Modulation of biodiversity-invasion relationships by resource availability

Commensal species defend invaders

in a changing world

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Modulation of biodiversity-invasion relationships by resource availability

Commensal species defend invaders in a changing world

Modulatie van biodiversiteit-invasie verhoudingen door resource beschikbaarheid

Commensale soorten verdedigen tegen indringers in een veranderende wereld

(met een samenvatting in het Nederlands)

Proefschrift

Ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G. J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 18 april des middags te 12.45 uur

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Chapter 1

General introduction

Tianjie Yang

Biological invasion and plant pathogens

Impacts of biological invasion

Species migration is an integral part of community assembly (Tilman 2004). In some cases, new species can enhance ecosystem functions, as exemplified by the enhancement of plant biomass and microbial respiration after large-dose microbial inoculations to soil (Singh et al. 2015). However, an introduced species can also disturb existing communities, especially in cases where introduced species become invasive. Indeed, biological invasion is one of the main threats to ecosystems (Mooney and Hobbs 2000) by affecting biodiversity, species interactions and ecosystem functioning (Heywood 1989, Galiana et al. 2014, Lurgi et al. 2014). Invasive species can outcompete native species or drive niche shifts (Medley 2010, Tingley et al. 2014), thereby impacting local ecosystem structure and functioning. Specifically, a successful invasion can profoundly reduce local biodiversity due to the competitive advantage held over local species (Kenis et al. 2008, Vilà et al. 2011). An invader can alter local resource profiles by exploiting resources or releasing substances, which can in turn lead to changes in the resident community composition (Gu et al. 2016).

Anthropogenic activities continue to increase the possibilities for species invasions across ecosystems. Furthermore, global change, including nutrient deposition and changes in temperature and precipitation, not only result in range shifts, but can affect native communities in such ways that make them more sensitive to invasion (Dukes and Mooney 1999, Stevens et al. 2004). Community invasibility is influenced by several factors (Levine et al. 2003, Weltzin et al. 2003), with biodiversity being accepted as one of the most important ones. A high diversity of the rhizosphere community can boost plant fitness and resistance to pathogens (Berendsen et al. 2012). However, the underlying mechanisms that dictate the relationship between biodiversity and susceptibility to invasion are not fully understood (Lonsdale 1999). Invasion success is species-dependent and is determined by traits of both the invader and the resident community (Lurgi et al. 2014). In order to better understand invasion patterns, it is necessary to disentangle characteristics of both the invader and the resident species or community.

Biological invasion in the rhizosphere

The rhizosphere is a hotspot for microbial activities (Bakker et al. 2013), where the functions of resident bacteria and fungi can have positive, neutral or negative impacts on plant growth (Lugtenberg and Kamilova 2009, Berendsen et al. 2012). Plant diseases directly reduce plant productivity, thereby threatening world food supply (Strange and Scott 2005). Soil-borne plant pathogens, including bacteria, fungi and oomycetes, are mainly introduced into local environments by water

pollution or anthropogenic activities. Once in the soil, recruitment from the bulk soil to the rhizosphere is necessary for a pathogen to impact root and plant health. For some pathogens, invasion is part of the disease processes (Loo 2009, Shanmugam et al. 2011). Pathogens first must enter root-associated communities, and subsequently the outcome of competition between pathogens and the resident community determines the success of pathogen invasion in rhizosphere. If plant pathogens can out-compete residents of the native community and increase their density, the resident community structure can collapse leading to serious declines in plant health (Figure 1).

Model invader: *Ralstonia solanacearum*

In this thesis, I use the plant pathogen *Ralstonia solanacearum* as an example of an important pathogenic invader. *R. solanacearum* is a soil-borne Gram-negative bacterium, which can cause bacterial wilt disease in over 50 plant families and more than 200 plant species, mostly in solanaceous plants (Hayward 1991, Yabuuchi et al. 1995). The pathogen causes enormous agricultural and economic losses due to its wide host range, complex subspecies and strong persistence (Denny and Hayward 2001, Elphinstone et al. 2005) (Figure 2). During the *R. solanacearum* infestation process, the pathogen multiplies in the plant inner cortex after invading through plant wounds. *R. solanacearum* then rapidly expands to the stem and crown through the vascular system (Vasse et al. 1995) by degrading plant cell walls. With the accumulation of *R. solanacearum*, the plant becomes stunted, wilted and ultimately dies (Tan et al. 2013a).

Biological control of *Ralstonia solanacearum*

Many strategies have been employed in attempts to suppress *R. solanacearum*. Traditionally, chemical fungicides have been used to control *R. solanacearum* and other pathogens, but such fungicides are being phased out due to their detrimental effects on the environment and potentially human health (Komárek et al. 2010). Pathogens can also be eradicated by soil sterilization, but such measures also destroy the resident communities, which also harbor beneficial microbes, including those that may inhibit pathogen invasion (Labeda et al. 1975). Plant breeding programs also offer potential for increasing plant defenses against pathogens, but such approaches have their limitations, which cannot find a suitable cultivar for every different arears (Chen et al. 2015).

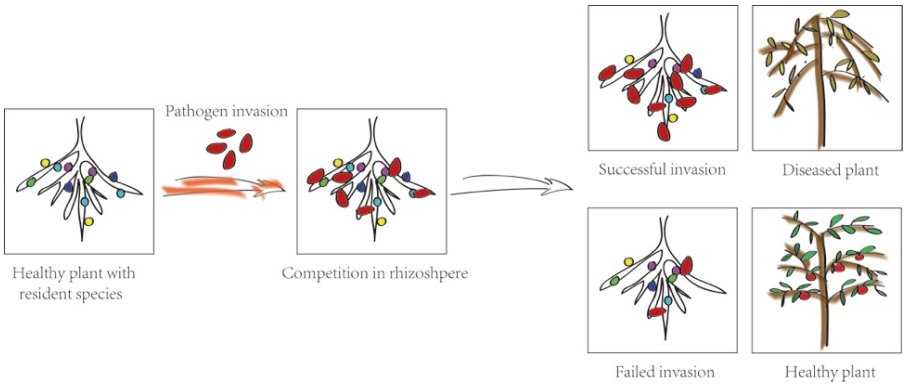

Figure 1. Mechanism by which pathogens cause plant disease by invading the rhizosphere. Pathogen invasion interferes with species interactions in the rhizosphere and can ultimately lead to plant disease. Whether a pathogen can out-compete the resident community dictates invasion success. If highly diverse resident communities can reduce plant pathogen success, plant health can be enhanced.

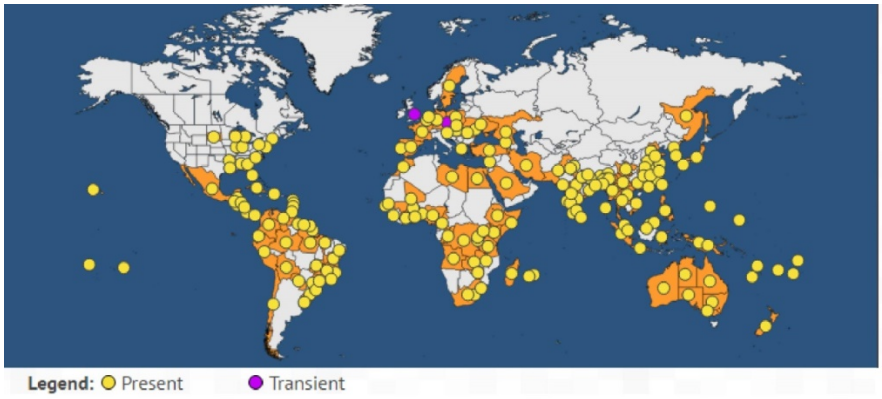

Figure 2. World distribution of *R. solanacearum* (Source: EPPO Global Database, https://gd.eppo.int/).

It is clear that more environmentally friendly and cost-effective strategies for the control of *R. solanacearum* are necessary. Biological strategies, for instance utilizing microbial antagonists of pathogens, have been studied intensively over the past two decades (Haas and Défago 2005, Pliego et al. 2011). The application of biocontrol agents can have several advantages over the use of chemical pesticides. For instance, a biological inoculum may be self-sustaining due the reproductive capacity the biocontrol agent. (Yuliar et al. 2015). Also, as may biocontrol agents are actual native to the soil systems in which they are used, they might be expected to induce less of an overall disturbance compared to foreign agents of control.

Several kinds of biocontrol agents have been used as plant protectors, including avirulent mutants (Etchebar et al. 1998, Chen et al. 2015), antagonistic species (Ji et al. 2008, Tan et al. 2015) and closely related species (Wei et al. 2013, 2015). In addition, the general biocontrol capabilities of soil microbiomes as a whole have been recognized in the development of suppressive soils (Haas and Défago 2005). The main aim behind the use of avirulent mutants is to create a new microbial strain without virulent genes by gene modification, such as a knockout. Mutants have been used to control pathogens in many fields, not only plant pathology, but also in control of mammalian diseases (Blander et al. 1989, Trigalet and Trigalet-Demery 1990, Tagg and Dierksen 2003). However, screening avirulent mutants can be technically demanding and expensive. However, such strategies demonstrate that avirulent strains closely related to important pathogens, including natural isolates, can inhibit pathogen success. From the perspective of competition, avirulent closely related species may share more common niches with a pathogen without harming the plant hosts (Wei et al. 2013, Peyraud et al. 2016). Although promising, the success of many biocontrol strategies has remained unpredictable. In order to develop more effective and reliable biocontrol agents, it is vital to decipher their interactions with pathogens.

Biodiversity and invasion relationships

Biodiversity plays as a significant role in invasion resistance (Kennedy et al. 2002). Theory predicts that communities with more diverse species are more resistant to invasion (Case 1990, Law and Morton 1996), because of the higher resource preemption of the community and less unconsumed resources available for invader establishment (Elton 1958). Previous observational and experimental studies using plant communities have supported this mechanism (Tilman 1997, Knops et al. 1999, Naeem et al. 2000, Levine 2000). Subsequently, similar mechanisms have been inferred in studies related to marine ecology and microbial ecology (Stachowicz et al. 1999, 2002, Van der Putten et al. 2007). From the gut to terrestrial ecosystems, biodiversity appears to help prevent invasion mainly due to functional diversity (Tilman 1997, Dillon et al. 2005, van Elsas et al. 2012). This

buffer against invasion can be due to resource use complementarity (Naeem et al. 2000, Hooper et al. 2005), antagonistic interactions (Postma et al. 2008) or the combined action of multiple functions (Symstad 2000, Hodgson et al. 2002). In addition, a highly diverse community has a greater possibility of containing species with specific negative interactions with the invading species(Wardle 2001, Fargione and Tilman 2005).

In the rhizosphere, biodiversity is one of the main determinants of resistance to invasion. However, the issue of biodiversity has generally been ignored in the development of microbial biocontrol strategies. Such strategies have typically developed by examining the traits and antagonistic qualities of specific isolates in one-on-one assays with the pathogen (Pliego et al. 2011, Zhao et al. 2013). Although such strategies can reveal interesting interactions between such isolates and plant pathogens, they generally ignore the fact that pathogens and antagonists both experience multiple interactions in the rhizosphere. This may explain why many single-species biocontrol agents can often be unreliable in their application to the field, even when *in situ* tests show great promise. It is necessary to consider the many interactions at play, including microbe-microbe, plant-microbe and soil-microbe interactions, that ultimately determine biocontrol effectiveness (Chaparro et al. 2012). As a whole, the microbiome plays an important role not only as a barrier to inhibit plant pathogens, but also by helping in plant nutrient uptake and growth promotion (Lugtenberg and Kamilova 2009, Bakker et al. 2013). It has been shown that reassembled soil communities can provide strong inhibitions to pathogenic invaders under laboratory conditions (De Boer et al. 2007, van Elsas et al. 2012). Furthermore, *in vivo* experiments have shown that complex microbial community inoculants can also reduce pathogen invasion in heterogeneous systems (Matos et al. 2005, Hu et al. 2016). These findings highlight the importance of diversity of the soil microbiome in resisting pathogen invasion. Thus, although a basic understanding of biodiversity-invasion relationships is emerging, additional studies should continue to be carried out to further disentangling the mechanisms behind these relationships (Levine et al. 2003, Fargione and Tilman 2005, Latz et al. 2016).

Mechanisms driving biodiversity-invasion relationships

Community richness enhances invasion resistance via niche overlap

A successful invasion proceeds via four stages; transport, colonization, establishment and landscape spread. The second and third stages of this process rely on the ability of the invader to survive given the resource availability and niche space of the recipient environment (Theoharides and Dukes 2007). Available resources allow invaders to establish and reproduce, eventually outcompeting resident species. In a broader sense, resource availability can be seen as an important element within the “niche” concept, with vacant niches providing room

for invader establishment. The definition of niche, as formulated by MacArthur (1968), Hutchinson (1957) and Levins (1968), represents the conditions under which a species can survive. If the resident community effectively occupies all available niches, introduced and potentially invasive species have no room to establish and proliferate (Tilman 2004, Mwangi et al. 2007). According to early landmark studies (Gause 1932, Macarthur and Levins 1967), niche overlap describes similarity in resource use among coexisting species (Colwell and Futuyma 1971). Species assemblages can reduce the abundance of invaders by high niche overlap (Wilson and Lindow 1994, Ji and Wilson 2002, Dianese et al. 2003).

Community richness enhances invasion resistance via biodiversity effects

In both synthesized and natural communities, functional diversity increases with the number of species present, leading to increased community performance. Such biodiversity effects can be explained via two main pathways: complementary and selection effects. In the complementary effect, species with niche differentiation or niche partitioning (Loreau 2000, Loreau and Hector 2001) allow for more total resource use of the community at high diversity. The selection effect entails that specific species with particular functions or traits are selected out of the community (Tilman et al. 1997, Loreau and Hector 2001). In terms of invasion, species which are more resistant to invasion contribute to the selection effect (Hodgson et al. 2002). These two biodiversity effects can also reduce invasion in combination (Fargione and Tilman 2005, Eisenhauer et al. 2012b, Byun et al. 2013).

Species interactions within communities

Allelopathy/antagonism. In most cases, diverse communities have a negative effect on invasion success as discussed above. However, highly diverse communities can also be more susceptible to invasion. In such cases, high overlap in resource use or functioning within a community may result in strong competition between resident community members (Becker et al. 2012). With the increase of diversity in the community, resource partitioning is constrained by limited resources, resulting in a completion between species for resources (Bell et al. 2009). Species may also overproduce toxic substance to increase their own fitness, thereby suppressing competition (Jackson and Buss 1975, Wardle et al. 1998). Antagonistic interactions within the community can thus reduce community ecosystem functioning (Jousset et al. 2011b, 2011c). Such examples of a negative biodiversity-invasion relationship illustrate that is important to take functional or genotypic dissimilarity into account when describing community structure in relation to resistance to invasion.

Facilitation. Besides negative interactions within community, positive interactions

can promote species growth and enhance functioning of the whole community. Facilitation is defined as an interaction occurring between species that benefits at least one species and does not harm either of them (Bruno et al. 2003). One species can facilitate neighboring species by providing resources, resulting in increasing resource availability and broadened niche breadth (Higashi 1993, Bertness et al. 1999). Such interactions can lead to changes or increased complexity within community structure (Hector et al. 2002). Community invasibility can be promoted or constrained by the existence of facilitators (Callaway and Walker 1997). Cross-feeding represents a very special case of facilitation (Wu et al. 2006, Bertin et al. 2011). Metabolic products can be produced and exchanged, benefiting to both of the species in a symbiotic way (Faust and Raes 2012, Pande et al. 2013). Species in the community can be partly fed by each other, thus the pressure of competition can be released, and diversity of the community may be maintained, especially in resource-limiting environments (Germerodt et al. 2016). This can promote the stability and resistance of the community, for instance to invasion. Thus, in order to predict invasion success, one must first understand the metabolic capabilities and interactions within the resident community.

Trophic characteristics within the community

It is not possible to predict community characteristics simply by adding up the individual interactions of specific interacting partners. Rather, performance of a given species, such as an invading species, is determined by a complex web of interactions that ultimately determines the performance of the target species. Trophic networks describe the topology of community interactions and offer insight into community responses to potential invaders. In this regard, there are two particularly important characteristics of trophic networks that can be used to help predict resistance to a specific potential invader, connectance and nestedness.

Connectance represents the proportion of realized ecological interactions out of all potential interactions (Martinez 1991, Poisot and Gravel 2014). This concept was originally used to describe how species connect with each other, but the definition can be extended to reflect species' average niche breadth. The distribution of species and community stability are modulated by connectance (May 1972, Thébault and Loreau 2005, Gravel et al. 2011, Poisot et al. 2013), thereby also altering ecosystem functioning. For example, connectance is a good predictor for invasion, by either promoting or constraining the invader from different trophic levels (Baiser et al. 2010). If community connectance is high, there is high possibility that species occupy niches in a complementary fashion (Poisot et al. 2013), thereby decreasing room for invasion (Romanuk et al. 2009). Alternatively, it has also been suggested that in some cases, higher connectance

can increase invasion success, due to an increase in competition and species loss in the community (Lurgi et al. 2014).

