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# Resource availability drives bacteria community resistance to pathogen invasion via altering bacterial pairwise interactions

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## Abstract

Microbial interactions within resident communities are a major determinant of resistance to pathogen invasion. Yet, interactions vary with environmental conditions, raising the question of how community composition and environments interactively shape invasion resistance. Here, we use resource availability (RA) as a model parameter altering the resistance of model bacterial communities to invasion by the plant pathogenic bacterium *Ralstonia solanacearum*. We found that at high RA, interactions between resident bacterial species were mainly driven by the direct antagonism, in terms of the means of invader inhibition. Consequently, the competitive resident communities with a higher production of antibacterial were invaded to a lesser degree than facilitative communities. At low RA, bacteria produced little direct antagonist potential, but facilitative communities reached a relatively higher community productivity, which showed higher resistance to pathogen invasion than competitive communities with lower productivities. This framework may lay the basis to understand complex microbial interactions and biological invasion as modulated by the dynamic changes of environmental resource availability.

## INTRODUCTION

Host-associated microbial communities can function as a line of defence against pathogens, thereby protecting their associated host organism (He et al., 2014; Wei et al., 2015). This process can also be viewed from the perspective of biological invasions where the members of resident communities can impact the ability or inability of invading species to establish in an ecosystem (Kurkjian et al., 2021; Vivant et al., 2013). The characteristics of resident communities are important for determining the outcomes of biological invasions (Li et al., 2019; Mickalide & Kuehn, 2019). For example, the interactions between microbes in resident communities can not only influence their survival, growth and contribution to community function (Ghoul & Mitri, 2016; Moons et al., 2009; Raes & Bork, 2008; Strom, 2008), but also impact the community

resistance to pathogen invasion. Microbes influence their direct environment for instance by consuming resources and excreting metabolites (Ratzke & Gore, 2018). These changes to the environment influence the growth and survival of both the microbe that originally altered the environment as well as other microbial species that are present, whether that be resident organisms or potential invaders. Facilitative interactions between residents can potentially increase the availability of resource niches via the production of secondary metabolites or public goods that can also be utilized by an invader (Bulleri et al., 2016; Mallon et al., 2015; Stachowicz, 2001). On the other hand, competing species can inhibit each other, for instance via the production of toxic metabolites, which may have negative effects on both resident and invading species (Becker et al., 2012; Hu et al., 2016).

However, microbial interactions are sensitive to a range of environmental factors, such as resource availability (Kinkel et al., 2011). Theoretical studies have suggested that certain mutualisms can become competitive under high nutrient conditions (Bull & Harcombe, 2009), and it has been shown that two yeast strains can interact in at least seven qualitatively different ways depending on the nutrient concentrations encountered (Hoek et al., 2016). In the highly fluctuating rhizosphere environment, where resource availability varies in space and time (Hu et al., 2018; Hu et al., 2020; Sauer et al., 2006), the same microbial community may be suppressive or conducive to pathogens. Although multiple studies have reported a shift in bacterial interactions in response to changes in resource availability levels, it is generally unknown how shifts in these interactions affect a community's susceptibility or resistance to pathogen invasion. We have previously demonstrated that the interactions within the resident bacterial community can reliably predict pathogen invasion both in lab microcosms and the plant rhizosphere (Li et al., 2019). Facilitative resident communities were more prone to invasions, while antagonistic resident communities were invaded to a lesser extent. In this study, we aimed to specifically explore if these relationships between bacterial interactions and community resistance to pathogen invasion were modulated by resource availability. Higher nutrient concentrations would be expected to allow bacterial populations to metabolize larger amount of growth substrates, like antimicrobial substance, thereby having a larger impact on their surrounding environment (Basan et al., 2015). Accordingly, we hypothesized that higher nutrient concentrations would lead to stronger antagonistic interactions and invader inhibition. Especially, competitive communities could also produce higher levels of antagonism than facilitative communities. Competitive communities should therefore provide greater resistance to the invader at high-resource availability (HRA) (Figure 1A). As opposed to conditions of HRA levels, resident communities at low-resource availability (LRA) conditions might produce lower levels of antagonism. We therefore expect that invasion success may, to some extent be driven by resident community productivity. If facilitative resident communities are able to reach higher population densities than antagonistic communities (Li et al., 2019), we expect that facilitative communities might better resist to the invader under these conditions (Figure 1A), as they may be able to occupy more niche space at LRA.

