**Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression**

**Running title:** **Microbial diversity and plant disease suppression**

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**ABSTRACT** Bacterial communities associated with plant roots play an important role in the suppression of soil-borne pathogens, and multispecies probiotic consortia may enhance disease suppression efficacy. Here we introduced defined *Pseudomonas* spp. consortia into naturally complex microbial communities and measured the importance of *Pseudomonas* community diversity for its survival and the suppression of the bacterial plant pathogen *Ralstonia solanacearum* in the tomato rhizosphere microbiome. Our results show that the survival of introduced *Pseudomonas* consortia increased with increasing diversity. Further, high *Pseudomonas* diversity reduced the pathogen growth in the tomato rhizosphere and decreased the disease incidence due to both intensified resource and interference competition with the pathogen. These results provide novel mechanistic insights into elevated pathogen suppression by diverse probiotic consortia in naturally diverse plant rhizosphere. Ecologically-based community assembly rules could thus play a key role in engineering functionally reliable microbiome applications.

**IMPORTANCE** The increasing demand for food supply requires more efficient control of plant diseases. The use of probiotics, naturally occurring bacterial antagonists and competitors that suppress pathogens, has recently re-emerged as a promising alternative to agrochemicals. It is however still unclear how many and which strains we should choose to construct effective probiotic consortia. Here we present a general ecological framework for assembling effective probiotic communities based on *in vitro* characterization of community functioning. Specifically, we show that increasing probiotic consortia diversity enhances the community survival in the naturally diverse rhizosphere microbiome leading to increased pathogen suppression via intensified resource and interference competition with the pathogen. We hope that these ecological guidelines can be put to test in microbiome engineering more widely in the future.

**KEYWORDS** Biodiversity-ecosystem functioning, interference competition, resource competition, microbial community ecology, *Pseudomonas* spp*.*, *Ralstonia solanacearum*

## INTRODUCTION

Biodiversity-ecosystem functioning (BEF) experiments suggest that species diversity provides various beneficial community-level benefits related to productivity ([1](#_ENREF_1), [2](#_ENREF_2)), cycling of nutrients, the rate of decomposition, resistance to environmental change, and resistance to species invasions. Such relationships are omnipresent, and in the case of microbes, play also an important role for the health of higher organisms by ensuring efficient functioning of the host-associated microbiome ([3](#_ENREF_3)). In the case of plant-microbe interactions, high bacterial diversity has been associated with increased resistance to pathogen invasions ([2](#_ENREF_2), [3](#_ENREF_3)) and plant infestation, for example via intensified resource competition ([4-6](#_ENREF_4)). Several studies have also shown that community composition and diversity can affect the invasion/colonization success of additional species ([4-6](#_ENREF_4)). Here we studied the potential beneficial effects of microbial diversity in the context of probiotic bacterial community performance. We hypothesized that diversity could affect the establishment, survival and functioning of introduced microbial consortia in the complex plant microbiome, and shape community ability to induce disease suppression.

Biodiversity effects could drive the functionality of introduced rhizosphere bacterial communities in different ways ([7](#_ENREF_7)). First, high species richness can increase the total number of resources species can collectively utilise as a community (niche breadth) ([5](#_ENREF_5)). This could improve community survival in the temporally and spatially fluctuating rhizosphere environment and ensure that at least one of the species will survive under the prevailing conditions ([8](#_ENREF_8)). Wide community niche breadth is also expected to intensify resource use in general, which could help bacteria to better colonize and persist in the rhizosphere ([9](#_ENREF_9), [10](#_ENREF_10)). Furthermore, wide niche breadth is likely to intensify the resource competition between the introduced bacterial community and a potential pathogen, which could lead to competitive exclusion of the pathogen ([5](#_ENREF_5), [11](#_ENREF_11)) and, in the present context, an elevated host plant protection.