Nestedness is defined as the degree to which the resources used by a certain species represent a subset of resources used by all species, as originally summarized from habitat nested subset theory (Young and Darlington 1957, Novak et al. 2011). In a mutualistic community, high nestedness was shown to enhance community stability (Thébault and Fontaine 2010, Rohr et al. 2014). A high degree of nestedness has also been shown to decrease persistence of the community (Thébault and Fontaine 2010), due to the competition between community members for similar subsets of resources. As competition for resources becomes more intensive, susceptibility of the community to invasion increases.

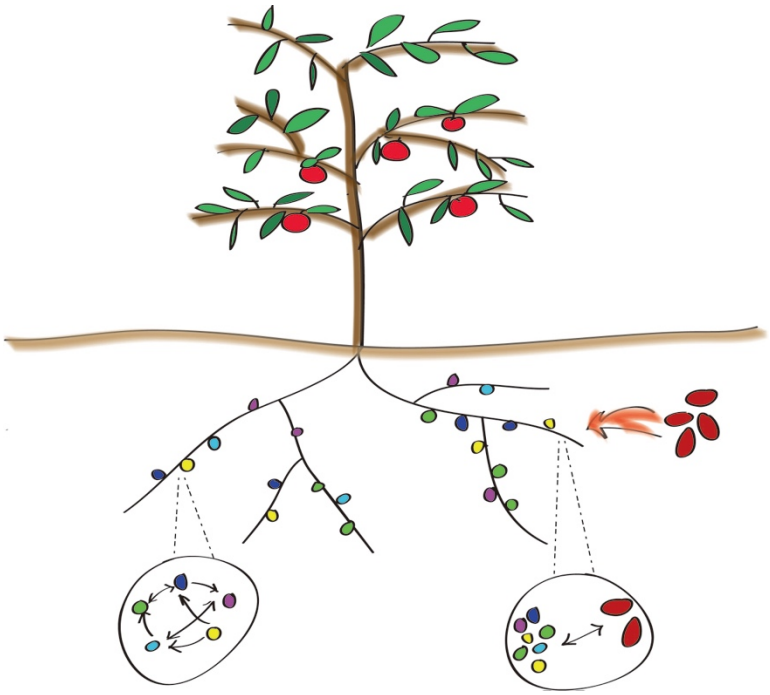

Figure 3. Rhizosphere microbiome interactions. Diverse resident communities colonizing the rhizosphere contain a complex network of interactions that may include competition, facilitation and cross-feeding (left circle). The introduction of an invasive species into the rhizosphere can lead to a perturbation of resident interaction networks (right circle).

Biodiversity / invasion relationships modulated by resource availability

Invasion mediated by resources at large scales

Statistical models have been used to examine both competition and resource availability (Byers and Noonburg 2003) when investigating diversity-resistance relationships. Negative biodiversity-invasion relationships can be explained by the decrease in resources left over for the invader after consumption by the resident community (Tilman 1997, Naeem et al. 2000). As an extrinsic factor, resource availability modulates invasion in both directly and indirectly.

Many studies have suggested that high resource availability can promote the establishment of invasive species. For instance, fertilization has been shown to favor the invasion of non-native plant species by reducing local species richness (Huenneke et al. 1990). Although fertilizer may relieve nutrient limitations, potentially leading to less competition between species, nutrient inputs typically result in uneven responses among resident species. As nutrient additions, unevenly select resident species, total diversity decreases. This creates less stable systems that more readily allow for invasion. Therefore, relationships between diversity and invasion success are modulated by resource availability (Stachowicz et al. 2002).

In principle, resource addition could promote invasion by creating vacant niches for invasive species. In the special case of resource pulses, invasion was facilitated by unconsumed resources favored by the invader (Li and Stevens 2012, Mallon et al. 2015). Fluctuating resource theory indeed suggests that resource pulses can weaken the resistance of community to invasion, because increasing resources cannot be fully consumed by the resident community (Davis et al. 2000).

Invasion mediated by resources in the rhizosphere

The effect of resource availability on modulating invasion is well-studied at relatively large scales. However, similar mechanisms may also be at play at a much smaller scale, for instance at the level of microbial interactions in the rhizosphere. Within the rhizosphere, the available resources are mostly root exudates, offering rich range of simple and complex chemical components (Bais et al. 2006). These low-molecular-weight compounds can modulate the community structure of microbiome (Badri et al. 2013) in the rhizosphere, and can be further related to plant disease defense (Carvalhais et al. 2015). Changes in root exudate profiles have been shown to cause large shifts in the rhizosphere microbiome (Marschner et al. 2002). The richness of exudate-mediated plant-microbe interactions, is also impacted by plant community structure and diversity (Garbeva et al. 2004, Latz et al. 2012). Moreover, root exudates enhance several microbial attributes that have been shown to be involved in resisting pathogen invades, including biofilm formation, root colonization, chemotaxis and expression of key functional genes

etc. (Jousset et al. 2011a, Tan et al. 2013b, Zhang et al. 2015). Stimulation of these traits has been shown to be closely correlated with the pathogen inhibition capacity in rhizosphere (Chin-A-Woeng et al. 2000, Webb et al. 2003). In addition to this indirect effect, some pathogens can be inhibited directly by root exudates (Bais et al. 2005). An example is the effect of phenolic acid on *Fusarium oxysporum* (Ling et al. 2013). Root exudates, and their impacts on the soil microbiome, are thus clearly critical to predictions of pathogen suppression.

Understanding biodiversity/invasion relationships from a genomic perspective

Via a range of *in vitro* and *in vivo* approaches, biodiversity-invasion relationships can be investigated by manipulating resident communities in a variety of ways. However, serious limitations remain. These include the fact that species express different phenotypes in response to various environmental conditions, which may be confounded in experimental designs or be highly variable in field experiments. Furthermore, most studies are designed to examine diversity via a suite of specific phenotypic traits. However, choice and definition of such traits can be somewhat arbitrary, and may not cover all relevant traits. Recent advances in genomics allow for a far more complete and unbiased examination of niche and metabolic relationships. Further major advances have been made recently in linking genotypes to phenotypes, for instance via metabolic reconstruction studies and complete transcriptomic analyses (Chen et al. 2009, Remenant et al. 2010, Garbeva et al. 2011, Zhang et al. 2015).

Genomic-scale metabolic modeling provides the ability to predict phenotypes by simulating specific conditions, thereby providing insight into the species interactions metabolic (inter)activities (Henry et al. 2010, Bordbar et al. 2014). Flux balance analysis (FBA) can be used to predict metabolic fluxes *in silico* by reconstructing genome data and mathematical models (Varma and Palsson 1994, Orth et al. 2010). Such approaches have proven successful in predicting metabolic patterns and species interactions (Feist et al. 2007, Mccloskey et al. 2013, Mee et al. 2014). For instance, studies have been reported that validated predicted FBA data by comparing such prediction to observed experimental data (Yus et al. 2009, Lewis et al. 2010). This consistency between predicted and observed data demonstrates the feasibility and possibility of simulating biodiversity-invasion relationships under various conditions. Currently, FBA has mostly used to predict the metabolic capacity of pathogens (Raghunathan et al. 2009, Peyraud et al. 2016), but such approaches can also be used to predict competitive interactions.

Outline of this thesis

Biological invasion has been identified as an important threat to global biodiversity (Heywood 1989, Mooney and Hobbs 2000). According to theoretical and empirical work, increasing biodiversity could profoundly reduce invasion by providing a more complete consumption of resources, thereby leaving limited niche space available for invader establishment (Fargione and Tilman 2005, Eisenhauer et al. 2012b, van Elsas et al. 2012). In addition, it is becoming increasingly clear that resource availability also modulates invasibility (Davis et al. 2000, Kuebbing et al. 2013, Mallon et al. 2015). However, there few studies have attempted to examine the interactive effect of biodiversity and resource availability on invasion success. Thus, the work in this thesis first seeks to determine whether biodiversity can reduce invasion and to examine the trophic characteristics of the resident community that best predict inhibition of invader growth. The work further seeks to examine the hypothesis that resource availability can modulate biodiversity-invasion relationships. Finally, in order to simulate biodiversity-invasion relationships under specific, genomic-scale metabolic models were used to predict the metabolic flux of each species and species interaction. This also allowed for a comparison between empirical and model results.

Throughout the course of this thesis research, *R. solanacearum* was used as a model pathogenic invader. Five closely related strains were chose as resident species and combined into 31 different communities (species richness from one to five, all possible combinations included). The model pathogen was co-cultured in each of the resident communities in a series of microplate microcosm and pot-based greenhouse experiments in **Chapter 2**, Figure 4). The advantages of microplate liquid culture system are that resources can be well-distributed and easily manipulated and experimental designs can incorporate a high degree of replication. Previous studies have shown that such platforms are ideal for examining biodiversity-ecosystem function relationships, including functions relevant to pathogen suppression (Jiang et al. 2011, Jousset et al. 2011b).

In **Chapter 2**, I investigated whether high species richness could reduce pathogen invasion by mimicking 20 kinds of rhizosphere environments in laboratory microcosms. In order to understand the underlying mechanism, I calculated trophic networks within microbial communities and between communities and invader by measuring resource use patterns of all the single strains in sole resource medium. I sought to determine the relationship between trophic networks and pathogen invasion (invasion success and invader growth). This study also served as a proof-of-principle for the *in vitro* sterile rhizosphere system used in this investigation. Proportions of wilted plants were used as a reflection of pathogen invasion in the plants. This study sought to address the

hypothesis that increased resident community diversity would reduce pathogen invasion. Trophic networks within the communities and between microbial communities and invader could be used as reliable predictors of invasion both *in vivo* and *in vitro*.

In **Chapter 3**, I continued to examine the biodiversity-invasion relationship, but now with several levels of resource availability. The same community combinations and invasion process were used in this chapter. Different resource availability was represented by diluting nutrient broth liquid medium. In this way, I could investigate whether and how the biodiversity-invasion relationship could be modulated by resource availability. If there would be negative relationship between biodiversity and invasion, it was also a goal to determine whether there were some species out-leading in the community, thereby boosting the resistance ability to invasion.

Not only are total amounts of resources potentially important in modulating community susceptibility to invasion, but resource stoichiometry may also be critical. **Chapter 4** therefore examined invasion as a function of different ratios of carbon, nitrogen and phosphorus (two kinds of nitrogen, 12 treatments in total). I hypothesized that resource ratio (stoichiometry) could also modulate biodiversity-invasion relationships. In addition, resource stoichiometry is a good predictor of ecosystem functioning (community productivity and invasion).

The work presented in Chapters 2 through 4, allowed me to examine whether observed resource use profiles could predict invasion, and if these biodiversity-invasion relationships are modulated by different resource conditions. The next goal was to fit these results into a more general ecosystem structure, using a more genomic perspective (**Chapter 5**). To this end, the genomes of all the strains used in this thesis were sequenced. The resulting genome data was used to build metabolic networks as related to specific resource conditions. Flux balance analysis was carried out by reconstructing metabolic networks. This work yielded predicted trophic traits based on this genomic-scale model, which could be compared to the phenotypic traits previously measured and used in the previous chapters. Moreover, former data from *vivo* and *vitro* experiments were validated using these predicted trophic traits. I hypothesized that predicted metabolic networks could be used to predict species interactions in the real world.

In **Chapter 6**, a synthesis and a general discussion are provided based on the results in this thesis and knowledge of biodiversity-invasion relationships. The discussion is mainly about mechanism of competition between resident community and invader and the driver, resource availability.

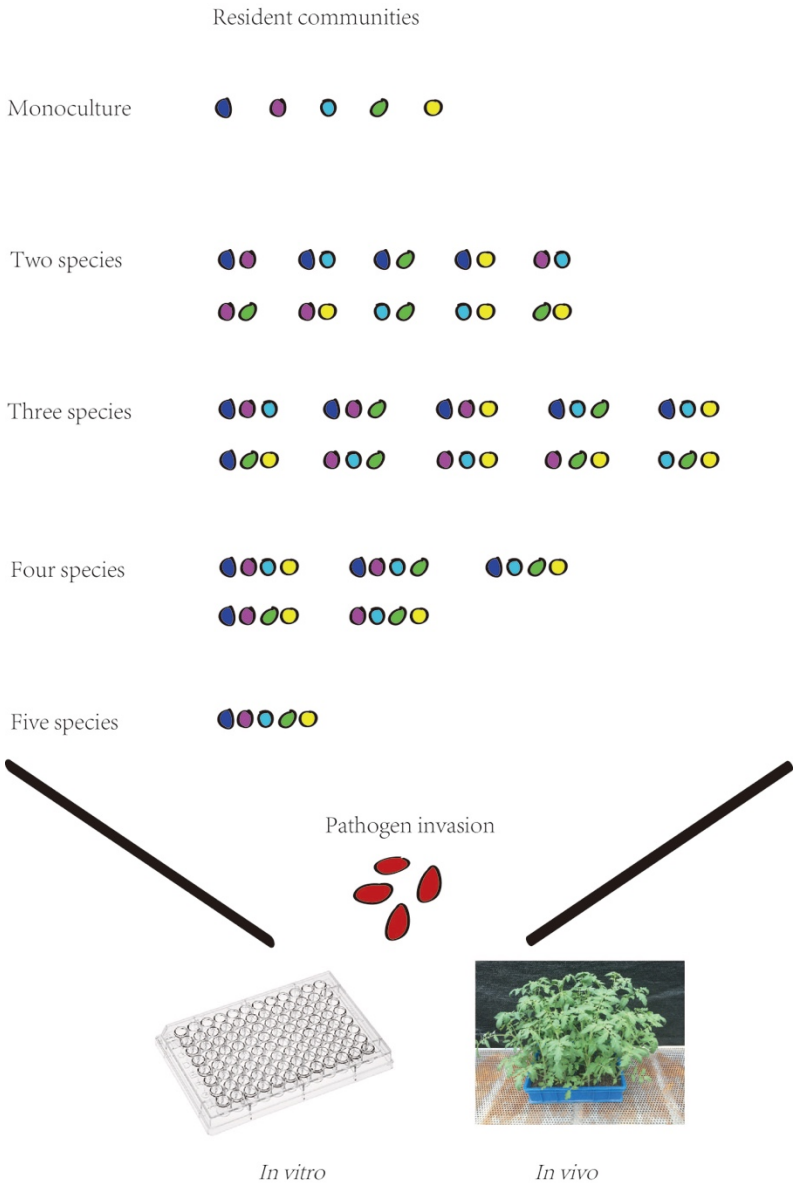

Figure 4. Scheme of experimental design. Five closely related *Ralstonia* strains were combined to construct resident communities (from one to five richness, all possible combinations included). The pathogen was co-cultured with all the communities. Subsequent experiments were performed used plant-soil systems in the greenhouse **(Chapter 2)**.

Chapter 2

Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health

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ABSTRACT

Host-associated bacterial communities can function as an important line of defense against pathogens in animals and plants. Empirical evidence and theoretical predictions suggest that species-rich communities are more resistant to pathogen invasions. Yet, the underlying mechanisms are unclear. Here, we experimentally test how the underlying resource competition networks of resident bacterial communities affect invasion resistance to the plant pathogen *Ralstonia solanacearum* in microcosms and in tomato plant rhizosphere. We find that bipartite resource competition networks are better predictors of invasion resistance compared to resident community diversity. Specifically, communities with a combination of stabilizing configurations (low nestedness and high connectance), and a clear niche overlap with the pathogen, reduce pathogen invasion success, constrain pathogen growth within invaded communities and have lower levels of diseased plants in greenhouse experiments. Bacterial resource competition network characteristics can thus be important in explaining positive diversity-invasion resistance relationships in bacterial rhizosphere communities.

INTRODUCTION

Bacterial communities that cover virtually all host surfaces from skin, gut and mucosa of animals (Grice and Segre 2011, Costello et al. 2012) to plant roots and leaves (Lundberg et al. 2012) or fungal hyphae (Scheublin et al. 2010) can function as a first line of defense against invading pathogens (Mendes et al. 2011, Shin et al. 2011, Berendsen et al. 2012, Bletz et al. 2013). While it has been found that highly diverse bacterial communities are often more resistant to pathogen invasions, having positive effects on associated host health (Dillon et al. 2005, van Elsas et al. 2012), the underlying mechanisms are still debated (Dillon et al. 2005, Bletz et al. 2013). The diversity resistance hypothesis argues that diverse communities are highly resistant to invasions due to high number of species interactions (Case 1990) and intensified competition for niche space (Kennedy et al. 2002, van Elsas et al. 2012). While this pattern is supported by both theory (Case 1990) and experiments (Tilman 1997, Knops et al. 1999, Stachowicz et al. 1999, Levine 2000, van Elsas et al. 2012), diversity-invasion resistance relationships are more complex (Levine and D'Antonio 1999) and can vary from positive to negative depending on the spatial scale (Matos et al. 2005, Irikiin et al. 2006) and resource heterogeneity (Kennedy et al. 2002). As a result, identifying underlying community properties that correlate with high species diversity could be important in explaining bacterial community resistance to pathogen invasions and help to develop synthetic communities that can efficiently suppress diseases.