To validate this hypothesis, we examined how resource availability impacted pairwise interactions within the resident bacterial community, and how these

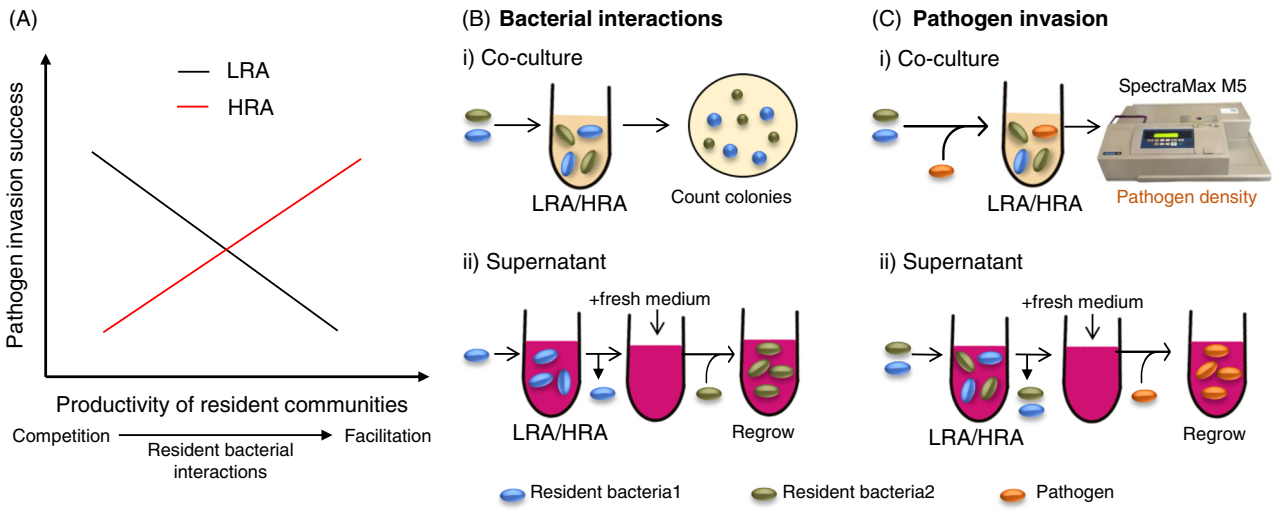


**FIGURE 1** Schematic figure depicting hypothesis and experimental design. (A) Invasion success as a function of interactions between resident species driven by resource availability, (B) pairwise interactions between resident bacteria by co-culture and supernatant assays, (C) two-species communities were confronted by a pathogen invasion, at low and high resource availability levels. LRA and HRA denote low resource availability and high resource availability, respectively

changes affected the resident community's resistance to the invasion of the plant pathogenic bacterium, *Ralstonia solanacearum*, the causal agent of bacterial wilt disease (Jiang et al., 2017; Salanoubat et al., 2002). Specifically, we first tested the pairwise interactions between resident bacteria by co-culture (cultured two bacteria together) and supernatant assays (cultured one bacterium in the presence of sterile supernatant from one of the other bacterial strains) at LRA and HRA levels (Figure 1B). Two-species communities were then confronted by a *R. solanacearum* invasion at either HRA or LRA, with invasion success being measured as the resulting density of *R. solanacearum* (co-culture assay in Figure 1C). To examine the toxicity of tested communities towards the *R. solanacearum*, we also tested the invasion success in the presence of sterile supernatant from each two-species community at LRA and HRA levels (supernatant assay in Figure 1C).

## EXPERIMENTAL PROCEDURES

### Bacterial strains and the assembly of pairwise resident communities

We used *R. solanacearum* strain QL-Rs1115 (Wei et al., 2015) tagged with the pYC12-mCherry plasmid (Tan et al., 2016) as the model invading pathogen in our experiments. Model resident communities were created using six bacterial strains isolated from the tomato rhizosphere from location from which the pathogen was also isolated (Qilin [118°57′E, 32°03′N], Nanjing, China). The strain collection used as model communities contained the isolates listed in Table S1 (*F. johnsoniae* WR4, *C. daecheongense* WR21, *D. acidovorans* WR42, *B. amyloliquefaciens* T-5, *L. sphaericus* HR92 and *R. pickettii* QL-A6), which have previously been shown to provide protection for associated host plants by inhibiting *R. solanacearum* pathogen growth via resource competition or direct toxin production (Huang et al., 2013; Huang et al., 2017; Li et al., 2019; Tan et al., 2016; Wei et al., 2015). These species can also be distinguished easily by colony morphology based on a previous study (Li et al., 2019), which makes it possible to test the pairwise interactions between each other. Moreover, these six taxa have high relative abundance in the rhizosphere microbiome (Gu et al., 2020; Lagos et al., 2015; Li et al., 2022). We have also previously demonstrated that the interactions within these resident bacterial community can reliably predict pathogen invasion both in vivo and in vitro (Li et al., 2019). In this study, we aimed to specifically further explore if these relationships between bacterial interactions and community resistance to pathogen invasion were modulated by resource availability. Therefore, model resident communities were constructed by using all six resident bacterial strains in all possible one- or two-species combinations (21 communities in total, Table S2). We used a substitutive design so that all communities were set up with the same initial total bacterial density ($10^5$ cells $ml^{-1}$) and evenness (i.e., multispecies communities had equal ratios of each species).