Biodiversity of the introduced rhizosphere bacterial communities could also affect interference competition with other microorganisms, including both the resident microbiota and pathogens. For example, previous studies have shown that the production of secondary metabolites that suppress pathogen growth ([12](#_ENREF_12), [13](#_ENREF_13)) can increase with the density and richness of the inoculated probiotic consortia ([14](#_ENREF_14), [15](#_ENREF_15)). As a result, diverse bacterial communities could be more effective at suppressing invading pathogens. Similarly, secondary metabolites may help the introduced microbial communities to compete with the indigenous microbiota, enhancing their survival. Furthermore, a combination of different bacterial secondary metabolites produced jointly by a diverse community could result in stronger antagonism towards the pathogen if they target different cellular functions ([16](#_ENREF_16)) – an idea analogous to mixing antibiotics from several antibiotic classes to achieve higher pathogen inhibition (and reduced resistance evolution) in clinical environments ([17](#_ENREF_17)). The interplay between bacterial strains in diverse bacterial community may also involve species-specific responses that trigger complex secretion systems leading to induction or upregulation of secondary metabolites or signal molecules that inhibit pathogen growth ([18](#_ENREF_18)). Surprisingly, despite of a growing interest for using microbial consortia in plant protection, there are hardly any studies investigating how the diversity and composition of introduced probiotic consortia may affect its functioning.

Here we used complementary laboratory and greenhouse experiments to study the mechanisms and importance of biodiversity of introduced plant growth promoting *Pseudomonas* spp*.* communities for disease suppression within the natural rhizosphere microbiome. Eight *Pseudomonas* spp*.* strainsproducing thebroad-spectrum antibiotics 2,4-diacetylphloroglucinol (DAPG) were used in this study. We assembled *Pseudomonas* communities in four richness levels as described previously ([19](#_ENREF_19), [20](#_ENREF_20)). We chose *Pseudomonas* bacteria due to their well-reported disease suppression abilities and widespread occurrence in the rhizosphere ([12](#_ENREF_12), [21](#_ENREF_21)). We first used simple *in vitro* experiments to quantify the relationship between *Pseudomonas* community strain richness and composition and traits linked to resource competition and antagonism. In order to bridge the gap between laboratory and the real world, we then assessed the ability of different *Pseudomonas* communities to survive *in vivo* in the naturally highly diverse tomato plant rhizosphere (homogenised natural soil), and to suppress the growth of *Ralstonia solanacearum* bacterial pathogen - the causative agent of global bacterial wilt disease epidemics ([22](#_ENREF_22)). We found that high biodiversity enabled the introduced *Pseudomonas* community to persist at high density in the rhizosphere throughout the experiment leading to dramatically increased pathogen suppression and lower disease incidence. These patterns matched well with the *in vitro* results: increasing *Pseudomonas* community diversity increased the intensity of both resource and interference competition, which in turn resulted in very low pathogen densities. Together these results suggest that BEF and competition theory could thus provide community assembly rules to engineer functionally reliable microbiome applications.

## RESULTS

**BEF relationships *in vitro.*** Increasing *Pseudomonas* community genotypic richness correlated positively with community niche breadth (R2 = 0.776, P < 0.0001, Fig. 1A), niche overlap with the pathogen (R2 = 0.709, P < 0.0001, Fig. 1B), and direct pathogen inhibition (R2 = 0.389, P < 0.0001, Fig. 1C) *in vitro*.

**BEF relationships *in vivo.*** Both disease incidence and pathogen density decreased significantly with increasing *Pseudomonas* community richness (Fig. 2A-B, Table 1). While all *Pseudomonas* monocultures reduced disease incidence to some extent, they offered only a partial protection against bacterial wilt disease. In contrast, the eight-strain community provided almost complete protection against bacterial wilt, and 2- and 4-strain communities provided intermediate levels of protection (Fig. 2A, Table 1). The effect of *Pseudomonas* community richness on disease suppression increased with time (Fig. 2B, significant richness × time interaction, Table 1): while community richness had no effect on disease suppression during the first 15 days after pathogen invasion, the 8-strain *Pseudomonas* community reduced pathogen density by 99% compared to the best performing monoculture on day 35 (Fig. 2B, Table 1).

