17, 170-176.

Smith-Ramesh, L.M., Moore, A.C. & Schmitz, O.J. (2017). Global synthesis suggests that food web connectance correlates to invasion resistance. *Global Change Biol*, 23, 465-473.

Stachowicz, J.J. (2001). Mutualism, Facilitation, and the Structure of Ecological Communities. *Bioscience*, 51, 235-246.

Stachowicz, J.J. & Byrnes, J.E. (2006). Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. *Mar Ecol Prog Ser*, 311, 251-262.

Stubbendieck, R.M., Vargas-Bautista, C. & Straight, P.D. (2016). Bacterial Communities: Interactions to Scale. *Front Microbiol*, 7.

Tan, S.Y., Gu, Y., Yang, C.L., Dong, Y., Mei, X.L., Shen, Q.R. *et al.* (2016). Bacillus amyloliquefaciens T-5 may prevent Ralstonia solanacearum infection through competitive exclusion. *Biol Fert Soils*, 52, 341-351.

Team, R.C. (2013). R: A language and environment for statistical computing.

Theoharides, K.A. & Dukes, J.S. (2007). Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist*, 176, 256-273.

Thorpe, A.S., Thelen, G.C., Diaconu, A. & Callaway, R.M. (2009). Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *J Ecol*, 97, 641-645.

Tilman, D. (1999). The ecological consequences of changes in biodiversity: A search for general principles. *Ecology*, 80, 1455-1474.

Tilman, D. (2004). Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *P Natl Acad Sci USA*, 101, 10854-10861.

Traveset, A. & Richardson, D.M. (2014). Mutualistic Interactions and Biological Invasions. *Annu Rev Ecol Evol S*, 45, 89-+.

Tuncil, Y.E., Xiao, Y., Porter, N.T., Reuhs, B.L., Martens, E.C. & Hamaker, B.R. (2017). Reciprocal Prioritization to Dietary Glycans by Gut Bacteria in a Competitive Environment Promotes Stable Coexistence. *Mbio*, 8.

van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V. & Salles, J.F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proc Natl Acad Sci U S A*, 109, 1159-1164.

Wang, L., Wu, Y.P. & Xu, Q. (2017a). Instability of spiky steady states for S-K-T biological competing model with cross-diffusion. *Nonlinear Anal-Theor*, 159, 424-457.

Wang, X., Wei, Z., Li, M., Wang, X., Shan, A., Mei, X. *et al.* (2017b). Parasites and competitors suppress bacterial pathogen synergistically due to evolutionary trade-offs. *Evolution*, 71, 733-746.

Wei, Z., Huang, J., Tan, S., Mei, X., Shen, Q. & Xu, Y. (2013). The congeneric strain Ralstonia pickettii QL-A6 of Ralstonia solanacearum as an effective biocontrol agent for bacterial wilt of tomato. *Biological Control*, 65, 278-285.

Wei, Z., Yang, T., Friman, V.P., Xu, Y., Shen, Q. & Jousset, A. (2015). Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health.

Nature communications, 6, 8413.