### Medium and bacterial culture

We set up two different resource availability treatments in this study: 100% NB medium (nutrient broth: 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) providing HRA, and 10% NB medium (by diluting 100% NB medium with sterile water, pH 7.0) providing lLRA.

Prior to each experiment, one colony of each strain, recovered from −80°C 20% glycerol stocks, was selected and grown in liquid NB with 170 r.p.m. agitation at 30°C for 12 h. Bacteria were then washed three times by centrifugation (5000 r.p.m for 5 min), resuspended in 0.85% NaCl and adjusted to a density of $10^7$ cells $ml^{-1}$ for each resident bacterium and $10^6$ cells $ml^{-1}$ for the invading pathogen.

### Determining pairwise interactions between resident community species at both HRA and LRA by co-culture assay

In order to investigate if and how the resource availability influenced the interaction between bacterial strains, we quantified the strength and direction of each pairwise interaction between resident species at HRA or LRA. To this end, 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 monocultures were inoculated with a starting density of $10^5$ cells $ml^{-1}$, and co-cultures were inoculated with half of this starting cell density for each species. Resident species were grown for 36 h in liquid 100% NB or 10% NB medium in 48-well plates (ending volume of 700 μl per well) at 30°C with shaking (170 r.p.m). Bacterial densities of each community were measured as optical density at 600 nm ($OD_{600}$) using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA, USA). Bacterial growth was measured as colony number units (CFU) per ml by serial dilution and plating on NB agar plates after 48 h growth.

To test the significance of the effects of resident bacteria on each other, the productivity of each species (CFU) was $\log_{10}$-transformed prior to a *t*-test to compare mean differences between each bacterium in co-culture as compared to monoculture, with *p*-values below 0.05 being considered statistically significant. The strength of pairwise interactions between two species (here *i* and *j*) was determined by comparing the

final productivity of each species ($CP_i$ and $CP_j$) in two-species co-cultures with their productivities ($MP_i$ and $MP_j$) in monocultures ($\log_{10}[CP_i / MP_i]$). We then determined the directionality of interaction. Interactions were considered facilitative if species *j* had a significant positive effect on species *i*, competitive if the effect was significantly negative and neutral if there was not significant effect. We also calculated the mean intensity of interaction (MIF) of co-cultures as an average of $\log_{10}$-transformed pairwise interactions using the following formula:

$$MIF_{ij} = \frac{1}{2}\left[\log\left(CP_i/MP_i\right) + \log\left(CP_j/MP_j\right)\right].$$

The two-species community was defined as facilitative when MIF > 0, competitive when MIF < 0.

## Determining pairwise interactions between resident community species at both HRA and LRA by supernatant assay

To assess the impact of resource availability on direct interference competition between resident community species (i.e., toxicity within two-species resident community), we compared the growth of each bacterial strain in the presence of supernatant from each of the other bacteria, which was collected from HRA (100% NB) and LRA (10% NB) medium, respectively. Briefly, after 36 h of growth in NB or 10% NB medium on a shaker at 170 r.p.m, 30°C, the 6 resident bacterial monocultures were filter-sterilized to remove living cells (0.22 μm filter). Subsequently, 20 μl of sterile supernatant from each strain's culture and 2 μl overnight culture of each resident bacterial strain (density of $10^7$ cells $ml^{-1}$) were inoculated into 180 μl of fresh NB medium (10× diluted, in order to better reflect the effect of the supernatant), respectively. Control treatments were inoculated with 20 μl of 10× diluted NB media instead of the bacterial supernatant. Each treatment was conducted in triplicate. All bacterial cultures were grown for 24 h at 30°C with shaking (170 r.p.m) before measuring $OD_{600}$ as resident bacterial density using a SpectraMax M5 plate reader.

To test the significance of direct toxicity of resident bacteria on each other, we conducted a *t*-test to compare mean differences between bacterial density ($OD_{600}$) from the treatment with exposure to bacterial supernatants and the control treatment, with *p*-values below 0.05 being considered statistically significant. We used the same method as described for the co-culture assay to determine the strength and directionality of direct toxicity of resident bacteria on each other. We also calculated mean intensity of interaction between resident species in supernatant assay (MIF_S) via the method used to calculate the mean intensity of interaction in the co-culture assay. Two-species communities were defined as facilitative when MIF_S > 0, antagonistic when MIF_S < 0.

## Measuring resident community toxicity towards *R. solanacearum* at HRA and LRA

In order to link pairwise interactions between resident species to resident community toxicity towards the pathogen at both HRA and LRA, we quantified pathogen growth in the presence of two-species resident community supernatants, which were collected after growth under the conditions of HRA (100% NB) or LRA (10% NB), respectively. Briefly, after 36 h of growth in 100% or 10% NB medium on a shaker at 170 r.p.m, 30°C, two-species resident community cultures were filter-sterilized to remove living cells (0.22 μm filter). Subsequently, 20 μl of sterile supernatant from each community's culture and 2 μl overnight culture of *R. solanacearum* (density of $10^6$ cells $ml^{-1}$) were added to 180 μl of fresh 10× diluted NB medium. Control treatments were inoculated with 20 μl of 10× diluted NB media instead of the community supernatant. Each treatment was conducted in triplicate. Bacteria were grown for 24 h (30°C, 170 r.p.m) before bacterial densities were measured as optical density at 600 nm with a SpectraMax M5 plate reader. Pathogen inhibition was defined as the percentage of reduction in pathogen growth compared to pathogen growth in the control treatment.