At initial stage, all *Pseudomonas* communities were able to colonize plant roots equally well regardless of the community diversity. However, only the 8-strains *Pseudomonas* communities were able to maintain high population densities in the rhizosphere throughout the whole experiment (Fig. 2C, significant Richness × Time interaction, Table 1), reaching circa 10 times higher densities compared to most productive monoculture at the end of the experiment [indicative of transgressive overyielding ([23](#_ENREF_23)), Table S1]. Interestingly, none of the *Pseudomonas* strains showed a particularly strong identity effect on pathogen suppression (Table S1). This suggests that high *Pseudomonas* community richness increased its ability to colonize the rhizosphere microbiome due to synergistic effects between community members instead of inclusion of one particularly efficiently colonizing *Pseudomonas* strain.

**Linking community performance *in vivo* to characteristics *in vitro***. We found that *Pseudomonas* community survival in the rhizosphere increased with increasing niche breadth of the community, while pathogen density correlated negatively with the increasing inhibition activity of *Pseudomonas* communities measured *in vitro* (Table 2). Pathogen invasion success in the rhizosphere depended also on the density of the *Pseudomonas* community (Table 2). We used a structural equation modelling approach to further study the relative importance of different mechanisms linking *Pseudomonas* community composition to the disease suppression. The final models fit the data well (both P > 0.05) and explained 72% of the variance in pathogen density and 37% of the variance in disease incidence at day 35 of the experiment (Fig. 3A-B). Pathogen density decreased with *in vitro* antagonistic activity against the pathogen, higher strain richness, and wider niche breadth of the *Pseudomonas* communities. Accordingly, disease incidence decreased with increasing richness of the *Pseudomonas* communities.

## DISCUSSION

Host-associated microbiomes play essential role in preventing diseases ([24](#_ENREF_24), [25](#_ENREF_25)). It is still however less clear how to manipulate and improve the functioning of host-associated microbiomes. While microbial diversity is known to enhance community resistance against pathogen invasions in general, BEF relationships are very variable ([5](#_ENREF_5), [19](#_ENREF_19), [26](#_ENREF_26)). We thus need to rethink what kind of guidelines to use for selecting species or strains that work best together in performing desired community level function. Here we show that amending complex rhizosphere microbiomes with carefully selected bacterial consortia based on microbial competitive interactions can improve key functions such as pathogen suppression. To this end, we used a combination of experiments to study how the diversity affected the survival and functioning of probiotic bacteria in naturally diverse tomato rhizosphere microbiome. Only the most diverse probiotic *Pseudomonas* communities (8-strains) were able to maintain high densities in the rhizosphere throughout the experiment and the pathogen densities correlated negatively with both the *Pseudomonas* density and diversity. The beneficial biodiversity effects on pathogen suppression could be explained via a two-step process where high *Pseudomonas* community diversity first improved the establishment and survival of the introduced probiotic community in the rhizosphere, which in turn ensured effective pathogen suppression at the later stages of infection. The positive relationship between *Pseudomonas* community diversity and the intensity of interference and resource competition thus likely helped the introduced community to compete with both non-pathogenic natural bacteria and the pathogen during the greenhouse experiment.

We found that increasing diversity increased both the number of resources the *Pseudomonas* community was able to use for its growth and the number of resources that were also used by the pathogen (niche overlap). While all *Pseudomonas* communities showed a comparable survival in the rhizosphere during the first two weeks of the experiment, only the most diverse *Pseudomonas* communities were able to persist at high densities and efficiently constrain pathogen invasion during the greenhouse experiment. One likely explanation for this is that only the diverse *Pseudomonas* communities were able to efficiently compete for resources with the pathogen and the already present natural bacterial communities. For example, plant-derived resources may have been readily available in the rhizosphere at the beginning of the experiment, allowing introduced *Pseudomonas* to reach high densities regardless of their diversity. However, increase in the pathogen and commensal bacteria could have intensified the resource competition towards the end of the experiment, leading to decline in *Pseudomonas* densities. These results suggest that high diversity of the introduced *Pseudomonas* community was beneficial likely due to improved survival in the presence of competitors ([9](#_ENREF_9), [10](#_ENREF_10)).