High diversity might increase community invasion resistance due to interactive effects on community stability (McCann 2000). For example, functionally diverse communities can be more resistant to invasions because some trophic interactions stabilize otherwise dominant competitive interactions within the community (Paine 1984). Diversity could also affect the antagonistic and facilitative trophic interaction networks between the different members of the resident community (Thébault and Fontaine 2010, Baiser et al. 2010, Kéfi et al. 2012). The structure of these kind of ecological networks, *i.e.* the configuration and the distribution of the different links among interacting species, can provide strong predictions on the function and stability of ecosystems (Thébault and Fontaine 2010). For instance, nestedness and connectance of species interaction networks can affect community stability and persistence (Thébault and Fontaine 2010, Rohr et al. 2014), and recent modeling studies have linked these interaction network structures to community invasion resistance (Romanuk et al. 2009, Galiana et al. 2014, Lurgi et al. 2014). As a result, interactions both within plant-associated rhizosphere communities and between the resident communities and the invading pathogen are likely to be important for plant health and fitness.

Here we directly examined the distribution of trophic links as underlying mechanisms to predict invasion resistance of plant root-associated bacterial

communities against an invading pathogen and the subsequent reduction of disease incidence. We focused on the important soil-borne bacterial pathogen *R. solanacearum*, the causative agent of bacterial wilt capable of infecting over 200 different plant species across 50 different families (Yamada et al. 2007). In order to cause disease, *R. solanacearum* pathogen must first invade the resident bacterial communities and reach a threshold density to become virulent (Von Bodman et al. 2003). We specifically focused on bacterial resource competition networks as a putative mechanism linking resident community composition, pathogen invasion success and subsequent spread of bacterial wilt disease in tomato plants (van Elsas et al. 2012).

Resident bacterial community was constructed by using five, phylogenetically related but non-virulent, *Ralstonia* spp. species (Wei et al. 2013), which were originally isolated from the tomato plant rhizosphere. The rationale behind using closely related but non-virulent species was to maximize the similarity in resource use between the resident community and the invading pathogen via close phylogenetic relationship (Wei et al. 2013). We first verified that the tested species did not show any direct antagonism towards each other in terms of growth inhibition (Wei et al. 2011), *i.e.* that these species interacted only via resource competition. We then characterized the resource consumption patterns of both the pathogen and each of the resident species on 48 labile carbon sources typically found in tomato plant rhizosphere (amino acids, organic acids and sugars). This information was then used to build bipartite networks reflecting bacterial interactions mediated by resource competition (Figure 1). In this approach all trophic links, measured by the ability of each consumer (bacteria) to grow on each resource (single carbon source), are summarized in matrices to characterize network properties of different communities. By using different combinations of bacterial species with varying species richness and community composition (31 different communities covering all possible combination of the five resident community species), we could define the niche overlap in resource competition patterns for the invading pathogen and each resident community, as well as the connectance and nestedness of the resource competition networks of the resident communities, chosen for their relevance for resource competition and prevalence in nature (Whitfeld et al. 2014) (Figure 1).

Three different network variables were used to describe 1) similarity in resource consumption between the resident community and the invader (niche overlap) and 2) the intensity of resource competition within resident communities (connectance and nestedness). In our study, niche overlap describes the ecological similarity between the invader and the resident community respective to their resource use. High niche overlap between the resident community and the invader is thus likely to be a decisive factor affecting invasion success (Ji and Wilson 2002, Tilman 2004) as high overlap will increase resource competition and

the probability of competitive exclusion of the invader. In our study, connectance describes the proportion of realized resource-consumer interactions from all possible resource-consumer interactions, and is thus reflective of the average niche breadth of the resident bacterial community. In highly connected communities all species of the resident community are able to use resources similarly, which could increase the consumption of every individual resource (Figure 1). High connectance could thus decrease pathogen invasion success via reduced opportunities for pathogen growth. While previous modeling work has shown that high connectance could either constrain (Romanuk et al. 2009) or promote (Lurgi et al. 2014) invasions depending on the relationship between species richness and connectance, food webs containing consumers with very diverse diet breadths (generalists) have been found to be more resistant to invasions (Lurgi et al. 2014). High connectance is thus likely to constrain pathogen invasion since all members of the resident community will efficiently exploit most resources. In general, nestedness describes trophic interactions between generalist and specialist resource consumers within the community. In highly nested networks, generalist species will have many resource-consumer links, which are shared by specialist species that have only few resource-consumer links (Figure 1). In mutualistic pollinator networks, nestedness has been shown to stabilize communities and enhance persistence (the number of species coexisting at equilibrium) (Thébault and Fontaine 2010, Rohr et al. 2014). However, in resource competition networks nestedness is likely to have a destabilizing effect (Thébault and Fontaine 2010) because specialists need to compete for subsets of resources that are used also by generalists, which will likely lead to decreased ranges of species' growth rates. As a result, communities with nested resource competition networks could be less stable and less even, and thus, more susceptible to invasions.

To test these hypotheses, we conducted replicated invasion experiments in laboratory microcosms (in 96-well microtiter plates) and in the tomato plant soil (rhizosphere) to link resident community composition, invasion resistance and spread of bacterial wilt plant disease. Our results show that bacterial resource competition networks are better at predicting invasion resistance than community diversity. Specifically, resident communities with stabilizing configurations (low nestedness and high connectance), and a clear niche overlap with the pathogen, reduce pathogen invasion success very efficiently leading to lowest levels of bacterial wilt plant disease. Bacterial resource competition network characteristics can thus be important in explaining positive diversity-invasion resistance relationships in bacterial rhizosphere communities.

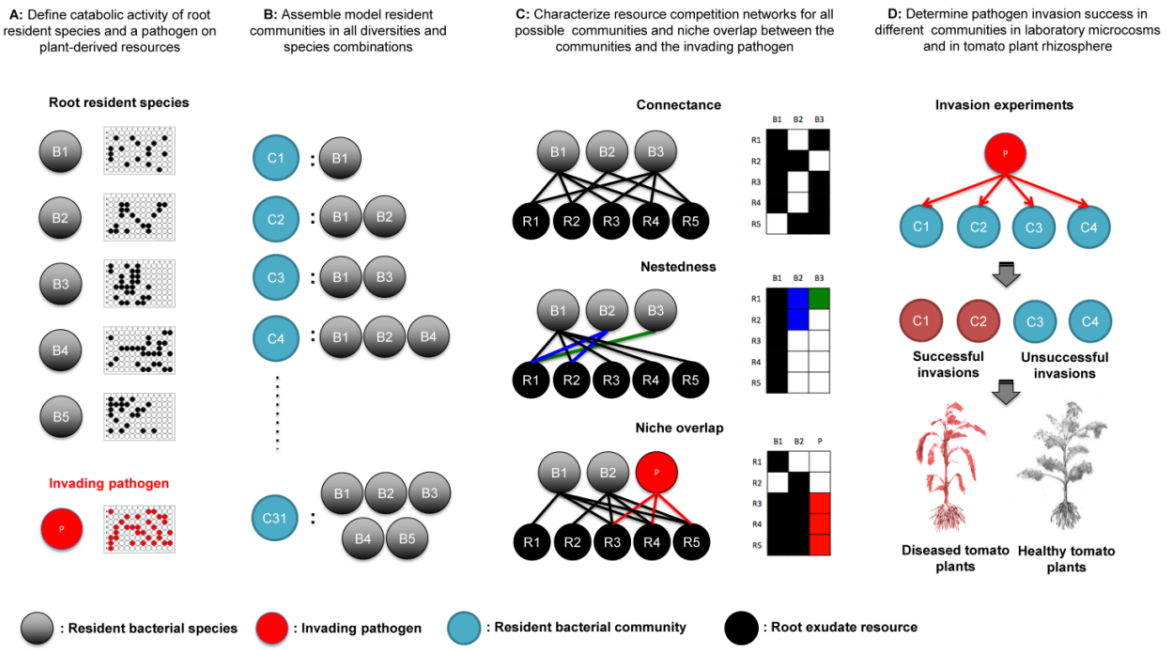

Figure 1. Conceptual framework and experimental design. We first characterized resource consumption patterns for both resident community species and the invading pathogen on carbon resources representative for conditions prevailing around tomato roots (panel A). We then assembled resident communities in all possible species combinations (panel B), and define resource competition netowrkd characteristics (connectance, nestedness and niche overlap) for all assembled communities (panel C). Finally, every assembled community was exposed to pathogen invasion in laboratory microcosms and tomato plant rhizosphere to link resident community invasion resistance with network characteristics (panel D). P denotes for pathogen, B1-B5 denotes different resident community members, C1-C31 denotes different possible resident community compositions and R1-R5 denotes different tomato roots exudates environments.

Table 1. Comparison of bipartite network-based and diversity-based general linear mixed models (GLM) for pathogen invasion success in microcosms and rhizosphere. The diversity GLM included the number of species of the resident community (continuous variable). The network GLM included bipartite network variables: nestedness, connectance, niche overlap and their first order interactions (continuous variables). The table lists the most parsimonious models selected on the base of the AIC information coefficient via backward and forward model selection.

		In vitro assays				Disease spread in greenhouse			
	Df.	Probability of invasion		Invader density		early (lag) phase		intermediate (exponential) phase)		late (assymptotic) phase	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Species richness											
Species richness	1	8.4	0.004 ↓	18.3	<0.0001 ↓	18.75	<0.0001 ↓	151.5	<0.0001 ↓	122.9	<0.0001 ↓
model summary		R^2 = 0.02 AIC: 389.8		R^2 = 0.23 AIC:265.5		R^2 = 0.23 AIC: 244.4		R^2 = 0.72 AIC: -356.6		R^2 = 067 AIC: -77.44	
Networks metrics											
Nestedness (NE)	1	19.6	<0.0001↑	*not retained*		5.03	0.03 ↓	139.34	<0.0001 ↑	119.1	<0.0001 ↑
Niche overlap (NO)	1	*not retained*		49	<0.0001 ↓	6.02	0.017 ↑	12.8	0.0007 ↓	26.5	<0.0001 ↓
Connectance (CO)	1	13.7	0.0002 ↓	*not retained*		18	<0.0001 ↑	11.07	0.002 ↑	1.32	0.255
NE*NO	1	*not retained*		*not retained*		*not retained*		2.32	0.133 ↑	4.1	0.047 ↓
NE*CO	1	8.7	<0.0001 ↓	*not retained*		15	0.0003 ↓	22.45	<0.0001 ↓	12.7	0.0007 ↓
NO*CO	1	*not retained*		*not retained*		5.9	0.018 ↑	*not retained*		*not retained*	
model summary		R^2 = 0.11 AIC: 360.3		R^2 = 0.46 AIC: 245.2		R^2 = 0.43 AIC: 229.9		R^2 = 0.753 AIC: -361.62		R^2 = 0.73 AIC: -78.90	

RESULTS

Pathogen invasion success in laboratory microcosms

Increasing resident community diversity reduced pathogen invasion success (Table 1). At the level of resource competition networks, resident communities with low nestedness and high connectance constrained pathogen invasion most efficiently (R^2 = 0.11, the effect of niche overlap non-significant, Figure 2, Table 1). Similar to pathogen invasion success, high resident community diversity reduced pathogen growth within successfully invaded communities (Table 1). At the network level, pathogen growth within successfully invaded communities was constrained only by high niche overlap in resource competition patterns between the invading pathogen and resident community (R^2 = 0.46, Figure 2; the effects of nestedness and connectance non-significant, Table 1). Crucially, the network-based model was a better predictor of both pathogen invasion success and subsequent growth within invaded resident communities than the diversity-based model.

Together these results suggest that the characteristics of bacterial resource competition networks could partially explain why more diverse communities were often resistant to pathogen invasions: while nestedness and connectance were important for predicting pathogen invasion probability, high niche overlap was the best predictor for subsequent pathogen growth within successfully invaded resident communities.

Pathogen invasion success in tomato plant rhizosphere

The proportion of wilted plants (clear symptom of *Ralstonia* infection, which correlates with pathogen density in the rhizosphere and thus with invasion success, Wei et al. 2011) increased with time and the bacterial wilt disease spread fitted well with a logistic regression (Figure 3; Table 1). Bacterial wilt disease incidence rapidly increased after a three-week initial lag phase before reaching equilibrium. The presence of resident communities reduced disease spread and this effect varied considerably between different communities. Increasing resident community diversity reduced the risk of tomato plant wilting at early, intermediate and late stages of infection (Figure 3, Table 1). Interestingly, the same network variables that predicted resident community resistance to pathogen invasion in microcosm experiments were also important in explaining

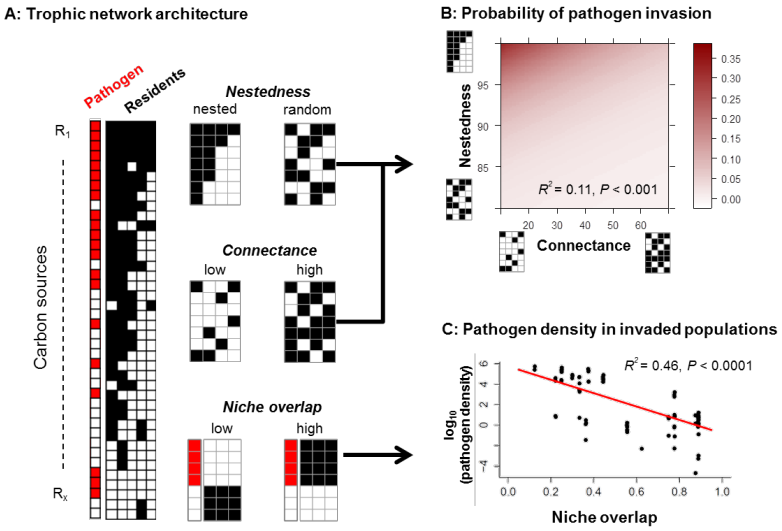

Figure 2. Pathogen invasion success measured in microcosm experiments. A schematic matrix capturing resource competition interactions between the pathogen (red boxes) and resident community species (black boxes); filled squares indicate that the given bacteria consume a given resource (panel A). Pathogen invasion success (probability of invader establishment, visualized as heatmap showing the results of the used GLM) was lowest in non-nested and highly connected resident communities (panel B). Pathogen growth in successfully invaded communities was constrained most when the resident communities had high niche overlap with the pathogen (panel C).

bacterial wilt spread in tomato plant rhizosphere. During the early stage of infection, resident communities with low nestedness, high connectance and high niche overlap with the pathogen, delayed the spread of bacterial wilt; in other words, the onset of disease spread took longer (R^2 = 0.43, Table 1, Figure 3). These relationships persisted throughout the rest of the invasion experiment, the network model being the best predictor of disease spread during the intermediate (R^2 = 0.75, Table 1, Figure 3) and late infection stages (R^2 = 0.73, Table 1, Figure 3). Note that high connectance promoted disease spread during the intermediate stage of infection before becoming non-significant during the late stage of infection (R^2 = 0.75, Table 1, Figure 3). We also found that network variables had interactive effects on disease spread. In general, nestedness and connectance reduced each other's effects of at every stage of infection (Table 1). Similarly, high niche overlap reduced the effect of nestedness during the late stage of infection, while niche overlap and connectance strengthened each other's effects during the early stage of infection (Table 1; correlated effects are analyzed in more detail in the next chapter). Alike to laboratory invasion experiments, the network-based model was a better predictor of tomato plant wilting compared to the diversity-based model (Table 1).

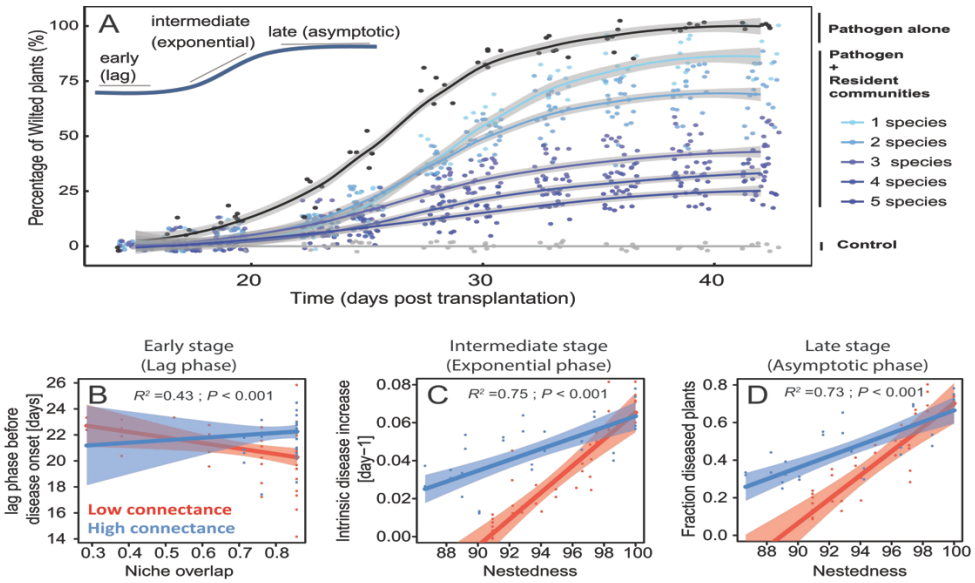

Figure 3. Pathogen invasion success measured in tomato plant rhizosphere. Spread of bacterial wilt plant disease in the absence and presence of resident communities (control denotes for treatment without ant bacteria). Disease spread was fitted with the data by using a logistic regression to obtain three variables describing the dynamics of disease dynamics: lag time before disease onset (early stage), the exponential rate of disease spread (intermediate stage) and the asymptotic disease saturation (late stage) (panel A). Main effects and interactions between connectance, niche overlap and nestedness on disease development during each stage of infection (panel B-D). The R^2 and P value refer to the most parsimonious model fitted for each disease stage.