## Measuring invasion success in microcosms at HRA and LRA

Bacterial communities were constructed according to the scheme provided in Table S2. Each resident community was first inoculated into media of the two resource availability treatments (with a starting density of $10^5$ cells $ml^{-1}$). The invader *R. solanacearum* QL-Rs1115 (tagged with the pYC12-mCherry plasmid) was subsequently introduced into all communities (with a starting density of $10^4$ cells $ml^{-1}$). Communities were incubated at 30°C with 170 r.p.m orbital agitation for 36 h (200 μl together with bacteria and medium per well of 96-well plate), a time chosen to allow all communities to reach stationary phase. Invader density was measured as the red mCherry protein fluorescence intensity (excitation: 587 nm, emission: 610 nm) using a SpectraMax M5 plate reader. Wells contained a total of 200 μl of liquid, including 196 μl of media, 2 μl inoculum of the constructed resident community and 2 μl inoculum of the invader. Control treatments were inoculated with 2 μl of sterile water instead of the constructed resident community. Each treatment was replicated four times. To control for the auto-fluorescence of resident community, we also grew each community at the two levels of resource availability and subtracted these values from those obtained from co-cultures with the invader.

## Statistical analyses

To test how resource availability influences the pairwise interactions between resident species, we used *t*-test to compare mean differences between resident bacterial interactions at HRA and LRA levels, with *p*-values below 0.05 being considered statistically significant. We used linear regression to test whether the secondary metabolites from each resident strain (mean intensity of interaction between resident species in supernatant) affected the pairwise interactions between resident species (mean intensity of interaction between resident species in co-culture) at HRA and LRA levels.

In order to determine how resource availability-modulated changes in pairwise interactions between resident species influence the community resistance to pathogen invasion (invader density), we used linear regression to analyse relationships between pairwise resident bacterial interactions (mean intensity of interaction between resident species in co-culture) and invader density at LRA and HRA levels. Furthermore, we also used linear regression to disentangle the mechanisms behind the impact of resident bacterial interactions on pathogen invasion at LRA and HRA levels. Specifically, we focused on exploring how changes in the pairwise interactions between resident species, as influenced by resource availability, affected pathogen density via effects on resident community productivity and toxicity towards pathogen.

To explore the identity effects (Yang et al., 2017) of the resident species on properties of resident communities, we conducted a model which expressed the productivity of resident communities, resident community toxicity towards invader and density of the invader as a function of the presence of each species (binary predictors), respectively. Before all analyses, pathogen density data were $\log_{10}$-transformed to fulfil the parametric model assumptions (i.e., linear regression). All data were analysed using the R 4.0.2 program (www.r-project.org).

# RESULTS

## Resource availability influences pairwise interactions between resident bacterial species

Each bacterial species exhibited a different pattern of positive, negative or neutral effects on the other strains, and the magnitude and directionality of these effects were differently influenced by resource availability (Figures 2A, B and S1). For example, strain *Ralstonia pickettii* QL-A6 (Rp) showed a stronger negative effect on strain *Lysinibacillus sphaericus* HR92 (Ls) at LRA as compared to HRA, and strain *Chryseobacterium daecheongense* WR21 (Cd) showed a negative effect on strain *Flavobacterium johnsoniae* WR4 (Fj) at LRA, but no significant effect on this strain at HRA. Production of toxic metabolites is another driver of bacterial interaction. Here, we found resident bacteria produced less toxic metabolites in the LRA conditions (Figure 2C), but their interactions were stronger in co-culture assays (Figure 2A). For example, the metabolites contained in the supernatant of strain *C. daecheongense* WR21 (Cd) did not significantly inhibit the growth of strain *Bacillus amyloliquefaciens* T-5 (Ba), but we observed strong inhibitory effect on strain *B. amyloliquefaciens* T-5 (Ba) in co-culture (Figure S1). In contrast, supernatants from high-resource media cultures showed stronger negative effects on the growth of other strains (Figure 2D). Moreover, there was a significant relationship between the mean intensity of interaction in co-culture and in supernatant at the HRA level ($R^2 = 0.59$, $p < 0.001$, dark blue in Figure 2E), with no relationship being observed at LRA ($R^2 = 0.09$, $p = 0.0519$, light blue in Figure 2E). These results suggest that the pairwise interactions between resident bacterial species were mostly driven by the production of secondary metabolites at HRA.