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

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

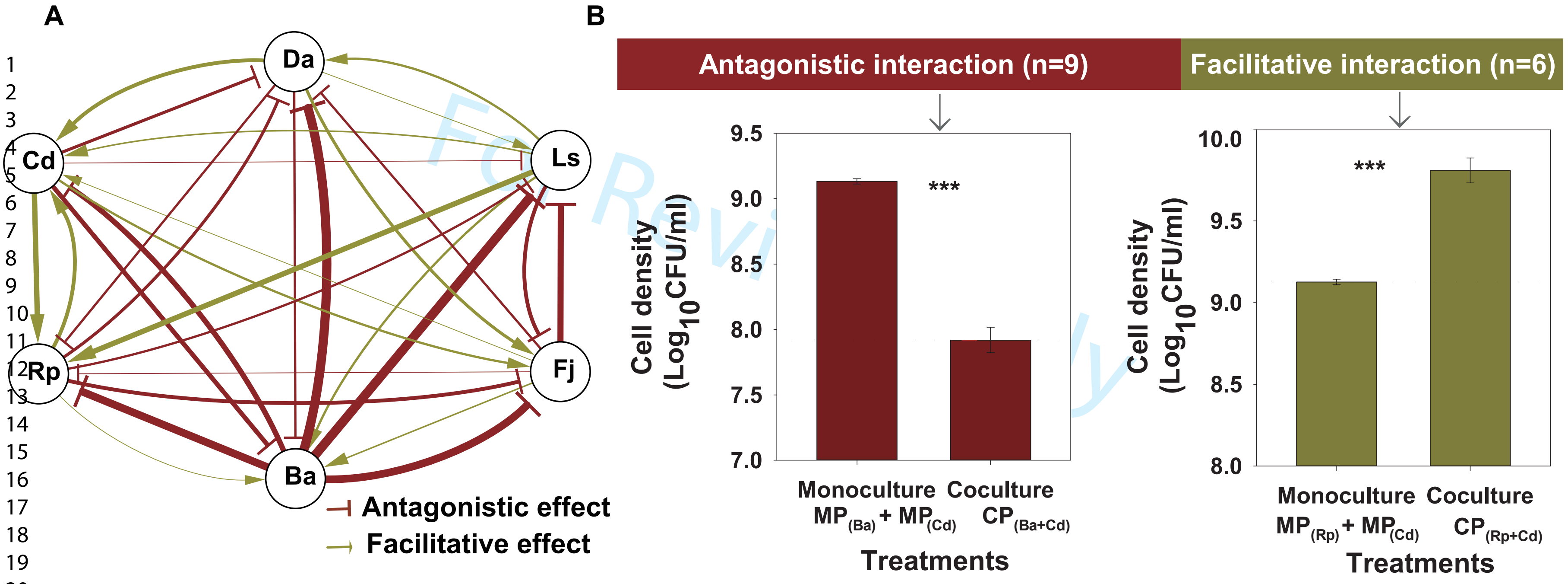
Figure legends

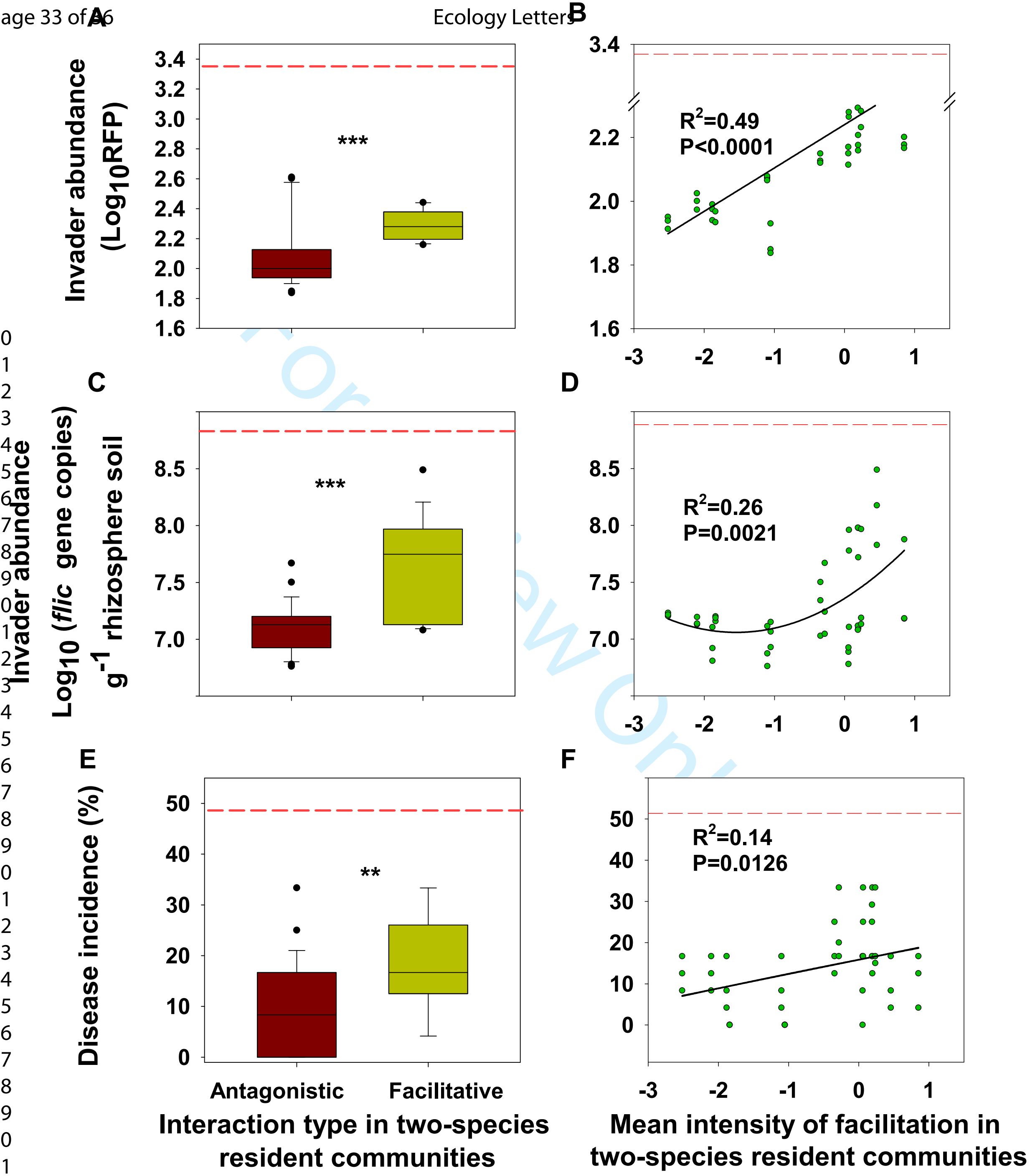
Figure 1. The type and relative strength of resident species pairwise interactions. (A) Network diagram showing the strength and directionality of all pairwise interactions between resident community species. The thickness of lines represents the strength and green and red color the facilitative or antagonistic effects between different species. (B) Nine of the fifteen pairwise interactions were on average antagonistic (co-culture density < monoculture density) and six facilitative (co-culture density > monoculture density). Panels show two examples: Left, antagonism between species Ba and Cd; Right, facilitation between species Rp and Cd. *** denotes for statistical significance at $p < 0.001$. All error bars denote for ± 1 s.e.m.

Figure 2. The type of pairwise resident community interactions predicts invasions *in vitro* and *in vivo*. (A) The *R. solanacearum* invader abundance in antagonistic and facilitative two-species resident communities measured *in vitro.* (B) The relationship between invader abundance and the mean intensity of facilitation in resident communities measured *in vitro*. (C) The relative invader abundance in antagonistic and facilitative two-species resident communities measured in the tomato rhizosphere 40 days after inoculation of the invader. (D) The relationship between invader abundance and the mean intensity of facilitation in resident communities measured *in vivo* in the tomato rhizosphere. (E) The bacterial wilt disease incidence (%) in antagonistic and facilitative pairwise resident communities 40 days after inoculation of the invader. (F) The relationship between disease incidence and the mean

intensity of facilitation in resident communities measured *in vivo* in the tomato rhizosphere. In all panels, the red dashed lines show the baseline for positive control treatments (invader-only). In panels, B, D and F, values below and above zero denote for antagonistic and antagonistic pairwise resident communities, respectively. Two and three stars denote for statistical significance at $p < 0.01$ and $p < 0.001$ significance levels, respectively. All the bars denote for ± 1 s.e.m.