 High probiotic community diversity could have also contributed to direct inhibition of the invading pathogen by stimulating secondary metabolite production ([27](#_ENREF_27)). In support for this, we found that mixing *Pseudomonas* supernatant from different monocultures increased pathogen suppression *in vitro*. This suggests that secondary metabolites produced by different *Pseudomonas* strains can synergistically suppress the pathogen. *Pseudomonas* bacteria produce a distinct set of secondary metabolites including polyketides, cyanide, lipopeptides , and exoenzymes, and all of these compounds vary in their molecular mechanisms and mode of action. Diverse *Pseudomonas* communities could thus produce a higher variety of toxins that could increase the total antibacterial activity of the *Pseudomonas* community. Increased pathogen inhibition correlated also positively with the *Pseudomonas* community survival in the rhizosphere, which suggests that more diverse communities could have exhibited elevated pathogen inhibition via density effects (higher the *Pseudomonas* population density, higher the amount of produced toxins). It should be noted that we did not quantify the antibacterial substances produced by *Pseudomonas* bacteria in our *in vitro* assay, and hence, further comparative genomics and/or metabolomics approaches are needed to unravel the mechanism underlying the toxicity of *Pseudomonas*. However, the filtration technique used in our assays is fast to perform and does not require prior knowledge of the molecular nature of the secreted compounds. Hence, this method could be generalised to other taxa and provide a valuable first-step screening tool to identify potential synergies between secondary metabolites, which could be further complemented with chemical analyses to gain more insight into specific mechanisms.

Even though it is difficult to disentangle the positive effects of resource competition and direct pathogen inhibition for the invasion resistance based on our data, structural equation modelling suggests that both modes of competition played significant roles. Especially, the niche breadth of the introduced *Pseudomonas* community was important by increasing the *Pseudomonas* and decreasing the pathogen densities. However, less clear patterns were found in the case of disease incidence, where only the *Pseudomonas* community richness seemed to significantly reduce disease development. This suggests that the high *Pseudomonas* community diversity increased plant pathogen suppression via some unidentified function. One such potential function could be bacterial cooperation ([15](#_ENREF_15)) or facilitation ([28](#_ENREF_28)). For example, it has been shown that bacteria that adapt to each other in diverse communities become more productive but also more dependent on each other ([28](#_ENREF_28)). *Pseudomonas* strains are also known to cooperate via production of siderophores that scavenge iron from the environment ([6](#_ENREF_6), [28](#_ENREF_28)). The extent to which these positive interactions affected the survival and the invasion resistance of the most diverse *Pseudomonas* communities in the present study is unknown. Moreover, bacterial diversity may also affect traits, such as biofilm formation or stress resistance, which are not captured in the measured parameters but may be important for function in the rhizosphere environment. This may explain why richness, but not the traits from the laboratory assays, predicted tomato disease. Regardless of these potential limitations, our data suggests that biodiversity-ecosystem functioning relationships are good indicators of the benefits of plant growth promoting bacterial communities to host plants.

Interestingly, diversity effects rather than the identity effects drove the functioning of the *Pseudomonas* communities once introduced into the natural rhizosphere microbiome: all strains performed better when grown in mixed communities compared to monocultures, and the invasion resistance was not systematically improved by the inclusion of any particular *Pseudomonas* strain. This suggests that pathogen suppression was an emergent and diversity-dependent community-level property. These findings have important implications for applied biology. Synthetic microbial communities are widely used in biotechnological processes due to their ability to provide functional properties that single microbial species or strain cannot offer ([29-31](#_ENREF_29)). Our findings suggest that biodiversity–ecosystem functioning theory can guide in assembling effective bacterial communities that reliably enhance microbiome function. We suggest that the present community-assembly principles can be transferred to other fields of microbiome research and biotechnology due to very general ecological mechanisms. Creating functionally diverse microbial consortia may increase the provisioning of focal functions particularly in complex environments, such as the rhizosphere ([32](#_ENREF_32)). Assemblages of different microorganisms combine properties unreachable by a single strain or species ([29](#_ENREF_29), [33](#_ENREF_33), [34](#_ENREF_34)) and have been proposed as a solution to improve industrial and agronomic processes ([31](#_ENREF_31), [35](#_ENREF_35), [36](#_ENREF_36)).