## Resource availability alters bacterial pairwise interactions with effects on productivity of resident community and its toxicity towards invader

The productivity of bacterial monoculture can affect pairwise interactions in a bacterial community, and the community productivity is the outcome of bacterial interaction. We found the productivity of bacterial monocultures and their pairwise consortia were significantly affected by resource availability (Figure S2). With no surprise, higher resource availability led to increased productivity of the resident community (Figure S2). Moreover, mean intensity of interaction between resident species was positively correlated with productivity of the resident community at both HRA ($R^2 = 0.47$, $p < 0.001$, dark blue in Figure 3A) and LRA ($R^2 = 0.39$, $p < 0.001$, light blue in Figure 3A). As a result, there was no interactive effect between resource availability and interactions within resident community on the productivity of the resident community (Table 1). However, different resident bacterial species affected the productivity of the resident community in resource-dependent manner (Table S3). For instance, the species Ba had strong negative effects on productivity of the resident community at HRA but did not appear to have clear effects on productivity of the resident community at LRA, the species Ls had positive effects on productivity of the resident community at HRA but had no effect on productivity of the resident community at LRA (Table S3).

We found that higher resource availability led to a greater level of resident community toxicity towards pathogen (Figure S3). Furthermore, mean intensity of interaction between resident species in co-culture had

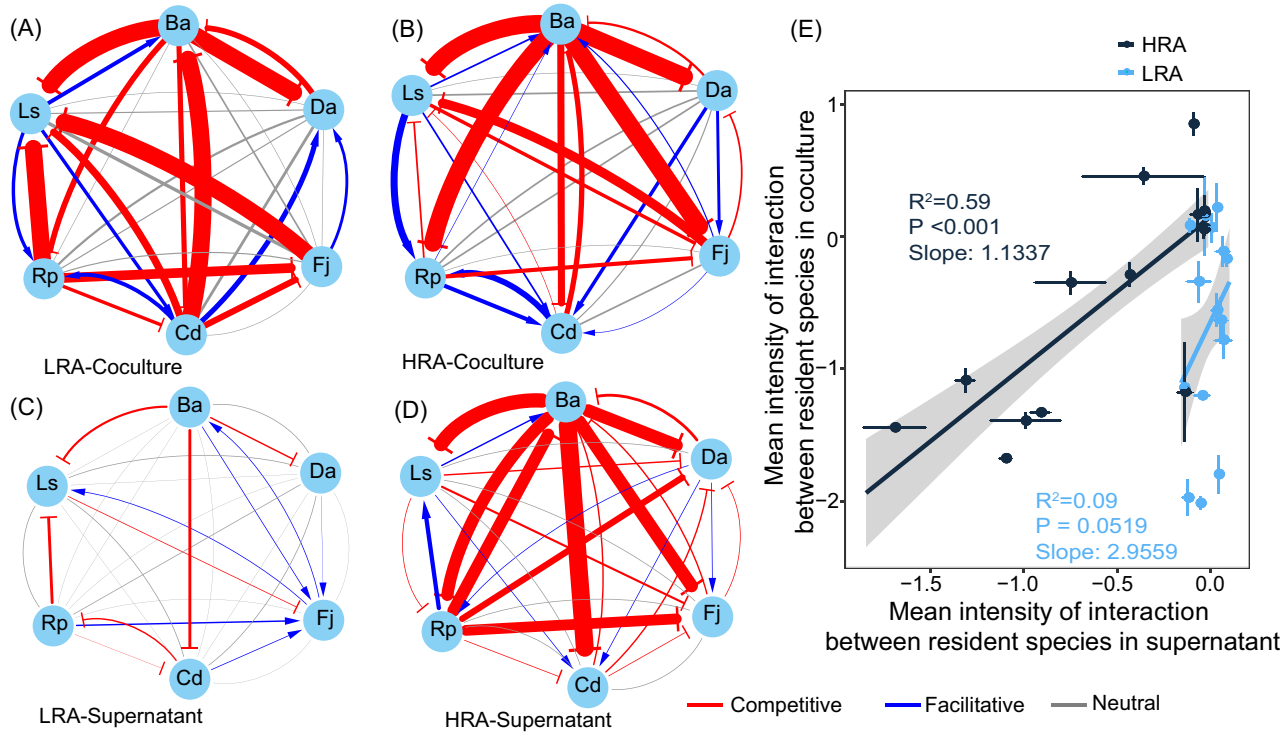


**FIGURE 2** Resource availability influences pairwise interactions between resident bacterial species. Pairwise interactions at low (A) and high (B) resource availability levels, and toxicity within resident communities at low (C) and high (D) resource availability levels. (E) Relationships between resident pairwise interactions (mean intensity of interaction) in supernatant and in co-culture at low and high resource availability levels. Grey shaded areas depict 95% confidence intervals of the logistic regression. Horizontal and vertical lines for each point indicate error bars, which denote mean ± 2 SEM. LRA and HRA denote low resource availability and high resource availability, respectively. Co-culture refers to the test of pairwise interactions between bacteria in co-culture. Supernatant denotes the effects of sterile supernatant from each strain on the other strains.