Figure 3. The relationship between invader abundance and disease incidence with predicted and observed mean intensities of facilitation within multispecies communities. (A-B) The relationship between invader abundance and the proportion of facilitative interactions in the resident communities measured *in vitro* and *in vivo*, respectively. (C) The relationship between bacterial wilt disease incidence (%) and the proportion of facilitative interactions in the resident communities. (D-E) The relationship between invader abundance and the predicted mean intensity of facilitation in the resident communities measured *in vitro* and *in vivo*, respectively. (F) The relationship between bacterial wilt disease incidence (%) and the predicted mean intensity of facilitation in the resident communities. (G-H) The relationship between invader abundance and the observed mean intensity of facilitation in the resident communities measured *in vitro* and *in vivo*, respectively. (I) The relationship between bacterial wilt disease incidence (%) and the observed mean intensity of facilitation in the resident communities. In all panels, red dashed lines show the baseline of invader densities in control treatments (invader-only). In panels D-I, values below and above zero denote for competitive and antagonistic resident communities, respectively.


A
Da
Cd
Ls
Rp
Fj
Ba
Antagonistic effect
Facilitative effect
B
Antagonistic interaction (n=9)
Facilitative interaction (n=6)
Cell density (Log10CFU/ml)
9.5
9.0
8.5
8.0
7.5
7.0
***
Monoculture MP(Ba) + MP(Cd)
Coculture CP(Ba+Cd)
Treatments
Cell density (Log10CFU/ml)
10.0
9.5
9.0
8.5
8.0
***
Monoculture MP(Rp) + MP(Cd)
Coculture CP(Rp+Cd)
Treatments


A
B
C
D
E
F
Invader abundance (Log10RFP)
Invader abundance
Log10 (flic gene copies) g-1 rhizosphere soil
Disease incidence (%)
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
***
R2=0.49
P<0.0001
-3
-2
-1
0
1
8.5
8.0
7.5
7.0
***
R2=0.26
P=0.0021
50
40
30
20
10
0
**
R2=0.14
P=0.0126
Antagonistic
Facilitative
Interaction type in two-species resident communities
Mean intensity of facilitation in two-species resident communities