These results suggest that the same network variables that predicted pathogen invasion success in simple laboratory microcosms can also predict pathogen invasion success in more complicated tomato plant rhizosphere. Low nestedness and high niche overlap were especially important in reducing the spread of bacterial wilt during the intermediate and late stages of pathogen invasion. While network variables had interactive effects on disease spread, their relative importance was generally smaller compared to main effects of network variables.

Linking diversity and networks for invasion resistance

We used structural equation modeling (path analysis) to describe the directed dependencies among diversity and different network variables. We first concentrated on pathogen invasion success in microtiter plate invasions assays. Only high community connectance had direct negative effect on pathogen invasion success (Figure 4, panel A). The effects of diversity and connectance were further channeled indirectly into reduction in resident community nestedness, which then directly constrained pathogen invasion success (Figure 4, panel A). Consistent with the GLMs on microtiter plate invasion, the effect of niche overlap

was non-significant. We next focused on pathogen growth in successfully invaded microcosm communities (Figure 4, panel B). The significant effects of diversity and connectance were channeled into increase in niche overlap, which led to reduction in the pathogen growth (Figure 4, panel C). Consistent with the GLM approach, the effect of nestedness was non-significant. Finally, we compared the relative importance of diversity and network variables of resident community for tomato plant survival. Both high connectance and high diversity directly reduced the levels of bacterial wilt disease (Figure 4, panel C). Moreover, both connectance and diversity had indirect effects on disease spread via nestedness (connectance increased and diversity decreased), which directly constrained disease spread (Figure 4, panel C). High connectance and diversity also indirectly increased the niche overlap with the pathogen. Even though retained in the final model, the effect of niche overlap itself was non-significant (Figure 4, panel C).

In conclusion, network variables were better predictors of resident community invasion resistance in microcosm experiments compared to diversity. However, this pattern was altered in the rhizosphere invasion experiments, where the mix of both indirect and direct effects of diversity and network variables best explained the pathogen invasion success and subsequent spread of tomato bacterial wilt disease.

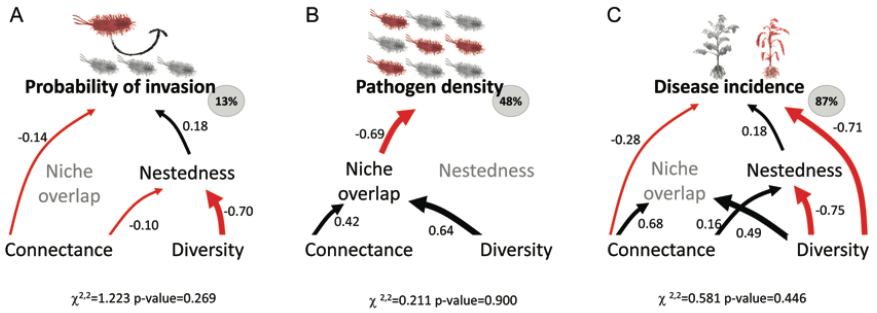

Figure 4. Structural equation models linking diversity and network variables with pathogen invasion success and subsequent spread of bacterial wilt plant disease. Results from microcosm invasion experiments (probability of invasion and pathogen density in invaded communities) (panel A, B). Results from rhizosphere invasion experiment (disease spread during the late stage of the infection) (panel C). Nestedness, connectance and niche overlap with the pathogen explained most of the invasion process in microcosm invasion experiments. In the rhizosphere invasion experiment, the direct effect of diversity was important for bacterial wilt disease spread. Grey circles left of each panel denote for the proportion of the total variance explained and the numbers on the arrows denote standardized correlation coefficients. Red arrows denote for negative effect on invasion process by the high value of the given variable, and black arrows denote for positive effect on invasion process by the high value of the given variable; arrow widths correspond with the relative effect size of each variable.

DISCUSSION

Here we used simple bipartite resource competition networks (Whitfeld et al. 2014) to predict resident bacterial resident community invasion resistance to the plant pathogen *Ralstonia solanacearum*. Biodiversity of microbial communities is increasingly recognized to constrain invasion by pathogens (van Elsas et al. 2012, Mallon et al. 2015). In the present study we concentrate on underlying mechanisms and show that the structure of resource competition networks provide a missing link between community composition and pathogen invasion. We show that resident community invasion resistance and its subsequent ability to prevent tomato bacterial wilt disease could be explained by using only few key resource competition network variables: connectance, nestedness and niche overlap with the pathogen. Crucially, network-based models had consistently better explanatory power compared to diversity-based models (Table 1). This suggests that diversity invasion resistance relationships could be mechanistically explained by the underlying interaction network architecture. Even though the network variables were the most important predictors of pathogen invasion success in microcosm experiment, resident community diversity also had direct effects on bacterial wilt disease spread in tomato plant rhizosphere. Both species interaction networks and species richness may thus be important for the invasion resistance of bacterial communities.

We hypothesized that pathogen invasion success should decrease with increasing niche overlap between the resident communities and invading pathogen due to intensified resource competition. While niche overlap had no effect on pathogen invasion probability in microcosm experiments, it constrained subsequent pathogen multiplication in successfully invaded resident communities. This result is in line with previous studies reporting that high catabolic similarity between the rhizosphere community and the invading pathogen reduces the community invasibility (Ji and Wilson 2002). Interestingly, the relative importance of niche overlap was low compared to the effects of nestedness and connectance in the rhizosphere invasion experiments. Together, these results suggest that invasion resistance is mediated not only by competitive exclusion of the pathogen due to similar niche breadth but is also affected by competitive interactions within the resident microbial community.

High connectance within rhizosphere communities could decrease pathogen invasion success if it leads to generally more efficient consumption of resources, and hence increased competition (Case 1990, Kennedy et al. 2002, van Elsas et al. 2012). Alternatively, high connectance could increase pathogen invasion success if the resource competition is stronger among the members of rhizosphere community than between the members of rhizosphere community and the invading pathogen. We found that highly connected resident communities

decreased the probability of pathogen invasion success in microcosm experiments. Mechanistically, this could have been due to more efficient consumption of resources by highly connected resident communities, which could have prevented pathogen establishment and subsequent growth. These patterns also fitted well to the disease spread in tomato plant rhizosphere, with the exception that connectance promoted disease spread during the intermediate stage of infection. The effects of connectance on resident community invasion resistance could depend on the spatial structure of the environment and could change during plant development. For example, it is possible that rhizosphere bacteria were able to consume resources more efficiently in spatially homogenous microcosm environment (liquid media) compared to spatially heterogeneous soil environment, where resource competition between rhizosphere community members was likely more intense at the local scale (Amarasekare 2003, Gómez and Buckling 2013). As a result, high connectance could have increased the resource competition within the rhizosphere community leading to community destabilization (May 1973) and successful pathogen invasion in the soil. This effect could have been stronger during the intermediate stages of infection making highly connected resident communities more susceptible for pathogen invasion. Interestingly, high connectance also reduced the negative effect of high nestedness on community invasion resistance, indicating that different network characteristics may together shape invasion resistance in rhizosphere bacterial communities.

Lastly, we predicted that low nestedness could increase resident community invasion resistance due to less intense resource competition among generalist and specialist members of resident communities. We found that highly nested resident communities were more susceptible to pathogen invasion and subsequent spread of bacterial wilt plant disease in the tomato plant rhizosphere. Nested resource competition patterns could destabilize rhizosphere communities because specialist species need to compete for a subset of the resources used by generalists (Thébault and Fontaine 2010), which could lead to a decrease in bacterial productivity in general (Poisot et al. 2013). In contrast to mutualistic nested networks, where generalists can facilitate the coexistence of specialists (Whitfeld et al. 2014, Rohr et al. 2014), generalists are likely to have negative effect on specialists via asymmetrical resource competition in highly nested exploitative interaction networks. Competition-mediated extinctions within the resident community (Thébault and Fontaine 2010) could thus lead to less efficient utilization of resource niche space and expose resident communities to invasions. Alternatively, when community assembly is niche-driven, random resource use patterns may result in a higher community evenness (Sugihara et al. 2003), which in turn could improve community resistance to invasion (De Roy et al. 2013, Taur and Pamer 2014). In this study, we used bacterial wilt disease symptoms as a proxy for both pathogen density changes in the greenhouse experiment: the

prevalence of bacterial wilt correlates directly with *R. solanacearum* densities in rhizosphere (Wei et al. 2011). Therefore, further experiments are needed to link the disease spread with changes in the density of resident species in soil.

Interestingly, diversity had direct positive effects on rhizosphere community invasion resistance in the tomato plant soil, which was not explained by resource competition network characteristics. There are multiple non-exclusive explanations for this. First, it is likely that some other factors in addition to resource competition patterns were important for resident community and pathogen fitness in the soil. For example, differences in species ability to colonize plant roots could have played significant role for the establishment of protective rhizosphere communities (Von Bodman et al. 2003). Moreover, some members of the resident community might have been able to induce systemic plant resistances against pathogens (Lugtenberg and Kamilova 2009) leading to increased invasion resistance. Unfortunately, it is impossible to disentangle these hypotheses on the basis of this data, and thus, further experiments are needed to validate these hypotheses.

Albeit being effective in demonstrating causal relationships between resident community interaction network structure, invasion resistance and subsequent disease spread in the soil, our simplified approach has some caveats that might lead to different invasion resistance patterns in more complex natural systems. Firstly, our diversity manipulation included only *Ralstonia* species that were closely related with the pathogen. As a result, resident community bacterial diversity could have different effect on invasion resistance depending on the genetic distance between the resident bacterial community and the invading pathogen. Further, our model focused only on resource-mediated competition. In a natural system, increasing bacterial diversity could also increase the strength of antagonistic interactions, which could potentially make communities more susceptible to pathogen invasion (Jousset et al. 2011c). Our simplified model rhizosphere bacterial community also contained only one trophic level: the resource-consuming bacteria. Natural soil rhizosphere communities are more complex having multiple trophic levels such as parasitic phages and predatory protists and nematodes (Hol et al. 2013). Thus, our approach could be extended in the future to a higher number of trophic levels and different types of species interactions, including antagonistic competition (Raaijmakers et al. 2009) and cross-feeding (Freilich et al. 2011). More complex food webs could be decomposed into a subset of bipartite networks (Kondoh et al. 2010) to improve the predictive power and correspondence with the more complex microbe-plant ecosystems (de Vries et al. 2013). Thirdly, the positive effects of resident bacterial communities on invasion resistance might be short-lived as rhizosphere bacterial communities, varying temporally during plant development (Chaparro et al. 2014) or growth season (Riley et al. 2001). Furthermore, bacterial interaction networks

and/or community composition are likely to change after crop plant removal in agricultural systems, which could affect the rhizosphere community invasion resistance on a longer term (Wei et al. 2011). Finally, it is possible that bacterial species embedded in resident community or the invading pathogen evolves. For example, *R. solanacearum* pathogen can rapidly evolve respective to its resource consumption patterns (Nakatsu et al. 1998, Riley et al. 2001) or host species (Guidot et al. 2014). However, it is not known if such adaptive processes could be driven or are affected by resource competition patterns with resident rhizosphere community members.

Soil-borne plant pathogens remain difficult to control because their populations are variable in time and space, and because they can often overcome plant resistance via evolution (Strange and Scott 2005). Currently an important part of global food production is lost to soil-borne plant diseases (Strange and Scott 2005). Thus, improving rhizosphere community invasion resistance could significantly improve crop yields across different agricultural systems. Here, we propose an ecological network approach to improve plant health by directly engineering the resource competition interaction networks of plant-associated bacterial communities. Instead of antagonizing the pathogen, rhizosphere invasion resistance could be optimized with interaction network structure that maximizes competition with the pathogen and simultaneously minimizes the competition within the resident bacterial communities. Even though our study is at least an order of magnitude simpler than any natural rhizosphere community, we propose that similar ecological mechanisms could predict the functioning of more complex microbiomes potentially affecting animal and human health (Berendsen et al. 2012, Bletz et al. 2013).

MATERIALS AND METHODS

Bacterial strains and plasmids

We used *Ralstonia solanacearum* QL-Rs1115 (GenBank accession GU390462) tagged with the pYC12-Mcherry plasmid as an invading bacterial pathogen (Wei et al. 2011). *R. solanacearum* is a soil-borne, gram-negative bacterium that invades plant roots and multiplies in the cortical tissue before invading the xylem (Von Bodman et al. 2003). In a matter of hours, the bacteria spread into the crown and stem, causing wilt, a generalized necrosis and plant death. Root colonization is the first step and prerequisite for plant infection (van Elsas et al. 2012). As roots are densely populated with diverse bacterial communities, interactions with the resident bacterial community could affect pathogen invasion success, and ultimately, host infection (Hibbing et al. 2010).

We used five non-virulent but closely related *Ralstonia* spp. strains to construct model resident bacterial communities that were exposed to pathogen

invasion. All resident community bacterial strains were isolated from the tomato rhizosphere and first grown on South Africa semi selective medium (SMSA-E) (Wei et al. 2013). Isolated *Ralstonia* colonies were identified on the basis of 16S rRNA gene sequence (27F and 1492R primers, Rochelle et al. 1992), deposited in GenBank under the accession number JN699058 (strain QL-A2), KJ780056 (strain QL-A3), HQ267096 (strain QL-A6), KJ780054 (strain QL-117) and KJ780055 (strain QL-140) and were cryopreserved at -80 ^{o}C in glycerol (20 % ending concentration). None of these strains were virulent and did not cause any bacterial wilt disease symptoms when injected into tomato stems (Wei et al. 2013). Moreover, none of the resident community strains showed direct antagonism towards each other or the pathogenic *R. solanacearum* QL-Rs1115 strain on soft agar overlay essays, which suggests that these strains interact indirectly via resource competition. All bacterial strains and plasmids are listed in Supplementary Table 1.

Construction of resident bacterial communities

We used a full factorial design to construct model resident bacterial communities covering all possible species richness's and community compositions (31 communities in total; Supplementary Table 2). Resident bacterial communities were then subjected to invasion by the pathogenic *R. solanacearum* QL-Rs1115 strain first in microtiter plates in simplified laboratory conditions, and second, in tomato plant rhizosphere in over one month-long greenhouse experiment. We used a substitutive design in all experiments, meaning that the initial resident community densities were adjusted to the same level in all community composition treatments (Gibson et al. 1999).

Characterization of bacterial resource competition networks

We used bacterial resource consumption patterns to determine indirect resource competition networks 1) between different members of resident communities and 2) between different resident communities and the invading pathogen. To this end, we first characterized the resource consumption pattern of each bacterial strain individually in single-species monocultures on 48 different single carbon resources representative of tomato root exudates. Briefly, overnight cultures were adjusted to an optical density (OD_{600}) of 0.1 and grown in microtiter plates in 150 µL OS minimal medium (Schnider-Keel et al. 2000) supplemented with 48 compounds representative of amino acids, organic acids and sugars found in tomato root exudates (Supplementary Table 3). After 48 h growth at 25 °C with agitation, optical density was recorded in a SpectraMax M5 Plate reader (Molecular Devices, Sunnyvale, CA, USA) and wells with an $OD_{600} > 0.05$ were scored as positive growth on given substrate. We then used these single-species and one-resource interactions to determine resource consumption patterns 1) between the different members of resident communities and 2) between different resident communities and the invading pathogen. We first defined niche overlap between resident

communities and the invading pathogen as the proportion of resources used by the pathogen and by at least one member of the given resident community (Ji and Wilson 2002). We the estimated two network variables that have been reported to be important for biological invasions (Romanuk et al. 2009, Galiana et al. 2014, Lurgi et al. 2014) for all resident communities: connectance and nestedness. In our system, connectance describes the proportion of realized trophic interactions from all possible trophic interactions and is thus reflective of species diet breadth: in highly connected communities all species would be able to use all resources while in poorly connected communities species use different subset of resources. Connectance was estimated as a proportion of realized links between species and resources they share in all given communities by using the formula $C = L / S^2$, where L denotes for links between species (number of resources all species can consume) and S denotes for species richness of the community. Nestedness describes the trophic interactions between generalist and specialist resource consumers. In highly nested networks, generalist species have many links, which are shared by specialist species that have only few links. Mathematically, nestedness was estimated by the system's "temperature" via binary matrixes in three steps by computing first an isocline of perfect order, reorganizing the matrix by permuting rows and columns in the way that maximizes its nestedness and comparing its normalized distance to the isocline (Rodriguez-Girones and Santamaria 2006). The temperature of the matrix is the sum of these distances, normalized in such a way that it ranges between 0 for a perfectly unnested (random) matrix and 100 for a maximally 'nested' matrix. Nestedness was computed with the function nested() of the R package bipartite using the binmatnest2 algorithm.