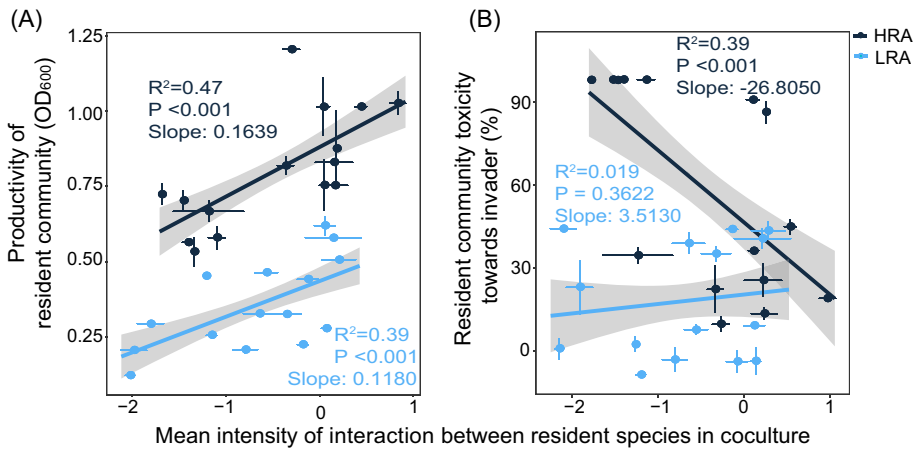


**FIGURE 3** Resource availability alters bacterial pairwise interactions with effects on productivity of resident community and its toxicity towards invader and productivity of resident community. The relationships between mean intensity of interaction in co-culture and (A) productivity of resident communities, (B) resident community toxicity towards pathogen at low and high resource availability. In x-axis of both panels A and B, values below and above zero denote for competitive and facilitative resident communities, respectively. LRA and HRA denote low resource availability and high resource availability, respectively. Grey shaded areas depict the 95% confidence interval of the logistic regression, horizontal and vertical lines in each dot in the figures are error bars, which denote for ±2 SEM

no effect on resident community toxicity towards pathogen at LRA ($R^2 = 0.019$, $p = 0.3622$, light blue in Figure 3B), but was negatively correlated with resident community toxicity towards pathogen ($R^2 = 0.39$, $p < 0.001$, dark blue in Figure 3B). Therefore, there was an interactive effect between resource availability and interaction within the resident community (mean intensity of interaction in co-culture) on resident

**TABLE 1** ANOVA table summarizing the interactive effects of pairwise interactions within the resident community and resource availability on the productivity of resident communities, resident community toxicity towards invader and density of the invader

| | Productivity of resident community | | | Resident community toxicity towards invader | | | Invader density | | |
|---|---|---|---|---|---|---|---|---|---|
| | *df* | *F* | *p* | *df* | *F* | *p* | *df* | *F* | *p* |
| Mean intensity of interaction in co-culture (MIF) | 1 | 108.32 | **<0.001**↑ | 1 | 7.30 | **0.008**↓ | 1 | 1.21 | 0.2741 |
| Resource availability (RA) | 1 | 230.08 | **<0.001**↑ | 1 | 65.96 | **<0.001**↑ | 1 | 145.06 | **<0.001**↓ |
| MIF * RA | 1 | 1.72 | 0.1938 | 1 | 22.55 | **<0.001**↓ | 1 | 39.32 | **<0.001**↑ |
| No. of residuals | 86 | | | 86 | | | 86 | | |

*Note*: Significant effects ($p < 0.05$) are highlighted in bold and the 'up' and 'down' arrows denote positive and negative effects, respectively.

community toxicity towards pathogen (Table 1). It also showed that the resident species identities had clear effects on the resident community toxicity towards pathogen (Table S3). At HRA, while the species Cd, Ba and Ls increased the resident community toxicity towards pathogen, the other resident species did not appear to have clear effects on resident community toxicity towards pathogen (Table S3). At LRA, the resident species Fj, Cd, Da and Ba had positive effects on resident community toxicity towards pathogen, but the species Ls and Rp had no effect on resident community toxicity towards pathogen (Table S3).

## Mechanisms by which resource availability modulates two-species bacterial community resistance to pathogen invasion

We examined model resident communities resistance to pathogen invasion as judged by final invader density. We found that higher resource availability led to a reduced invasion success of pathogen (Figure S4). In addition, invader success correlated positively with the mean intensity of interaction between resident species at high ($R^2 = 0.35$, $p < 0.001$, dark blue in Figure 4A) resource availability, but an opposite correlation was observed at LRA ($R^2 = 0.4$, $p < 0.001$, light blue in Figure 4A). Thus, there was an interactive effect between resource availability and interaction within the resident community (mean intensity of interaction in co-culture) on invasion success of pathogen (Table 1). Compared to positive controls (*R. solanacearum*-only: dark and light blue dashed lines in Figure 3), pathogen densities were lower in the presence of resident species at both HRA and LRA. Furthermore, the resident species identities also had clear effects on the density of the invader (Table S3). At HRA, while the species Fj, Da and Ba had negative effects on invader density, the other resident species did not show significant effects on invader density (Table S3). At LRA, the resident species Fj and Da had negative effects on invader density, the species Cd, Ba and Ls had no effect on invader density, but Rp had a slightly positive effect on invader density (Table S3).