## MATERIALS AND METHODS

**Bacterial study strains.** We used eight fluorescent pseudomonad strains (CHA0, PF5, Q2-87, Q8R1-96, 1M1-96, MVP1-4, F113 and Phl1C2) as described previously ([20](#_ENREF_20)); for more information see Table S2. All strains were stored at -80°C. Prior to experiments, one single colony of each strain was selected randomly, grown overnight in lysogenic broth (LB), washed three times in 0.85% NaCl and adjusted to an OD600 of 0.5 using a spectrophotometer (Spectra Max M5, Molecular Devices, Sunnyvale, CA, USA). We used *Ralstonia solanacearum* QL-Rs1115 strain (Race 1 and Biovar 3) as a pathogen. This strain was originally isolated from tomato rhizosphere in Qilin (118°57' E, 32°03' N), Nanjing, China, is highly virulent, and able to cause wilting of tomato, eggplant, pepper, and potato ([13](#_ENREF_13)).

**Assembly of *Pseudomonas* communities.** We created 48 communities out of eight different *Pseudomonas* strains, which we combined following a substitutive design as described previously ([19](#_ENREF_19)) to obtain initial richness levels of 1, 2, 4, and 8 strains (Table S3). The diversity gradient was assembled so that each strain was drawn randomly, allowing disentangling the effects of strain identity and community diversity. We used a substitutive design so that the total biomass of every *Pseudomonas* community inoculant was kept the same in all treatments but the proportion of every single strain decreased with increasing community richness (100%, 50%, 25%, and 12.5% for 1, 2, 4, and 8 strain communities, respectively).

**Characterizing BEF relationships *in vitro*.** In order to link biodiversity effects to bacterial resource competition, we assessed the resource use of the eight *Pseudomonas spp.* and *R. solanacearum* strainson 48 different single carbon resources (Table S4) representative of tomato root exudates ([5](#_ENREF_5)). Briefly, bacteria grown overnight in tryptic soy broth (TSB, tryptone 15 g L-1, soy peptone 5 g L-1, NaCl 5 g L-1) were pelleted by centrifugation (4000 g, 3 min), washed three times in 0.85% NaCl before measuring their growth on 96-well microtiter plates containing OS minimal medium ([37](#_ENREF_37)) supplemented with 10 mM of single resource representative of amino acids, organic acids, and sugars found in tomato root exudates ([5](#_ENREF_5)). We used a total of 48 different single compounds as listed in Table S4. All microplate wells were inoculated with equal amounts of the specified bacterial mixtures (start OD600 = 0.05) and incubated for 48 h with agitation (170 rpm) at 30°C. Optical density (600 nm) was recorded at regular intervals with a spectrophotometer (Spectra Max M5, Molecular Devices, Sunnyvale, CA, USA). Community-level resource use metrics were characterized using two indices, niche breadth and niche overlap index, defined as a number of resources consumed by the *Pseudomonas* communities and the proportion of resource used by both *R. solanacearum* and the *Pseudomonas* community, respectively. Wells with an OD600 > 0.05 were scored as positive growth on any given substrate.