Pathogen invasion success in laboratory microcosms

All species of resident bacterial communities were first grown alone in liquid nutrient medium (NA, glucose 10.0 g L^{-1}, peptone 5.0 g L^{-1}, yeast extract 0.5 g L^{-1}, beef extract 3.0 g L^{-1}, pH 7.0) on a shaker at 170 rpm, 30 °C for 12 h, after which 31 resident communities were constructed with a starting density of 10^7 cells mL^{-1}. All resident communities were inoculated to 20 different resources environments (total of 620 individual populations), each of which were composed of OS minimal medium supplemented with a random mixture of 20 carbons selected from the total pool of 48 resources (Supplementary Table 4). As a result, the network variables (connectance, nestedness and niche overlap between resident community and pathogen) varied depending on the resource environment. Model resident communities were then subsequently exposed to invasion by mCherry-tagged *R. solanacearum* QL-Rs1115 strain (10^5 cells mL^{-1}) or grown alone in the absence of pathogen in 96 wells microtiter plates (200 µL $well^{-1}$) at 30 °C with agitation. Resident community growth was recorded after 48h on the basis of optical density (OD_{600}) and pathogen growth on the basis of the mCherry

fluorescence signal (excitation: 587 nm, emission: 610 nm). The invasion process was broken into two steps. First, the probability of successful invasion was measured as a binary variable for each resident community-resource environment combination: invasion was scored as successful if the fluorescence signal was higher, and unsuccessful if it was lower, than the background signal of the corresponding control treatment. Second, we quantified the pathogen invasion success within successfully invaded resident communities as pathogen density based on the level of fluorescence signal (bacterial autofluorescence signal of corresponding control treatment were used as a blank (Eisenhauer et al. 2012b).

Disease spread in tomato

The spread of *Ralstonia* wilt in tomato plants previously inoculated with the different resident bacterial communities was tested in a 42 days long greenhouse experiment (Supplementary Figure 1). As disease incidence is directly correlated to pathogen density around plant roots (Wei et al. 2013), we used wilting symptoms as proxy for following pathogen invasion of rhizosphere communities. Similar to microcosm invasion experiments, we used the same 31 non-virulent resident communities (2 replicates each), a positive control containing only the pathogen (3 replicates) and a negative control without any bacteria (3 replicates). 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). Tomato plants at the three-leaf stage (11 days after sowing) were transplanted to seedling trays (supplementary figure 1) containing the same seedling substrate as describe above and inoculated with resident bacterial communities by drenching method (ending concentration of 10^8 CFU g^{-1} soil) (Wei et al. 2013). The invasion experiment was initiated one week later by inoculating resident bacterial communities with the pathogen (ending concentration of 10^6 CFU g^{-1} soil). Tomato plants were grown for 42 days after transplantation in a greenhouse with natural temperature variation ranging from 25 °C to 35 °C and watered with sterile water regularly. Note that the plants from individual single seedling plates were not considered as independent replicates, but instead the number of wilted plants per seedling plate was used as a disease index. Seedling plates were arranged in randomized order and rearranged randomly every two days. The number of wilted plants per seedling plate was recorded at daily basis after 17th day post transplantation (no symptoms were observed before day 17). As we were unable to quantify available carbon resources in the rhizosphere directly, we made the assumption that all 48 resources used to characterize bacterial resource consumption patterns were available in the rhizosphere.

Statistical analyses

Pathogen invasion pattern in microcosm experiment was analyzed with generalized linear models (GLM) assuming binomial data distribution for invasion success analysis and lognormal data distribution for invader density increase analysis. For greenhouse invasions experiments, disease spread over time was assessed as a logistic growth curve spanning the percentage of wilted plants as a function of time during whole duration of the experiment. The curves were fitted individually for each tray, in an approach very similar to mortality curves. This fit produced three variables: the lag phase [days after pathogen addition] before disease onset, the intrinsic increase of wilted plants [day^{-1}] and the asymptotic disease prevalence [% of wilted plants]; later referred to as early, intermediate and late stages of infection, respectively. Curves were fitted individually for each tray - an approach very similar to survival analysis—and the resulting variables used as dependent variables in independent GLMs, weighted by $-\log_{10}(SE)$ of the parameter estimates in order to account for the quality of the curve fitting. Due to potential correlation between connectance and nestedness, sequential analysis was used to uncover the most parsimonious GLM's. To this end, we used stepwise model selection using the Akaike information criteria (AIC) to select the model with best explanatory power (step() function in R). We used both a backward elimination (starting with a full model including nestedness, connectance and niche overlap and their first-order interactions) and forward-selection model (from simple to more complex) to avoid selecting a local AIC minimum (Latz et al. 2012). The explanatory power of network models was further compared with classical biodiversity-ecosystem functioning models, which explained pathogen invasion success and disease spread as a function of diversity (continuous) or the presence-absence of each species.

Finally, we used structural equation modeling (SEM) approach to study the direct and indirect effects of diversity and resource competition network variables for the probability of pathogen invasion and growth in microcosm invasion experiments, and for the asymptotic spread of bacterial wilt in the greenhouse invasion experiment. Briefly, we based our approach on a recent work (Thébault and Fontaine 2010) and used diversity and connectance of the resident community as exogenous variables and nestedness and niche overlap as endogenous variables to disentangle the direct and indirect effects of each variable on pathogen invasion success and spread of bacterial wilt disease. Please note that the SEM approach does not allow us to include interactions into the model, and thus the results might slightly differ from the models presented in Table 1. The most parsimonious model was derived from the full model by removing non-significant pathways using Bayesian information criterion and chi-square tests. Standard error of means was computed with the lavaan package in R 3.0.3.

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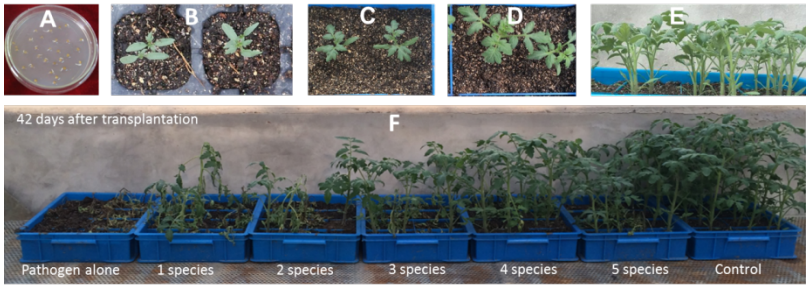
SUPPLEMENTARY INFORMATION



Supplementary Figure 1. Overview of the greenhouse experiment. Surface-sterilized tomato seeds (*Lycopersicon esculentum*, cultivar "Jiangshu") were first germinated on water-agar plates for 3 days (A) before sowing into seedling plates (B) containing Cobalt -60-sterilized seedling substrate (Huainong, Huaian soil and fertilizer Institute, Huaian, China). After 11 days of growth on seedling plates, tomato plants (at three-leaf stage) were transplanted to seedling trays (350mm×250mm×100mm) containing the same growth substrate as described above. Sixteen seedlings were transplanted into one seedling tray (every two plants grown in a separate cell) and trays were then subsequently inoculated with different resident bacterial communities by drenching method (final concentration of 10^{8} CFU g^{-1} soil, C; Wei *et al.* 2011). Control treatments without resident bacterial communities were also set up at this stage. Invasion experiment was initiated one week later by adding *Ralstonia solanacearum* (final concentration of 10^{6} CFU g^{-1} soil, D) to all plants. Tomato plants were grown in a greenhouse with natural 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 at daily basis after 17 days post transplantation: no symptoms were observed before the day 17 (E). Invasion experiment was ended 42 days after transplantation when the control plants reached the maximal size supported by seedling trays. F) Seedling trays representative for disease incidence in plants inoculated with resident communities of increasing species richness.

Supplementary Table 1. Bacterial strains and plasmid used in this study.

Strain		Close match	GenBank No.	Source
Commensal species	QL-A2	*Ralstonia mannitolilytica*	JN699058	Wei et al. 2013
	QL-A3	*Ralstonia mannitolilytica*	KJ780056	Wei et al. 2013
	QL-A6	*Ralstonia pickettii*	HQ267096	Wei et al. 2013
	QL -117	*Ralstonia taiwanensis*	KJ780054	This study
	QL-140	*Ralstonia* sp.	KJ780055	This study
Pathogen	QL-Rs1115	*Ralstonia solanacearum*	GU390462	Wei et al. 2011
Plasmid	PYC12-M*	mCherry insert in the pYC12, under control of the constitutive Ptac promoter, gm^R		This study

*Generation of pYC12-M: The *mCherry* gene from the pTCV89 plasmid was cloned into pYC12 under the constitutive promoter P_{tac} (Wang et al. 2008). Both plasmids were kindly donated by Associate Professor Zhong Zengtao from key lab of microbiological engineering of agricultural environment, Nanjing Agricultural University, Nanjing, China. The *mCherry* gene bordered by *Nde* I and *EcoR* I sites was amplified from plasmid pTCV89 using F1 (5'-ATG GCT CATATG GTG AGC AAG GGC GAG GAG-3') and R1 primers (5'-GTC GAATTC AGG GCG AAT TGG AGC TCC TTA C-3'). The fragment was cloned into the plasmid pYC12 to obtain the recombinant plasmid pYC12-M. The plasmid pYC12-M was introduced into the cells of *R. solanacearum* by electroporation, transformants were selected on NAg (NA medium containing 30 µg mL^{-1} Gm) plates and fluorescence checked by fluorescence microscopy.

Supplementary Table 2. All possible community compositions of biocontrol bacterial communities.

Community	QL-A2	QL-A3	QL-A6	QL-117	QL-140	Richness
Control	0	0	0	0	0	0
1	1	0	0	0	0	1
2	0	1	0	0	0	1
3	0	0	1	0	0	1
4	0	0	0	1	0	1
5	0	0	0	0	1	1
6	1	1	0	0	0	2
7	1	0	1	0	0	2
8	1	0	0	1	0	2
9	1	0	0	0	1	2
10	0	1	1	0	0	2
11	0	1	0	1	0	2
12	0	1	0	0	1	2
13	0	0	1	1	0	2
14	0	0	1	0	1	2
15	0	0	0	1	1	2
16	1	1	1	0	0	3
17	1	1	0	1	0	3
18	1	1	0	0	1	3
19	0	1	1	1	0	3
20	0	1	1	0	1	3
21	0	0	1	1	1	3
22	1	0	1	1	0	3
23	1	0	0	1	1	3
24	0	1	0	1	1	3
25	1	0	1	0	1	3
26	1	1	1	1	0	4
27	1	0	1	1	1	4
28	0	1	1	1	1	4
29	1	1	0	1	1	4
30	1	1	1	0	1	4
31	1	1	1	1	1	5

Supplementary Table 3. Classification of 48 carbons used to compose synthetic tomato exudates.

Resource name and abbreviation	Resource classification	Resource code
Acetic aicd (Ace)	organic acid	1
L-Alanine (Ala)	Amino acid	2
β-Alanine (Bala)	Amino acid	3
L-Arginine (Arg)	Amino acid	4
Ascorbic acid (Asc)	organic acid	5
L-Asparagine (Asn)	Amino acid	6
γ-Aminobutyric acid (Ami)	Amino acid	7
Citric acid (Cit)	organic acid	8
Citrulline (Cin)	Amino acid	9
Ethanolamine (Eth)	other	10
Formic acid (For)	organic acid	11
Fructose (Fruc)	sugar	12
Galacturonic acid (Galac)	organic acid	13
Glucose (Glu)	sugar	14
L-Glutamine (Gln)	Amino acid	15
Glutaric acid (Glut)	organic acid	16
L-Glycine (Gly)	Amino acid	17
Glycolic acid (Glyc)	organic acid	18
L-Histidine (His)	Amino acid	19
Isoleucine (Iso)	organic acid	20
Lactic acid (Lac)	organic acid	21
L-Lysine (Lys)	organic acid	22
L-Leucine (Leu)	organic acid	23
Maleic acid (Male)	organic acid	24
Malic acid (Mal)	organic acid	25
Malonic acid (Malon)	organic acid	26
L-Methionine (Met)	organic acid	27
Myoinositol (Mino)	other	28
2-Oxoglutaric (Oxo)	organic acid	29
L-Phenyalanine (Phe)	organic acid	30
L-Proline (Pro)	organic acid	31
Pyruvic acid (Pyr)	organic acid	32
L-Serine (Ser)	organic acid	33
Succinic acid (Succ)	organic acid	34
Sucrose (Sucr)	sugar	35

Resource name and abbreviation	Resource classification	Resource code
Tartaric acid (Tar)	organic acid	36
L-Threonine (Thr)	organic acid	37
L-Tryptophan (Try)	organic acid	38
L-Valine (Val)	organic acid	39
Maltose (Mal)	sugar	40
L-Arabinose (Ara)	sugar	41
D-Galactose (Gal)	sugar	42
D-Mannose (Man)	sugar	43
D-Xylose (Xyl)	sugar	44
D-Ribose (Rib)	sugar	45
D-Mannitol (Mann)	sugar	46
Inosine (Ino)	other	47
Oxalic acid(Oxa)	organic acid	48

Supplementary Table 4. Twenty different resource combinations (C1-C20) each consisting of 20 randomly selected resources. In all combinations, one indicates the presence of the given resource and zero indicates the absence of the given resource.

Resource code	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
1	0	0	0	1	1	0	0	1	0	1	1	1	1	1	0	0	1	1	0	1
2	1	0	1	1	0	1	1	0	1	0	0	0	0	0	1	1	0	1	1	0
3	1	1	0	0	1	0	1	1	0	0	0	0	0	1	1	1	0	1	1	0
4	0	0	1	0	1	0	0	0	0	1	1	1	1	1	0	0	1	1	1	1
5	1	1	0	0	1	1	1	0	0	0	1	1	0	0	0	1	0	1	0	0
6	0	1	1	1	0	0	1	1	0	1	0	0	1	1	0	0	1	0	0	0
7	0	0	1	1	0	0	1	0	1	1	0	0	1	0	1	0	0	0	0	1
8	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1
9	0	1	1	0	0	0	0	1	1	1	0	1	0	1	0	1	1	0	0	1
10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
11	0	1	0	0	0	0	1	0	1	0	1	1	0	1	1	1	0	1	0	0
12	0	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0
13	0	1	1	0	1	0	0	0	0	1	0	1	1	0	0	0	1	0	0	1
14	1	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	1
15	0	0	0	1	1	0	0	1	0	0	1	1	1	1	1	1	0	0	0	1
16	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
17	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	1	1	0	0	0
18	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0
19	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0
20	0	0	1	0	0	1	0	1	0	1	1	0	1	1	0	0	0	1	1	0
21	0	0	1	0	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0
22	0	0	1	1	0	1	0	0	0	0	1	0	1	0	0	1	1	1	1	1
23	0	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	1
24	1	0	1	0	1	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1
25	0	1	0	1	0	1	0	1	1	0	0	0	0	0	1	0	1	1	1	1
26	1	0	1	1	1	1	0	1	1	0	1	0	1	0	0	1	0	0	0	0
27	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1
28	1	0	0	1	0	1	0	0	0	1	1	1	0	0	0	0	1	1	1	0
29	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0	0	1	0	1	1
30	0	0	1	0	1	1	0	1	0	1	0	1	1	1	0	0	0	0	0	0
31	1	0	1	0	0	0	1	0	1	1	1	0	0	0	0	0	1	0	0	1
32	1	1	0	0	0	1	1	0	0	1	1	0	0	1	0	0	1	1	0	0
33	1	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0

Resource code	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
34	0	0	0	1	1	1	0	1	1	1	0	1	0	0	0	1	0	1	0	0
35	0	0	0	1	1	1	1	1	0	0	0	1	0	0	1	1	0	0	0	1
36	1	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	0	0	1	0
37	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
38	1	1	1	1	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0
39	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0
40	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	1	1	0
41	1	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0	1	1	0	1
42	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0
43	1	0	1	1	1	0	1	0	1	0	0	1	0	0	0	1	1	0	0	0
44	1	0	1	0	1	0	0	1	1	0	1	1	0	1	0	1	0	1	0	1
45	1	1	1	1	1	0	1	0	0	1	0	1	1	0	1	1	1	0	1	1
46	1	1	0	1	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	1
47	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0
48	0	0	1	0	1	0	1	1	1	0	0	0	0	1	0	0	1	0	1	0

freedom evo
TECAN

Chapter 3

Resource availability modulates biodiversity-invasion relationships by altering competitive interactions

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ABSTRACT

Community diversity affects the survival of newly introduced species by increasing resource competition. Competitive interactions can be modulated by resource availability, and we hypothesized this may alter biodiversity-invasion relationships. We assessed the growth of an invading pathogen, *Ralstonia solanacearum,* introduced into communities of one to five closely related resident species under different resource concentrations. We examined invader growth as a function of resident community diversity and keystone species and resource availability. We found that the invader growth was reduced by increasing resident community diversity and resource availability. Mechanistically, this could be explained by changes in the competitive interactions between resident and pathogen species along the resource availability gradient. At low resource availability, resident species with a high catabolic similarity with the invader efficiently controlled the invader growth, while at high levels of resource availability, fast-growing resident species became more important for the pathogen suppression. These results indicate that the relative importance of different resident species can change dynamically along to resource availability gradient. Diverse communities could be thus more robust to pathogen invasions by providing a set of keystone species that can take suppressive roles across different environments.