At LRA, invader density decreased with increasing productivity of resident community ($R^2 = 0.33$, $p < 0.001$, light blue in Figure 4B) and resident community toxicity towards pathogen ($R^2 = 0.13$, $p = 0.017$, light blue in Figure 4C). However, we found that productivity of resident community provided better prediction of invader density at LRA, as compared to resident community toxicity towards pathogen (Table 2). In HRA conditions, invader density was positively linked with productivity of resident community ($R^2 = 0.4$, $p < 0.001$, dark blue in Figure 4B), but negatively linked with resident community toxicity towards pathogen ($R^2 = 0.33$, $p < 0.001$, dark blue in Figure 4C). Mechanistically, this could be explained by a trade-off relationship between productivity and secondary metabolites producing of bacterial community at HRA as suggested by a negative correlation between the productivity of the resident community and its toxicity towards pathogen at HRA ($R^2 = 0.27$, $p < 0.001$, dark blue in Figure S5), but not at LRA (light blue in Figure S5). Moreover, we found invader density was influenced more by resident community toxicity towards pathogen than productivity at HRA (Table 2).

Together, these results suggest that facilitative communities that reached higher total densities suppressed the invader more efficiently in LRA conditions. HRA conditions yielded a contrasting pattern in which competitive communities that achieve lower total community densities, but produced more direct antagonist compounds, provided a stronger inhibition of the pathogen growth.

## DISCUSSION

Microbial communities are structured by interactions between their constituent species in the context of their abiotic environment (Lax et al., 2020). Interactions within a resident community can have important consequences for ecosystem functions such as the ability of the community to resist biological invasions (Li et al., 2019; Mickalide & Kuehn, 2019). For example, it has been observed that communities with a greater proportion of competitive interactions are better able to constrain invader growth than more facilitative

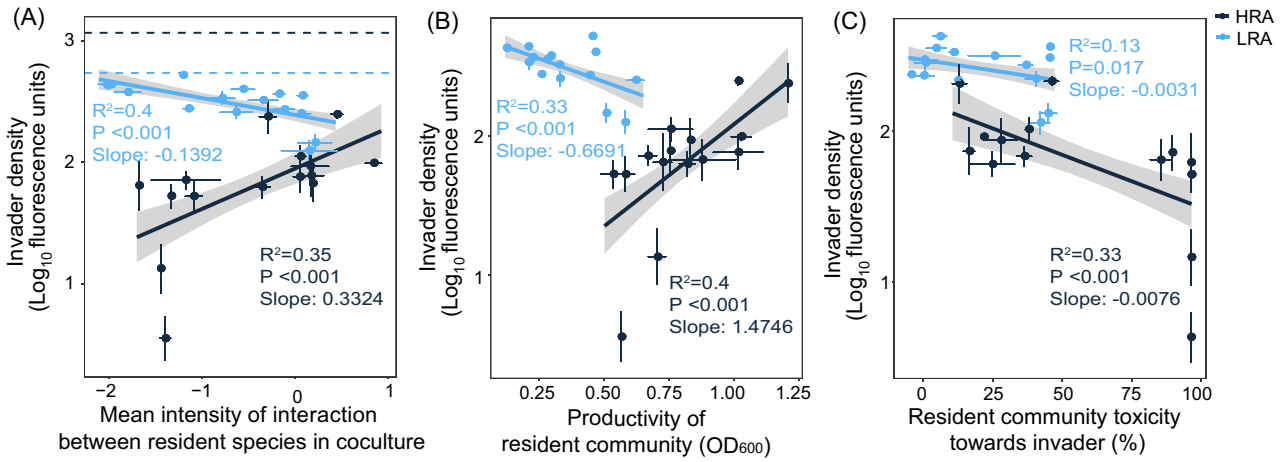


**FIGURE 4** Resource availability alters bacterial pairwise interactions with effects on community resistance to pathogen invasion. The relationship between (A) mean intensity of interaction between resident species in co-culture, (B) productivity of resident community, (C) resident community toxicity towards invaders and invader density at low and high resource availability, respectively. Dashed lines show the baseline invader densities in control treatments (invader-only). For the x-axis in panel A, values below and above zero denote for competitive and facilitative resident communities, respectively. LRA and HRA denote low resource availability and high resource availability, respectively. Grey shaded areas depict 95% confidence intervals of the logistic regression, and horizontal and vertical lines for each point indicate error bars, which denote mean ± 2 SEM