 In order to link biodiversity effects to direct inhibition of the pathogen, we quantified the pathogen growth in the presence of *Pseudomonas* supernatants. To avoid biases due to competition or facilitation between different *Pseudomonas* strains, we grew all the eight *Pseudomonas* strainsindividually in nutrient broth for 30 h (30°C, 170 rpm), after which cells were pelleted by centrifugation (4000 g, 3 min). Cell-free supernatants were then mixed in proportions matching the diversity gradient of the communities (1, 2, 4, and 8 strains richness levels, Table S3), and inhibition experiments started immediately. Briefly, 20 μl of supernatant mix was added to a fresh culture (180 µl, OD600 = 0.05) of the pathogen *R. solanacearum* in M-SMSA media ([38](#_ENREF_38)). Control treatments received 20 µl M-SMSA media. Bacteria were grown for 24 h (30°C, 170 rpm) before measuring bacterial densities as optical density at 600 nm using a spectrophotometer (Spectra Max M5 Plate reader, Molecular Devices, Sunnyvale, CA, USA). Pathogen inhibition was defined as the percentage of reduction in pathogen growth compared to pathogen growth in the control treatment.

**Validating BEF relationships in a greenhouse experiment.** The biocontrol efficiency of *Pseudomonas* bacterial communities was assessed in a 50-day long greenhouse experiment (overview of the protocol presented in Fig. S1). The soil was collected from a tomato field in Qilin town of Nanjing, China ([13](#_ENREF_13)), sieved at 5 mm, and homogenized. Please note, that the homogenised soil contained the natural microbial community. Similar to the *in vitro* experiments, we used the same 48 *Pseudomonas* community combinations (Table S3). Surface-sterilized tomato seeds (*Lycopersicon esculentum,* cultivar “*Jiangshu*”) were germinated on water-agar plates for three days before sowing into seedling plates containing Cobalt-60-sterilized seedling substrate (Huainong, Huaian soil and fertilizer Institute, Huaian, China). Germinated tomato plants were transplanted to seedling trays containing natural, non-sterile soil at the three-leaf stage (12 days after sowing). Twenty-four seedlings were transplanted into one seedling tray with 8 cells; each of which contained 500 g soil planted with three seedlings. Each tray was treated as one biological replicate. Two replicate seedling plates were used for all communities (and four replicate plates for positive control). After 10 days of growth, plants were inoculated with *Pseudomonas* communities by root drenching methods with final concentration of 5.0 × 107 CFU of bacteria g-1 soil ([39](#_ENREF_39)). After five days post inoculation of *Pseudomonas* communities, the pathogen *R. solanacearum* was inoculatedat a final concentration of 106 CFU of bacteria g-1 soil. Tomato plants were then grown for 35 days in a greenhouse (natural temperature variation ranging from 25°C to 35°C) and watered regularly with sterile water. Disease incidence per seedling plate was used as a disease index ([13](#_ENREF_13)). Seedling plates were rearranged randomly every two days. Disease progression was monitored daily after the pathogen inoculation. The experiment was terminated 35 days after pathogen inoculation when all the plants of positive control treatment showed symptoms of wilting.

**Tomato rhizosphere sampling and DNA extraction.** We performed a destructive sampling to estimate pathogen and introduced *Pseudomonas* abundances 5, 15, 25, and 35 days after the pathogen inoculation. We removed two randomly chosen plants per community at every time point from one of the replicate seedling plates (total of 416 rhizosphere samples). Rhizosphere soil was collected by first gently removing the plants from the pots before shaking off excess soil and collecting the soil attached to the roots. Samples were stored at -80°C for DNA extraction. Microbial DNA was extracted using Power Soil DNA Isolation Kit (Mo bio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. DNA quality was checked by running samples on 1% sodium boric acid agarose gel electrophoresis, and determining DNA concentration by using NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Extracted DNA was stored at -80°C for bacterial density analyses.