INTRODUCTION

Species migrations between local populations are an essential driver of biodiversity and ecosystem functioning (Lundberg and Moberg 2003, Venail et al. 2008, Lindström and Östman 2011, Lindström and Langenheder 2012). Different communities are typically interconnected allowing species to disperse from one community to another. New migrating species can change community functioning by introducing for instance metabolic capabilities (Singh et al. 2015) or creating new niches (Tilman 2004). However, in some cases, effects of new species on community functioning may be seen as undesirable. This is true for instance for pathogens, which must first establish themselves in the host-associated microbial communities in order to cause a disease. A better understanding of the drivers that affect pathogen invasion success could thus help developing better disease management strategies (Jousset et al. 2014, Wei et al. 2015).

The success of invaders depends to a great extent on the prevailing competitive interactions within the resident community, which could be in turn affected by community diversity and environmental factors such as resource availability. For instance, functionally diverse communities may be able to occupy more niches thereby outcompeting invaders (Elton 1958, Romanuk et al. 2009, van Elsas et al. 2012). Identity of the species that comprise the resident community could also affect the growth of the invader. For example, highly competitive resident species could efficiently inhibit invader by consuming the resources that would be otherwise consumed by the invader (Jiang *et al.*, 2011; Eisenhauer *et al.*, 2012; Fargione *et al.*, 2003; Fargione and Tilman, 2005; Hector *et al.*, 2002). As a result, some resident species could be relatively more important for the invasion resistance than the others, *i.e.* 'keystone' species (McClean et al. 2015). In the current study, we addressed the combined effect of resident community diversity and the composition (the relative importance of species identity effects) on the pathogen invasion success across resource availability gradients.

Changes in resource availability could have important effects on invader growth. For example, increase in resource availability might facilitate pathogen invasion (Li and Stevens 2012) if the resident community is not able to efficiently consume all the resources thereby leaving free niche space for the invader (Davis et al. 2000, Mallon et al. 2015). However, there are hardly any studies looking how resource availability and resident community diversity interactively shape ecosystem susceptibility to pathogen invasions (Romanuk and Kolasa 2005).

In this study, we addressed the interplay between diversity (species richness) and resource availability on the invasion resistance of bacterial communities. We specifically sought to explain pathogen invasion within the context of resource competition by exposing resident bacterial communities with

varying diversities and species composition to the invasion of the plant pathogen *Ralstonia solanacearum* (Yabuuchi et al. 1995) in liquid microcosms. We have previously demonstrated that the diversity of the resident bacterial communities can predict disease incidence in the plant rhizosphere (Wei et al. 2015). In the soil, resource availability is highly variable and influenced for instance by the species vicinity to plant roots or organic material content. In the present study, we examined how changes in resource availability may alter the relationship between diversity, species identity and the pathogen invasion. We hypothesized that increasing the resident community diversity could constrain pathogen invasion by increasing the strength of resource competition. However, this effect could rely on the presence of specific resident species in the community. Moreover, it is possible that changes in resource availability could change the relative importance of different resident species for the invasion process.

RESULTS

Pathogen invasion success in microcosms

Increasing resident community species richness and resource availability reduced pathogen relative abundance (GLM, Table 1, ***Model 1-"richness and resources"***, Figure 1). No interactive effect between resource availability and diversity on pathogen relative abundance was found (Table 1, ***Model 1-"richness and resources"***).

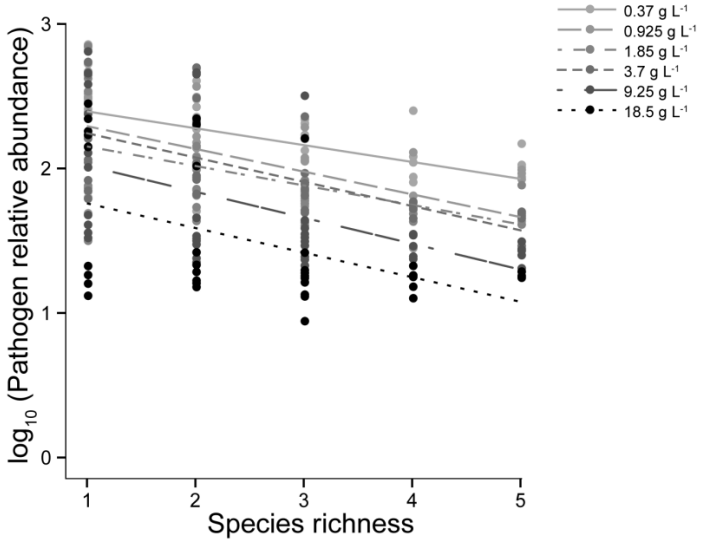

Figure 1. The effect of resident diversity and resource availability on the growth of the invading pathogen (the log10-transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 48h incubation. Different lines show diversity-invasion resistance relationship at different levels of resource availability.

Crucially, resource availability changed the relative importance of the resident community species identity on the pathogen density during the invasion. While the importance of species QL-A2 and QL-A3 became more significant with increasing resource availability (slope decreased, Table 1, ***Model 2-"identity effects"*** and explanatory power increased, Figure 2), the effect of the rest of the species (QL-A6, QL-117 and QL-140) became less significant with increasing resource availability (slope increased, Table 1, ***Model 2-"identity effects"*** and explanatory power decreased, Figure 2).

As a result, different strains affected the pathogen invasion success in resource-dependent manner (see ANOVA table in Supplementary Table 5). At low resource availability (NB concentration of 0.37 g L^{-1} and 0.925 g L^{-1}), communities with QL-A6 species explained most of the variance (Figure 2, Supplementary Table 5). In contrast, at high resource availability (NB concentration more than 1.85 g L^{-1}), communities with QL-A2 or QL-A3 had relatively large effect on the pathogen invasion (Figure 2, Supplementary Table 5). Together these results suggested that resource availability altered the functional role of the resident species: communities with species QL-A6 reduced pathogen densities especially at low resource availability levels, whereas communities with species QL-A2 and QL-A3 reduced pathogen densities when the resource availability was high.

Impact of resource use metrics of resident communities on pathogen invasion

In order to investigate the cause behind the changing species identity effect along resource availability levels, we fitted a linear model between the resource use metrics of the resident communities (catabolic similarity and growth rate) with the pathogen relative abundance. Both catabolic similarity and growth rates had significant negative effects on the pathogen densities (Table 2). More specifically, increasing resource availability gradually decreased the effect of catabolic similarity for the pathogen invasion, whereas the relative importance of resident community growth rate increased with increasing resource availability (slope of the catabolic similarity increased and slope of the growth rate decreased, Table 2).

In addition to summarizing interactive effects of catabolic similarity and growth rate on pathogen relative abundance, we also assessed the explanatory power (R^2 value of each linear model) of these resource use metrics on the pathogen invasion success. In line with the previous results, the explanatory power of the resident community growth rate linearly increased with the increasing resource availability, while the explanatory power of catabolic similarity with increasing resource availability levels (Figure 3).

In summary, communities that contained species with high catabolic similarity with the pathogen suppressed pathogen invasion at low resource availability levels, whereas communities that contained quickly growing species

controlled pathogen invasion at high resource availability levels.

Table 1. ANOVA table summarizing the interactive effects of diversity and resource availability (***Model 1-"richness and resources"***) and species identity and resource availability (***Model 2-"identity effects"***) on the relative abundance of the pathogen (the log10-transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 48h of incubation). The significant effects ($P < 0.05$) are highlighted in bold and the 'up' and 'down' arrows denote for positive or negative effects, respectively.

	Df	F	*P*
Model 1-richness and resources			
Diversity (SR)	1	89.72	**< 0.001 ↓**
Resource availability (RA)	1	109.89	**< 0.001 ↓**
SR * RA	Non-significant and thus not retained in the final model		
Residuals	234		
Model summary	AIC: 144.31, R^2 = 0.46		
Model 2-identity effects			
QL-A2	1	84.59	**< 0.001 ↓**
QL-A3	1	122.67	**< 0.001 ↓**
QL-A6	1	81.62	**< 0.001 ↓**
QL-117	1	0.27	0.60
QL-140	1	1.28	0.26
Resource availability (RA)	1	221.07	**< 0.001 ↓**
QL-A2 * RA	1	40.52	**< 0.001 ↓**
QL-A3 * RA	1	40.55	**< 0.001 ↓**
QL-A6 * RA	1	42.55	**< 0.001 ↑**
QL-117 * RA	1	5.18	**0.024 ↑**
QL-140 * RA	1	7.45	**0.0068 ↑**
Residuals	225		
Model summary	AIC: -12.93, R^2 = 0.74		

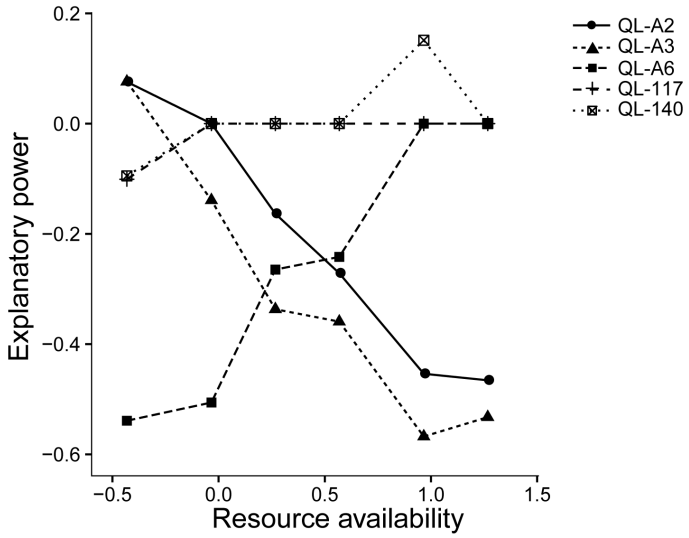

Figure 2. The relationship between resource availability and explanatory power of the presence of each resident species on the relative abundance of the pathogen (the log-transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}). The explanatory power was defined as the general coefficient of the presence of a given species in the resident community. A value lower than 0 means negative effect of certain species on pathogen relative abundance, more than 0 means positive effect, 0 means neutral effect. Separates analyses were performed at each resource availability level and the resource availability values were log_{10}-transformed.

Table 2. ANOVA table summarizing the interactive effects of catabolic similarity and growth rate of resident communities on the relative abundance of the pathogen (transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}). The significant effects ($P < 0.05$) are highlighted in bold and the 'up' and 'down' arrows denote for positive or negative effects, respectively.

	Df	F	*P*
Model 3-resource use metrics			
Catabolic similarity	1	38.75	**< 0.001 ↓**
Growth rate	1	178.43	**< 0.001 ↓**
Resource availability (RA)	1	0.061	0.80
Catabolic similarity * RA	1	28.54	**< 0.001 ↑**
Growth rate * RA	1	6.02	**0.015 ↓**
Residuals	231		
Model summary	AIC: 121.70	$R^2 = 0.52$	

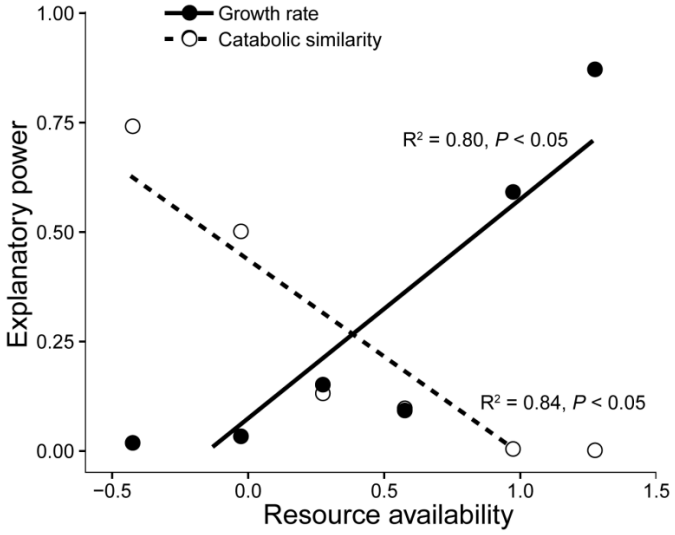

Figure 3. Correlation between resource availability and catabolic similarity (open symbols, dashed line) and growth rate (closed symbols, plain line) with the explanatory power of these resource use metrics on pathogen growth at each resource availability level. Values of resource availability levels were $\log_{10}$-transformed.

DISCUSSION

Resource competition is an important component of species interactions having important effects on biodiversity-invasion relationships (Mallon et al. 2015).While species-rich communities may constrain invader growth via intense resource competition (Tilman 1999, van Elsas et al. 2012, Mallon et al. 2015), it is less clear how biodiversity-invasion relationships are modulated by resource heterogeneity in space and time. In this study, we explored how changes in resource availability affect biodiversity-invasion relationships and if these changes could be explained by changes in the competitive interactions between the resident community species and the invading pathogen. We found that resident diversity and resource availability both had a negative effect on the invader growth. Mechanistically, these results could be explained by dynamic changes in the competitive asymmetry and the relative contribution of different resident species on the pathogen invasion success along the resource gradient. First, increasing the resource availability favored fast-growing resident community species over the pathogen leading to less successful invasions. Second, increasing the diversity increased the likelihood that resident communities contained species that were good at competing across the whole resource gradient leading to less successful invasions also when the resource availability was low. Understanding the role of species identity effects across a range of environmental conditions is thus important for predicting the dynamics and outcomes of biological invasions in a

changing world.

We found that resident diversity and resource availability both had a negative effect on the invader growth. The observed negative relationship between resident diversity and the invader growth is in line with some previous studies (Naeem et al. 2000, Roscher et al. 2009, Byun et al. 2013) and theoretical models (Elton 1958, Tilman 1999). However, the reduction of invader growth at high levels of resource availability is in contrast with some other recent findings (Davis et al. 2000, Mallon et al. 2015). For example, Mallon *et al.* (2015) recently showed that increasing resource availability can promote invasions due to relaxed resource competition. In our case, this discrepancy may be explained by the fact that bacteria were likely able to consume all the resources during the experiment without addition of new external resources. As a result, even though increase in resource availability boosted community abundance it unlikely opened up vacant niches for the invader because the experimental system was "closed" (Kuebbing et al. 2013). Interestingly, no interactive effect was found between the resident community diversity and resource availability on invader growth. This suggests that diversity independently constrained pathogen growth regardless of the resource availability (and vice versa).

To study the effect of resident community diversity on the pathogen invasion more closely we looked how the relative contribution of each resident community species (*e.g.* species identity effect) changed across the resource availability gradient (*i.e.* the inclusion of given strains in a community affecting invader growth). We found that the presence of certain three species (QL-A2, QL-A3 and QL-A6) had especially important role for constraining pathogen invasions. This in line with previous studies that have shown that species identity effects can play a fundamental role for invasions in communities and that species-specific traits may be important in invasion suppression (Eisenhauer et al. 2012b, Byun et al. 2013). The two other species (QL-117 and QL-140) had no significant effect on the invader growth. This result could be explained by the species' relatively poor growth ability under the microcosm conditions. The NB medium contains a large amount of glucose (approximate 54 % of the total dry weight), and QL-117 and QL-140 utilize this resource more poorly than the other three species (Supplementary Table 3). Such asymmetries in resource use efficiency has previously been shown to be important for the competition with invaders (Tilman 1982). Crucially, while the QL-117 and QL-140 strains had weak effects on the invasion regardless of the resource availability, the QL-A2, QL-A3 and QL-A6 strains played an important role even though their relative contribution changed along the resource availability gradient.