**TABLE 2** ANOVA table summarizing the effect of resident community toxicity towards invader and productivity of resident community on invader density

| | Invader density at high resource availability | | | Invader density at low resource availability | | |
|---|---|---|---|---|---|---|
| | ***df*** | ***F*** | ***p*** | ***df*** | ***F*** | ***p*** |
| Resident community toxicity towards invader | 1 | 27.03 | **5.567e-06*****↓ | 1 | 8.99 | **0.004551****↑ |
| Productivity of resident community | 1 | 12.14 | **0.001169 ****↑ | 1 | 20.86 | **4.278e-05*****↑ |
| No. of residuals | 42 | | | 42 | | |

*Note*: The 'up' and 'down' arrows denote positive and negative effects respectively.

communities (Li et al., 2019). However, the nature of microbial interactions is impacted by environmental conditions such as the level of nutrient availability, and how such impacts are related to community resistance to biological invasion is generally unknown. In this study, we explored how changes in resource availability affect relationships between interactions within model resident communities and their ability to resist pathogen invasion. At HRA, we found that more competitive resident communities produced more direct antagonism and subsequently better-resisted pathogen invasion than more facilitative communities. At LRA, facilitative communities that reached higher total productivity were more resistant to pathogen invasion than more competitive communities. Understanding how resource availability influences the interactions between resident bacteria is thus important for predicting the dynamics and outcomes of biological invasions.

In line with a recent study, we found that interactions between resident bacterial species were mostly driven by the production of secondary metabolites in HRA conditions (Ratzke et al., 2020). Given the results of our assays (supernatant assays) involving exposure to spent media, it appeared that the observed microbial interactions involved modification of the environment, for instance, via the production of inhibitory compounds. In HRA conditions, microbes have sufficient substrates to produce relatively large amounts of inhibitory compounds, thereby leading to a greater level of influence on other microbial strains (Estrela et al., 2019; Goldford et al., 2018; Niehaus et al., 2019). Interestingly, we found resident bacteria produced little toxic metabolites in LRA conditions, but there were strong competitive interactions between them in co-culture assays. This suggests that interactions at LRA were not driven by the production of toxic metabolites but by competition for resources, presumably because there were not sufficient resources to allow for large investment into the production of such secondary metabolites (Westhoff et al., 2021).

Resource availability showed a strong negative effect on the density of the model pathogen invader (Table 1), which contrasts with other studies (Davis et al., 2000; Kuebbing et al., 2013; Mallon et al., 2015). For example, Mallon et al. found that increasing resource availability may promote *E. coli* invasions due

to reled resource competition (Mallon et al., 2015). This discrepancy may be explained by the fact that bacteria were likely able to consume all the available resources during our experiments without addition of new external resources (Yang et al., 2017). Furthermore, resident communities produced inhibitory compounds that reduced the growth of invader *R. solanacearum* under conditions of HRA as compared to LRA conditions (Figure S3).

We also observed an interactive effect between the pairwise interactions within the resident community and resource availability conditions on invader density. Mechanistically, the greater resistance to pathogen invasion of competitive communities at HRA level was linked to direct inhibition of the invader by antagonistic communities (Figures 2B and 4C). However, facilitative communities were more resistance to pathogen invasion at the LRA level, which can be explained by the fact that facilitative communities reached higher total community productivity (Figures 2A and 4B). Our results (Table 2) suggest that under HRA conditions, antagonism is an important determinant of community invasion resistance (Li et al., 2019; Ratzke et al., 2020). This result is also 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). As our results suggested that interactions in LRA conditions were driven by competition for resources, communities that showed higher productivity may be able to better occupy niche spaces, thereby outcompeting invaders (Elton, 1958; Romanuk et al., 2009; van Elsas et al., 2012).

We conclude that resource availability can modulate bacterial community resistance to pathogen invasion by changing pairwise interactions within the resident community. In many cases, microbial interactions may not be driven by a single parameter—resource availability in our case—but by a set of multiple parameters such as pH, temperature or other environmental factors. However, also in these cases, interactions are mediated by modifying and reacting to the environment (Pacheco et al., 2019; Ratzke & Gore, 2018), which can influence the community functions.

It should be noted that natural plant rhizosphere is far more complex than the simple artificial systems we have used in this study. Referring to natural conditions, more species in the synthetic community would represent more the real circumstance. However, it shows that more species diversity would increase the antagonistic interactions in the synthetic community and the community would collapse (Becker et al., 2012). Moreover, experiments conducted within one trophic level suggest that pairwise bacterial interactions can predict three-species bacterial interactions with as high as 90% accuracy (Friedman et al., 2017). While predicting interactions 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 interactional outcomes of up to 8-species communities (Friedman et al., 2017). Although our framework need to be expandable to more multivariate and more complex natural systems, it laid the basis for understanding how more complex microbial interactions, and the functions they yield at the community level, are impacted by changing environmental conditions.

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## CONFLICT OF INTEREST

There is no conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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