**Pathogen and *Pseudomonas* bacterial densities in the rhizosphere.** We used qPCR to quantify the abundance of the introduced *Pseudomonas* bacteria and the pathogen in the rhizosphere soil. *Pseudomonas* bacterial density was estimated with primers B2BF: 5'-ACC CAC CGC AGC ATC GTT TAT GAG C-3' and B2BR3: 5'-AGC AGA GCG ACG AGA ACT CCA GGG A-3' targeting the *phlD* gene ([40](#_ENREF_40)), which is part of the *phl* operon responsible for the synthesis of the broad spectrum antibiotics 2,4-diacetylphloroglucinol (DAPG). We use this gene as a reference as it is shared by all the used *Pseudomonas* strains, while being present only at a low background concentration in the reference soil (the background level is shown in all figures as red, dashed line). Pathogen density was quantified by using specific primers (forward: 5'-GAA CGC CAA CGG TGC GAA CT-3' and reverse: 5'-GGC GGC CTT CAG GGA GGT C-3') targeting the *fliC* gene coding the flagella subunit ([41](#_ENREF_41)). The qPCR analyses were carried out with Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, CA, USA) using SYBR Green I fluorescent dye detection in 20-μl volumes containing 10 μl of SYBR Premix Ex Taq (TaKaRa Biotech. Co., Japan), 2 μl of template, and 0.4 μl of both forward and reverse primers (10 mM each). The PCR was performed by initially denaturing at 95°C for 30 s, cycling 40 times with a 5 s denaturizing step at 95°C following 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 sample was replicated three times.

**Statistical analyses.** *In vitro* experiments: we used generalized linear models (GLM) to test whether *Pseudomonas* community richness affects niche breadth, niche overlap with the pathogen, and direct pathogen inhibition.

*Greenhouse experiment*: data was analysed in three ways. First, we used separate GLMs expressing disease incidence as well as pathogen and *Pseudomonas* community abundances as a function of the interactive effects of time and *Pseudomonas* community richness. Bacterial abundance data were log10-transformed and disease incidence data square arcsine-transformed prior to analysis. Second, we attempted to link the dependent variables to changes in the characteristics of the *Pseudomonas* community, including resource competition metrics (niche breadth and niche overlap), direct pathogen inhibition (toxicity), and *Pseudomonas* community density in the rhizosphere. Due to potential correlations between different explanatory variables, a sequential analysis was used to uncover the most parsimonious GLMs. To this end, we used stepwise model selection based on Akaike information criteria (AIC) to choose the model with best explanatory power (step () function in R). We used both a backward elimination starting with the full model and forward-selection model (from simple to full model) to avoid selecting a local AIC minimum ([42](#_ENREF_42)). Finally, we used structural equation modeling (SEM) to shed light on the mechanisms of disease incidence in tomato plants by accounting for multiple potentially correlated effect pathways. SEM analysis was chosen because it can disentangle the direct and indirect effects ([43](#_ENREF_43)) of diversity and community characteristic parameters *in vitro* for the survival of *Pseudomonas* communities, pathogen density in tomato rhizosphere, and for the disease incidence in the greenhouse experiment. The initial model was based on previous knowledge ([44](#_ENREF_44)) assigning the exogenous variable “richness” and the endogenous variables “niche breadth”, “niche overlap”, “toxin production”, “*Pseudomonas* density”, “pathogen density”, and “disease incidence”. Due to the relatively low level of replication and the complex structural equation model, we ran separate models for “pathogen density” and “disease incidence”. The adequacy of the models was determined via chi²-tests, AIC, and RMSEA ([44](#_ENREF_44)). Model modification indices and stepwise removal of non-significant relationships were used to improve the models; however, only scientifically sound relationships were considered ([43](#_ENREF_43)). Structural equation modeling was performed using Amos 5 (Amos Development Corporation, Crawfordville, FL, USA).

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at

Table S1, DOCX file, 24 KB.

Table S2, DOCX file, 17 KB.

Table S3, DOCX file, 21 KB.

Table S4, DOCX file, 16 KB.

Figure S1, DOCX file, 2227 KB.