We could well predict which species would constrain invasion at each resource availability level based on simple resource use metrics. For instance,

species with a high catabolic similarity with the invader showed the strongest effect on its growth at low resource availability (QL-A6 especially important). Catabolic similarity between two species is a good proxy of the intensity of the resource competition (Bøhn and Amundsen 2001, Ji and Wilson 2002). The observed negative correlation between the catabolic similarity and the invader growth confirms that resident species with highly similar resource use patterns to the invader are likely to increase community invasion resistance at low resource availability levels (Wilson and Lindow 1994, Ji and Wilson 2002, Dianese et al. 2003). However, the effect of catabolic similarity became less significant with increasing resource availability, whereas the significance of fast-growing species (QL-A2 and QL-A3 especially important) became more important. This result is expected as bacterial ability to grow fast will likely increase its fitness most when the resource availability is periodically high (Pekkonen et al. 2013, Mallon et al. 2015, Fierer et al. 2007, Ramirez et al. 2012). Together these results suggest that the beneficial diversity effects are likely driven by functional redundancy of the resident community: diverse communities simply more likely contain resident species that are able to efficiently compete for resources with the pathogen in both low and high resource availability environments compared to less diverse communities. Understanding the functional variation across resource availability gradient is thus likely important for the development of predictive biodiversity-invasion relationship framework in changing environments (Fargione and Tilman 2005; Wardle 2001, Hodgson et al. 2002).

We conclude that resource availability can modulate biodiversity-invasion relationships by changing the relative influence of the different resident species on the invasion process. Our key finding is that the functioning of invasion-resistant communities may be stable if different species are able to adopt similar roles under different environmental conditions. A potential application of this finding would be the design of functionally dynamic and redundant microbial communities that can efficiently out-compete pathogens under temporally and spatially changing environmental conditions.

MATERIALS AND METHODS

Bacterial strains and plasmids

We used *Ralstonia solanacearum* QL-Rs1115 (GenBank accession GU390462) constitutively tagged with mCherry, using the stable plasmid pYC12-mCherry, as the invading pathogen (Wei et al. 2011). We built resident communities comprised of between one to five avirulent, but closely related *Ralstonia* species, deposited under the accession number JN699058 (strain QL-A2, *Ralstonia mannitolilytica*), KJ780056 (strain QL-A3, *Ralstonia mannitolilytica*), HQ267096 (strain QL-A6, *Ralstonia pickettii*), KJ780054 (strain QL-117, *Cupriavidus taiwanensis*, formerly *Ralstonia sp*.), and KJ780055 (strain QL-140, *Cupriavidus sp*. , formerly *Ralstonia*

sp.) (Wei et al. 2015). None of the bacteria showed antagonism towards each other or *R. solanacearum* QL-Rs1115 on nutrient agar plates, suggesting that resource competition was a main driver of competitive interactions with the pathogen. All information on the bacterial strains and plasmid can be found in Supplementary Table 1.

Resident community set up

All five avirulent species were used to construct resident communities, which included one to five resident species (diversity level) in all possible different combinations (31 communities in total, Supplementary Table 2). All resident communities were then invaded by pathogen *R. solanacearum* QL-Rs1115 while a set of same communities were grown as controls without a pathogen.

Pathogen invasion in microcosms

Prior to each experiment, one colony of each strain, recovered from -80 ℃ 20 % glycerol stocks, was selected and grown in nutrient broth (NB, glucose 10.0 g L^{-1}, tryptone 5.0 g L^{-1}, beef extract 3.0 g L^{-1}, yeast extract 0.5 g L^{-1}, pH 7.0) with 170 r.p.m. agitation at 30 ℃ for 12 h. Bacteria were then washed three times by centrifugation (6000 rpm, 5 min), resuspended in 0.85% NaCl and adjusted to a density of 10^7 cells mL^{-1} and bacterial communities constructed according to the scheme provided in Supplementary Table 2.

We set up six different resource availability treatments by diluting NB to a concentration of 18.5, 9.25, 3.7, 1.85, 0.925 and 0.37 g L^{-1} with sterile water in 96-well microtiter plates (6 resource availability treatments). Each resident community was first inoculated to each resource availability treatment, and pathogen *R. solanacearum* QL-Rs1115 (tagged with the pYC12-mCherry plasmid) was subsequently introduced into all communities (with final concentration of 10^5 cells mL^{-1}). Communities were incubated at 30 ℃ with 170 r.p.m. orbital agitation for 48 h (200 µL together with bacteria and medium per well), a time chosen to allow all communities to reach stationary phase. Total bacterial growth was then estimated by measuring the optical density (OD_{600}) of communities. Pathogen abundance was estimated on the base of the mCherry fluorescence signal (Excitation: 587 nm, Emission: 610 nm) with a SpectraMax M5 Plate reader (Molecular Devices, Sunnyvale, CA, USA). The relative density of the pathogen relative was defined as the mCherry relative fluorescence unit (RFU, mCherry/OD_{600}) at the end of the experiment. We used communities without pathogen to blank the fluorescence signal and control wells containing culture medium only to blank the OD_{600}.

Determining resident bacterial resource use metrics

We selected catabolic similarity and growth rate as explanatory variables to infer bacterial competitive interactions. Both variables are representative for resource

use across several taxa, ensuring the generalizability of the results. Catabolic similarity was used as a proxy for the potential nutrient competition between resident species and the invading pathogen (Wilson and Lindow 1994, Ji and Wilson 2002). Based on previous studies (Ji and Wilson 2002, Wei et al. 2015), catabolic similarity was defined as resource use similarity between the resident community and the pathogen (*R. solanacearum* QL-Rs1115 with mCherry).

We determined bacterial resource use patterns by growing each species separately on minimal medium supplemented by one out of 48 different resources (Supplementary Table 3) representative for nutrients prevailing around tomato roots, the original habitat of these bacteria as described previously (Wei et al. 2015). Briefly, overnight cultures of each strain were washed three times by 0.85 % NaCl, adjusted to a density of 10^7 cells $mL^{-1}$and grown on OS minimal medium (Schnider-Keel et al. 2000) with single resource from 48 resources in 96-well microtiter plates. Optical density (OD_{600}) of each well was measured in plate reader after incubation for 48 h at 30 ℃ with agitation. Resource use was scored positive in all wells with an $OD_{600} > 0.05$. Catabolic similarity was calculated based on resource use patterns of all the communities and the pathogen (*R. solanacearum* QL-Rs1115 with mCherry). In a previous study, we demonstrated that these measured patterns provide a robust estimate of competitive interactions in various conditions ranging from liquid microcosms to soil (Wei et al. 2015). Since catabolic similarity was calculated as bacterial growth across many different media, same value was used for different resource availabilities. Values of catabolic similarity were shown in Supplementary Table 4.

We further determined the intrinsic growth rate of each single species and all the communities in mono- and polycultures. All resident communities were combined as described (31 communities in total, Supplementary Table 2) with an initial cell density of 10^7 per mL^{-1} and inoculated in 96-well microtiter plates. All communities were grown in triplicates under each of the six tested resource availability levels (18.5, 9.25, 3.7, 1.85, 0.925 and 0.37 g L^{-1} NB) at 30 ℃ with agitation (170 r.p.m.) for 48 h. To obtain bacterial growth rates, we used OD_{600} value from 13 time points to fit in Logistics model function ("gcFitModel" in package "grofit" in R 3.3.1) , which fits both gram positive and negative bacterial growth (Kacena et al. 1999). The Maximum slope (μ) in Logistic model was regarded as estimated growth rate (Kahm et al. 2010) (with unit h^{-1}) and mean growth rate of triplicates were used in the further analysis.

Statistical analyses

We log_{10}–transformed pathogen relative abundance (RFU, mCherry/OD_{600}) and resource availability prior to further analyses. In the first step, we set up three separate general linear models (GLM) expressing pathogen relative abundance as a function of the interactive effects of resident community composition and

resource availability. The first model (*Model 1-"richness and resources"*) expressed pathogen relative abundance as a function of interactive effect of diversity (continuous predictor) and resource availability (continuous predictor). The second model (*Model 2-"identity effects"*) expressed pathogen abundance as a function of the presence of each species (binary predictors) and resource availability (continuous predictor). The third model (*Model 3-"resource use metrics"*) expressed pathogen relative abundance as a function of the growth rate and catabolic similarity of the communities.

In the second step, we sought to explain the contribution of each species to pathogen relative abundance, catabolic similarity and growth rate separately at each resource availability level. The overlap approach was used to remove overlapping effects among each resident species. We used a function "sAICfun" in the library "devtools" (Byrnes et al. 2014) to examine independent coefficient values and the effect of single species presence in communities on the pathogen relative abundance. The explanatory power here was defined as the general coefficient of the single species presence in the community. Similarly, we investigated the identity effect on growth rate at each resource availability level. . Identity effect on catabolic similarity was also analyzed via same method. Model analyses and figures showing identity effect on growth rate and catabolic similarity were presented in Supplementary information respectively (identity effect on growth rates: Supplementary Table 6 and Supplementary Figure 1, identity effect on catabolic similarity: Supplementary Figure 2).

In the third step, in order to summarize how contribution of resource use metrics on pathogen relative abundance changed across resource gradients, we made separate linear models to make correlation between resource use metrics (growth rate and catabolic similarity) and pathogen relative abundance at each resource availability level. The explanatory power was defined as R^2 of the variable at each resource availability level. All analyses were performed with R 3.3.1.

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SUPPLEMENTARY INFORMATION

Supplementary Table 1. Information of the bacterial strains and plasmid used in this study.

Strain		Close match	GenBank No.	Source
Resident species	QL-A2	*Ralstonia mannitolilytica*	JN699058	(Wei et al. 2013)
	QL-A3	*Ralstonia mannitolilytica*	KJ780056	(Wei et al. 2013)
	QL-A6	*Ralstonia pickettii*	HQ267096	(Wei et al. 2013)
	QL-117	*Ralstonia taiwanensis*	KJ780054	(Wei et al. 2015)
	QL-140	*Ralstonia sp.*	KJ780055	(Wei et al. 2015)
Invader	QL-Rs1115	*Ralstonia solanacearum*	GU390462	(Wei et al. 2011)
Plasmid	PYC12-M	mCherry inserted in the pYC12, under control of the constitutive Ptac promotor, gm^R		(Wei et al. 2015)

Supplementary Table 2. The composition of resident species communities used in the study. In the table, "1" denotes for the presence of the species in the community and "0" for the absence of species in the community. Diversity denotes for species richness.

Community	QL-A2	QL-A3	QL-A6	QL-117	QL-140	Diversity
1	1	0	0	0	0	1
2	0	1	0	0	0	1
3	0	0	1	0	0	1
4	0	0	0	1	0	1
5	0	0	0	0	1	1
6	1	1	0	0	0	2
7	1	0	1	0	0	2
8	1	0	0	1	0	2
9	1	0	0	0	1	2
10	0	1	1	0	0	2
11	0	1	0	1	0	2
12	0	1	0	0	1	2
13	0	0	1	1	0	2
14	0	0	1	0	1	2
15	0	0	0	1	1	2
16	1	1	1	0	0	3
17	1	1	0	1	0	3
18	1	1	0	0	1	3
19	0	1	1	1	0	3
20	0	1	1	0	1	3
21	0	0	1	1	1	3
22	1	0	1	1	0	3
23	1	0	0	1	1	3
24	0	1	0	1	1	3
25	1	0	1	0	1	3
26	1	1	1	1	0	4
27	1	0	1	1	1	4
28	0	1	1	1	1	4
29	1	1	0	1	1	4
30	1	1	1	0	1	4
31	1	1	1	1	1	5

Supplementary Table 3. The resource use patterns of each resident species and the pathogen on 48 different resources. The plus sign denotes for species ability to grow on specific resources.

No.	Resources	QL-A2	QL-A3	QL-A6	QL-117	QL-140	QL-Rs1115
1	Acetic aicd(Ace)			+		+	+
2	L-Alanine(Ala)			+	+	+	+
3	β-Alanine(Ala)		+	+	+	+	
4	L-Arginine(Arg)						
5	Ascorbic acid(Asc)					+	
6	L-Asparagine(Asn)	+	+	+	+	+	+
7	γ-Aminobutyric acid(Ami)	+	+	+		+	+
8	Citric acid(Cit)			+		+	+
9	Citrulline(Cin)			+	+	+	+
10	Ethanolamine(Eth)						
11	Formic acid(For)						
12	Fructose(Fruc)			+		+	
13	Galacturonic acid(Galac)			+			+
14	Glucose(Glu)	+	+	+			+
15	L-Glutamine(Gln)	+	+	+	+	+	+
16	Glutaric acid(Glut)					+	
17	L-Glycine(Gly)			+		+	
18	Glycolic acid(Glyc)			+	+	+	
19	L-Histidine(His)		+	+	+	+	+
20	Isoleucine(Iso)	+			+	+	
21	Lactic acid(Lac)	+	+	+	+	+	+
22	L-Lysine(Lys)						
23	L-Leucine(Leu)				+	+	
24	Maleic acid(Male)					+	
25	Malic acid(Mal)			+	+	+	+
26	Malonic acid(Malon)			+	+	+	
27	L-Methionine(Met)						
28	Myoinositol(Ino)						+
29	2-Oxoglutaric(Oxo)			+		+	+
30	L-Phenyalanine(Phe)			+	+	+	+
31	L-Proline(Pro)	+	+	+	+	+	+
32	Pyruvic acid(Pyr)		+	+	+	+	+
34	Succinic acid(Succ)			+	+	+	+
35	Sucrose(Sucs)						+
36	Tartaric acid(Tar)						+
37	L-Threonine(Thr)			+	+	+	
38	L-Tryptophan(Try)			+	+	+	
39	L-Valine(Val)		+	+	+	+	
40	Maltose(Mal)			+		+	
41	L-Arabinose(Ara)			+		+	
42	D-Galactose(Gal)		+	+			
43	D-Mannose(Man)		+	+			
44	D-Xylose(Xyl)						
45	D-Ribose(Rib)						
46	D-Mannitol		+				
47	Inosine(I)		+				
48	Oxalic acid(Oxal)						

Supplementary Table 4. The catabolic similarity (number of resources both pathogen and at least one resident community species can utilize) between the pathogen and the resident communities. High index values denote for high catabolic similarity.

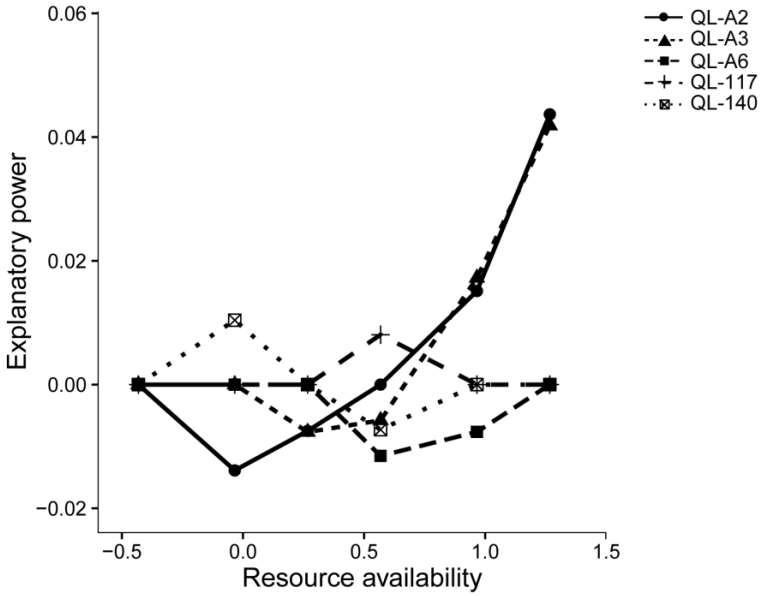
QL-A2	QL-A3	QL-A6	QL-117	QL-140	QL-Rs1115	Diversity	Catabolic similarity
1	0	0	0	0	1	1	0.285714
1	0	0	0	0	1	1	0.285714
0	1	0	0	0	1	1	0.380952
0	1	0	0	0	1	1	0.380952
0	0	1	0	0	1	1	0.857143
0	0	1	0	0	1	1	0.857143
0	0	0	1	0	1	1	0.52381
0	0	0	1	0	1	1	0.52381
0	0	0	0	1	1	1	0.714286
0	0	0	0	1	1	1	0.714286
1	1	0	0	0	1	2	0.380952
1	0	1	0	0	1	2	0.857143
1	0	0	1	0	1	2	0.619048
1	0	0	0	1	1	2	0.761905
0	1	1	0	0	1	2	0.857143
0	1	0	1	0	1	2	0.619048
0	1	0	0	1	1	2	0.761905
0	0	1	1	0	1	2	0.857143
0	0	1	0	1	1	2	0.857143
0	0	0	1	1	1	2	0.714286
1	1	1	0	0	1	3	0.857143
1	1	0	1	0	1	3	0.619048
1	1	0	0	1	1	3	0.761905
0	1	1	1	0	1	3	0.857143
0	1	1	0	1	1	3	0.857143
0	0	1	1	1	1	3	0.857143
1	0	1	1	0	1	3	0.857143
1	0	0	1	1	1	3	0.761905
0	1	0	1	1	1	3	0.761905
1	0	1	0	1	1	3	0.857143
1	1	1	1	0	1	4	0.857143
1	0	1	1	1	1	4	0.857143
0	1	1	1	1	1	4	0.857143
1	1	0	1	1	1	4	0.761905
1	1	1	0	1	1	4	0.857143
1	1	1	1	1	1	5	0.857143
1	1	1	1	1	1	5	0.857143
1	1	1	1	1	1	5	0.857143
1	1	1	1	1	1	5	0.857143
1	1	1	1	1	1	5	0.857143

Supplementary Table 5. Table summarizing the effects of resource availability and resident species identity effects on the pathogen invasion success (growth) at each resource availability level. On the effect column, the "1" denotes for a positive effect, "0" for a neutral effect, and "-1" for a negative effect. Coefficient was calculated by function "sAICfun" in R 3.3.1.