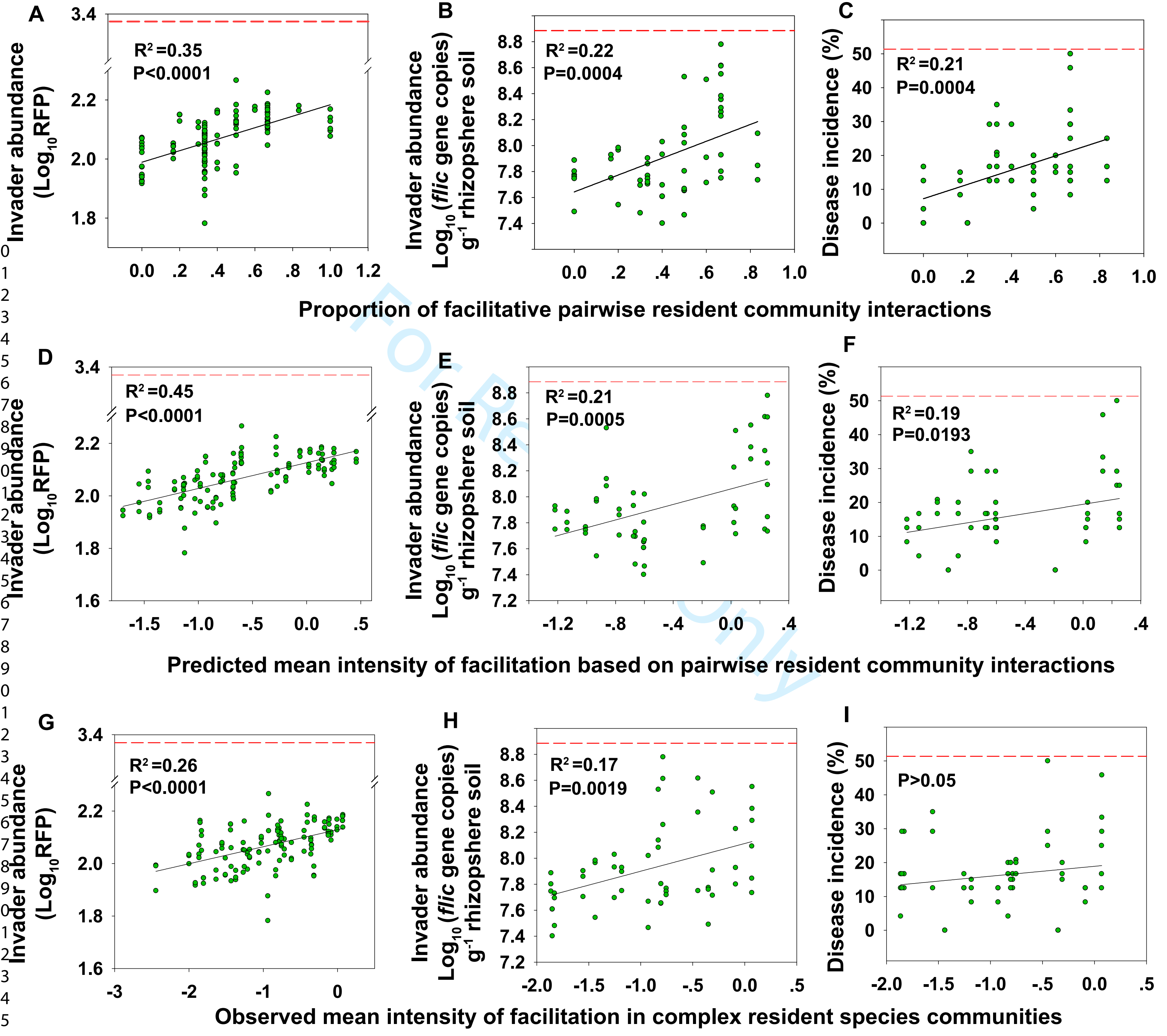

A
Invader abundance (Log$_{10}$RFP)
3.4
2.2
2.0
1.8
R² =0.35
P<0.0001
0.0 .2 .4 .6 .8 1.0 1.2
B
Invader abundance Log$_{10}$(*flic* gene copies) g^{-1} rhizopshere soil
8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4
R² =0.22
P=0.0004
0.0 .2 .4 .6 .8 1.0
C
Disease incidence (%)
50 40 30 20 10 0
R² =0.21
P=0.0004
0.0 .2 .4 .6 .8 1.0
Proportion of facilitative pairwise resident community interactions
D
Invader abundance (Log$_{10}$RFP)
3.4
2.2 2.0 1.8 1.6
R² =0.45
P<0.0001
-1.5 -1.0 -.5 0.0 .5
E
Invader abundance Log$_{10}$(*flic* gene copies) g^{-1} rhizopshere soil
8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2
R² =0.21
P=0.0005
-1.2 -.8 -.4 0.0 .4
F
Disease incidence (%)
50 40 30 20 10 0
R² =0.19
P=0.0193
-1.2 -.8 -.4 0.0 .4
Predicted mean intensity of facilitation based on pairwise resident community interactions
G
Invader abundance (Log$_{10}$RFP)
3.4
2.2 2.0 1.8 1.6
R² =0.26
P<0.0001
-3 -2 -1 0
H
Invader abundance Log$_{10}$(*flic* gene copies) g^{-1} rhizopshere soil
8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2
R² =0.17
P=0.0019
-2.0 -1.5 -1.0 -.5 0.0 .5
I
Disease incidence (%)
50 40 30 20 10 0
P>0.05
-2.0 -1.5 -1.0 -.5 0.0 .5
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.