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## Additional information

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## Figure Legends

**Figure 1. Characterization of biodiversity–ecosystem functioning relationships *in vitro*.**Panel (A): *Pseudomonas* community niche breadth was defined as the number of carbon sources used by at least one of the members of *Pseudomonas* community (detailed information on resources can be found in Table S4). Panel (B): *Pseudomonas* community niche overlap with the pathogen was defined as similarity in resource consumption between the resident community and the pathogen. Panel (C): Antibacterial activity of *Pseudomonas* community was determined as the reduction in pathogen density in the presence of *Pseudomonas* bacterial supernatants; all supernatants were derived from monocultures and mixed together when testing the synergistic effects.

**Figure 2. Characterization of biodiversity–ecosystem functioning relationships *in vivo****.* Panel (A): The dynamics of bacterial wilt disease incidence in *Pseudomonas* communities at different richness levels and at different points in time. Panel (B): Pathogen density dynamics as affected by *Pseudomonas* communities with different richness levels. Panel (C): *Pseudomonas* density dynamics in communities with different richness levels. Panel columns denote for 5 days, 15 days, 25 days, and 35 days post pathogen inoculation (dpi). The red dotted lines show the baseline for control treatments: in panels A and B, red dotted lines denote for disease incidence and pathogen density in the absence of *Pseudomonas* bacteria, and in panel C, for *Pseudomonas*-specific *phlD* gene density in natural soil in the absence of introduced *Pseudomonas* bacteria.

**Figure 3. Structural equation models testing the mechanistic links between *Pseudomonas* community richness and pathogen density (A) and disease incidence (B) 35 days after pathogen inoculation.** Panel (A): direct and indirect (via *Pseudomonas* community niche breadth and *Pseudomonas* community toxicity) richness effects on pathogen density. Panel (B): disease incidence was explained only by a direct richness effect. Blue circles in both panels denote for the proportion of the total variance explained. Blue arrows indicate negative relationships and red arrows indicate positive relationships; double-headed, dashed arrows indicate undirected correlations between different variables (no hypothesis tested), and grey arrows indicate non-significant relationships between different variables. Arrow widths indicate the relative effect size and the numbers beside the arrows show standardized correlation coefficients (relative effect sizes of non-significant correlations are not shown).

Figure S1. Overview of the greenhouse experiment. Surface-sterilized tomato seeds (*Lycopersicon esculentum*, cultivar “Jiangshu”) were germinated on water-agar plates for three days (A) before sowing into seedling plates (B) containing Cobalt -60-sterilized seedling substrate (Huainong, Huaian soil and fertilizer Institute, Huaian, China). At the three-leaf stage (12 days after sowing), tomato plants were transplanted to seedling trays (350mm×250mm×100mm) containing the same natural soil as described in the materials and methods (C). Sixteen seedlings were transplanted into one seedling tray with 8 cells with each containing two seedlings. Tomato plants were first inoculated with *Pseudomonas* bacterial communities by drenching method (Wei et al. 2011) ten days after the transplantation (with ending *Pseudomonas* density of 5.0 × 107 CFU g -1 soil). Pathogen was inoculated five days later (ending *R. solanacearum* density of 106 CFU g-1 soil). Tomato plants were grown in a greenhouse with natural daily temperature variation ranging from 25 °C to 35 °C and watered regularly with sterile water. The number of wilted plants per seedling plate was recorded on daily basis after the pathogen inoculation (D-E): red flags represent the number of wilted and infected tomato plants. The experiment was ended 50 days after the transplantation when all the plants in the control treatment (*R. solanacearum* only) showed disease symptoms.

Table S1. Analysis of variance showing the effect of *Pseudomonas* strains’ identity on disease incidence, pathogen and *Pseudomonas* community abundance, and transgressive overyielding (*Pseudomonas* strain abundances when grown in polycultures versus monocultures) in *Pseudomonas* communities at 5 days, 15 days, 25 days and 35 days post pathogen inoculation (dpi).

Table S2. List of the bacterial species and strains used in this study.

Table S3. Composition of the *Pseudomonas* bacterial communities used in this study (0 and 1 denote for the absence and presence of *Pseudomonas* strains in given community, respectively).

Table S4. Carbon resources used to quantify pathogen and *Pseudomonas* community resource use metrics (niche breadth and niche overlap).