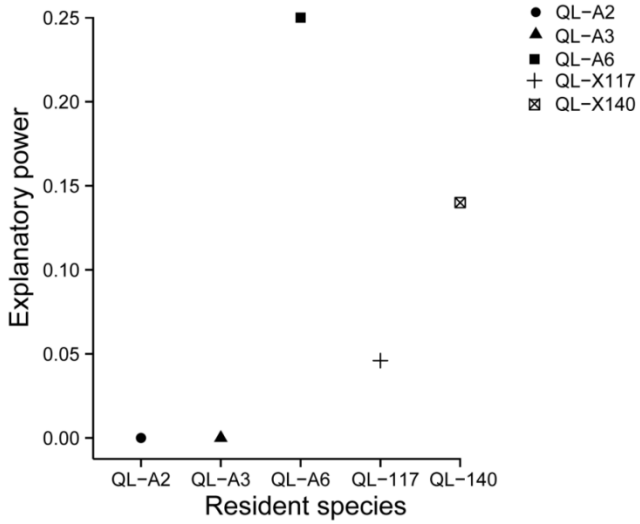
Strain	Resource availability											
	0.37 g L^{-1}		0.925 g L^{-1}		1.85 g L^{-1}		3.7 g L^{-1}		9.25 g L^{-1}		18.5 g L^{-1}	
	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient
QL-A2	1	0.076	0	0	-1	-0.16	-1	-0.27	-1	-0.45	-1	-0.47
QL-A3	1	0.076	-1	-0.14	-1	-0.34	-1	-0.36	-1	-0.57	-1	-0.53
QL-A6	-1	-0.54	-1	-0.51	-1	-0.26	-1	-0.24	0	0	0	0
QL-117	-1	-0.10	0	0	0	0	0	0	0	0	0	0
QL-140	-1	-0.095	0	0	0	0	0	0	1	2.18	0	0

Supplementary Table 6. Table summarizing the effects of resource availability and resident species identity effects on the growth rate of resident communities at each resource availability. On the effect column, the "1" denotes for a positive effect, "0" for neutral effect, and "-1" for a negative effect. Coefficient was calculated by function "sAICfun" in R 3.3.1.

Strain	Resource availability											
	0.37 g L^{-1}		0.925 g L^{-1}		1.85 g L^{-1}		3.7 g L^{-1}		9.25 g L^{-1}		18.5 g L^{-1}	
	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient	Effect	Coefficient
QL-A2	0	0	-1	-0.014	-1	-0.0075	0	0	1	0.015	1	0.044
QL-A3	0	0	0	0	-1	-0.007 7	-1	-0.005 8	1	0.017	1	0.042
QL-A6	0	0	0	0	0	0	-1	-0.012	-1	-0.007 6	0	0
QL-117	0	0	0	0	0	0	1	0.008 1	0	0	0	0
QL-140	0	0	1	0.010	0	0	-1	-0.007 3	0	0	0	0



Supplementary Figure 1. Explanatory power of the presence of each resident species on the growth rate of all the communities at all resource availability levels. Explanatory power is defined as the general coefficient of the presence of a given resident species in the community (values lower than "0" denote for a negative effect of certain species on pathogen relative abundance, values higher than "0" denote for a positive effect and "0" denote for a neutral effect). Separates analyses were performed at each resource availability level.



Supplementary Figure 2. The effect of resident species identity effects (the presence in the community) on the catabolic similarity with the pathogen. Explanatory power is defined as the general coefficient of the presence of a given resident species in the community (values lower than "0" denote for a negative effect of certain species on pathogen relative abundance, values higher than "0" denote for a positive effect and "0" denote for a neutral effect).

Chapter 4

The effect of resource stoichiometry on biodiversity-invasion relationships

Tianjie Yang, Zhong Wei, Ville-Petri Friman, George A. Kowalchuk, Yangchun Xu, Qirong Shen, Alexandre Jousset

ABSTRACT

Biodiversity is important for ecosystem functioning and protecting against biological invasions. Resource availability, including resource stoichiometry (relative quantities of nutritional resources), may also impact the between resident communities and invaders. We hypothesized that biodiversity-ecosystem functioning relationships would be affected by resource stoichiometry. To test this hypothesis, we constructed microbial communities composed of between one and five bacterial species growing at different ratios of carbon, nitrogen and phosphorus. We assessed community productivity and the ability to inhibit the invasion by the plant pathogen *Ralstonia solanacearum*. Relative abundance of N and P made community function essentially dependent on the presence of specific species (identity effects). Increasing the N:P and C:N ratios had negative effects on community functions, while increasing C:P ratio had positive effect. Our findings demonstrate that alteration in resource stoichiometric balance can affect microbial community function and susceptibility to pathogen invasion.

INTRODUCTION

Carbon, phosphorus and nitrogen are the main elements in living organisms. As such, their cycling and availability plays an important role in global ecosystems (Pregitzer and Euskirchen 2004, Bradshaw et al. 2012, Powers et al. 2015). These nutrient elements can separately affect ecosystem functioning. Nitrogen is essential for the synthesis of proteins and therefore the enzymatic functioning and growth of all living cells (Harder and Dijkhuizen 1983, Magasanik 1993). Phosphorus is especially vital for the production of nucleic acids, and phosphorus limitation impedes ribosome formation (Elser et al. 2003) and cell growth rate (Hood and Sterner 2014). In soil, element ratios are largely driven by the input of plant-derived carbon, for instance in the form of root exudates or other plant-derived organic matter (Kuzyakov 2002, Kuzyakov and Xu 2013). Mineralized nitrogen (ammonium and nitrate) or small simple organic forms of nitrogen are required for a variety of cellular processes across all the domains of life. There are of course numerous elements that can be limited in terrestrial ecosystems, (Harder and Dijkhuizen 1983), C, P and N most typically limit terrestrial ecosystem primary production and functioning (Vitousek and Howarth 1991, Elser et al. 2007). However, these elements are not required in equal proportions, and their stoichiometry is also an important determinant of metabolism efficiency. In addition, increasing anthropogenic activities (e.g. greenhouse gases emission, over-fertilization, P-mining) are not only increasing nutrient loads in many environments, but also inducing nutrient imbalances that have important consequences for global nutrient cycles (Mosier et al. 1998, Mack et al. 2004). Changes in element ratios can affect both the functioning of individual species as well as the relationship between biodiversity and ecosystem functioning.

Several studies have highlighted the importance of resource stoichiometry on ecosystem structure and processes in community ecology (Hillebrand et al. 2001, Carnicer et al. 2015). Resource stoichiometry can affect species coexistence (Grover 2004) and competitive interactions (Jeyasingh et al. 2009), thereby modulating community functions. Furthermore, numerous studies have shown how resource stoichiometry can alter ecosystem functioning. A particular ecosystem function of interest in this respect is community susceptibility to invasion. Based on a conceptual model and meta-analyses, it appear that resource stoichiometry affects invasion, principally due to the impacts of resource stoichiometry on specific species biomass stoichiometry and growth (González et al. 2010). However, few studies have been able to link species interactions with ecosystem functioning under the changes in resource stoichiometry. Given the continued changes in environmental resource stoichiometry, it is necessary to understand the impact of elemental balance on the biodiversity-ecosystem function relationship.

Biodiversity can promote ecosystem functioning by providing sets that cannot be combined within a single species (Loreau and Hector 2001, Eisenhauer et al. 2012a). Species richness can also buffer communities against biological invasion (Levine 2000, Kennedy et al. 2002) and enhance community productivity (Naeem et al. 1995, Griffin et al. 2009). Biodiversity-ecosystem functioning (BEF) relationships may however also be modulated by environmental conditions, such as resource availability (Fridley 2002, Mallon et al. 2015). In order to better understand microbial community assembly, it is necessary to both community structure and resource conditions. In the context of global eutrophication by human activities, multi-resource availability rather than one resource may be a key factor currently affecting ecosystem functioning (Limpens et al. 2003, Didham et al. 2007).

In this study, we investigated whether alterations in resource stoichiometry change BEF relationships in model microbial communities. We set up microcosm experiments in which we subjected resident microbial communities of varying species richness and composition to invasion by a reference invader. We used the plant pathogen *Ralstonia solanacearum,* a major threat to global food production, as the model invader. To reach the host, this pathogen must first invade plant root-associated microbial communities. Controlling this invasion process may be the key to plants protection. We manipulated C:N:P ratios around the "Redfield" ratio (C:N:P = 106:16:1). This reference ratio was first defined from planktonic biomass and marine water (Redfield 1958) and later found to be good reflection of element composition in terrestrial ecosystems (Cleveland and Liptzin 2007, Tian et al. 2010). Previous studies showed that resident species communities can efficiently suppress invasion both *in vitro* and *in vivo* due to resource competition (Wei et al. 2015). However, in the soil, the content of C, N and P is variable and may change for instance as a function of fertilization, soil type or the presence of plant-derived carbon. We hypothesized that community functions (community productivity and inhibition of pathogen invasion) would be affected by species richness, resource stoichiometry and their interaction. We expected that resource stoichiometry would be a better predictor of invader growth and community productivity than resource concentrations.

RESULTS

Invader growth and community productivity

Both productivity of uninvaded communities and relative abundance of the invader were affected by richness. Species richness promoted community productivity at all resource levels (Figure 1, panel A).

Invader growth was generally reduced by higher species richness (Figure 1, panel B). The level of invader growth showed a very large degree of variation

under conditions of high P intermediate N concentrations.

Biodiversity-invasion relationships modulated by resource stoichiometry

In order to examine biodiversity effects at each elemental resource level more closely, separate analyses were conducted to determine the explanatory power of diversity versus identity effects. Relatively low diversity effects were observed across all the resource conditions examined. However, identity effects appeared to show higher explanatory power for predicting both community productivity and invader growth than diversity effect (Figure 2). Especially when P concentration is high and N concentration is intermediate (resource availability HM in Figure 2), identity was a strong predictor of invader growth (Figure 2). This is in line with the large variation in invader growth observed for this treatment (Figure 1).

We set up four different models to compare: (1) the effect of absolute resource concentration and stoichiometry on invader growth and community productivity and (2) how biodiversity effects changed with absolute resource concentration and stoichiometry.

We first analyzed how resource concentration affected the relationship between species richness and community productivity, and species richness and invader growth. Richness promoted community productivity (Table2, ***Model 1-"Richness-concentration"*)**. Higher phosphorus concentrations also promoted community productivity (main effect of P concentration on community productivity, Table 2, ***Model 1-"Richness-concentration"***). Consistent with the results presented in Figure 1, species richness had a positive effect on community productivity (main effect of richness on community productivity, Table 2, ***Model 1-"Richness-concentration"***). There was no interactive effect between resource concentration and species richness on community productivity. In terms of invader growth, nitrogen (NH_4^+) concentration constrained invader relative abundance (main effect of N concentration on invader growth, Table 2, ***Model 2-"Richness-ratio"***). There was a negative correlation between species richness and invader growth (main effect of richness on invader growth, Table 2, ***Model 2-"Richness-ratio"***), without any interactive effect with any resource concentration.

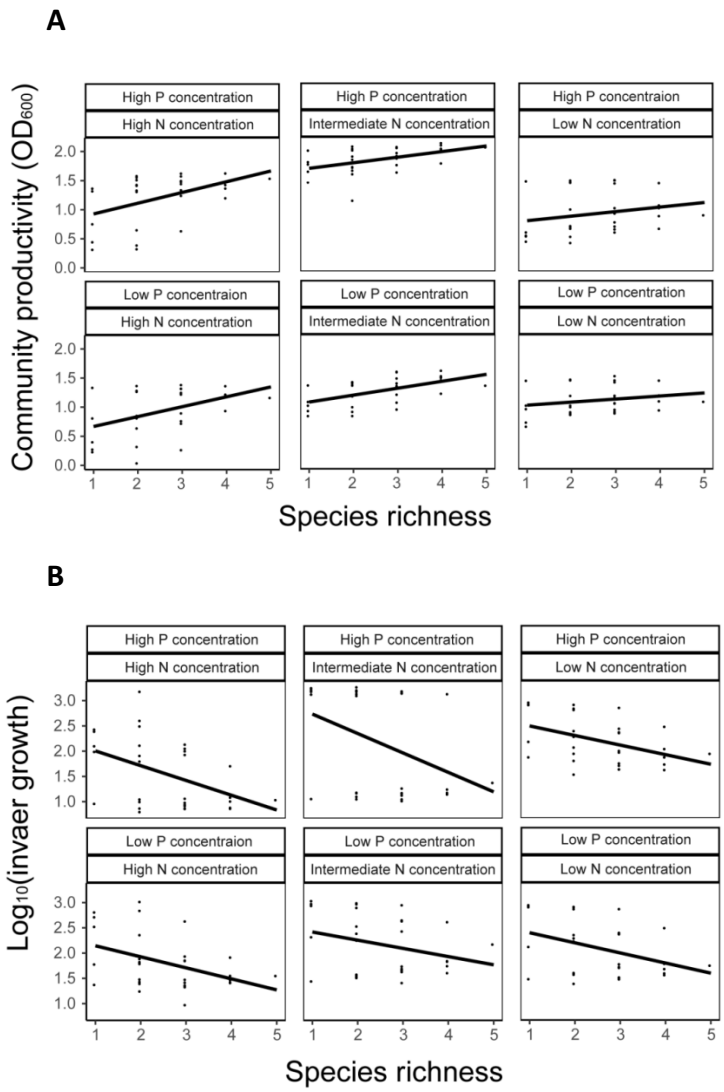


Figure 1. Relationship between resident community richness and productivity of uninvaded communities (panel A) and invader success (panel B) (for media containing NH_4^+ as N source). Community productivity was defined as optical density (OD_{600}) of each community combination. Invader relative abundance was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Both panels present data based on microcosms containing ammonium as sole nitrogen source.

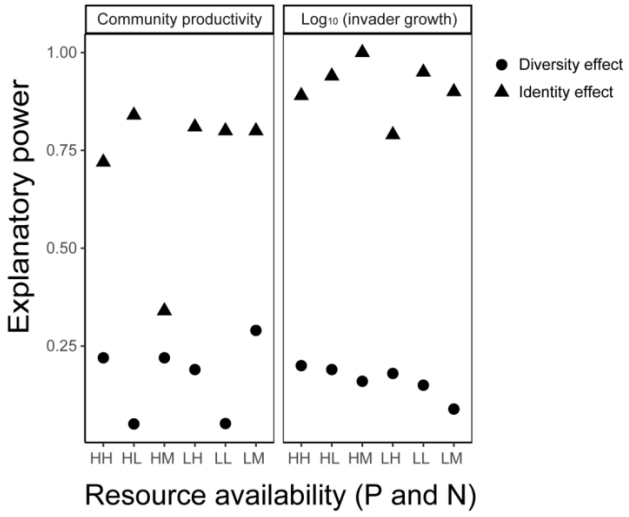


Figure 2. The explanatory power of diversity (round points) and identity (triangular points) effects on community productivity and invader growth at each elemental resource availability level (for media containing NH_4^+ as N source). Community productivity was defined as optical density (OD_{600}) of each community combination. Invader relative abundance was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Both panels present the data from microcosms containing ammonium as sole nitrogen source. Explanatory power stood for R^2 between biodiversity effect and community functions. HH: high P concentration and high N concentration, HL: high P concentration and low N concentration, HM: high P concentration and intermediate N concentration, LH: low P concentration and high N concentration, LL: low P concentration and low N concentration, LM: low P concentration and intermediate N concentration. Separate analyses were performed for each of the elemental resource levels. The explanatory power was defined as R^2 of the biodiversity effect (diversity effect versus identity effect) when fitted to community productivity and invader growth.

We then built a model to predict community productivity and invader growth based on resource ratios. Community productivity was still promoted by high richness (main effect of richness on community productivity, Table 2, ***Model 2-"Richness-ratio"***). C:N had a negative main effect on community productivity, while C:P had a positive effect (Table 2, ***Model 2-"Richness-ratio"***). In this model, species richness reduced invader growth as shown above (main effect of richness on invader growth, Table 2, ***Model 2-"Richness-ratio"*** and Figure 1). Similarly, C:N and C:P had the same effect on invader growth when compared with effect on community productivity (Table 2, ***Model 2-"Richness-ratio"***). In addition, N:P negatively affected invader growth (main effect of NP on invader growth, Table 2, ***Model 2-"Richness-ratio"***).

We did not observe any interactive effects between any resource availability and species richness through all the models, which indicated that the diversity effect on community functions was not be altered by either resource concentration or resource ratios.

Table 2. ANOVA table summarizing the interactive effect of resource availability and richness on invader growth and community productivity (for media containing NH_4^+ as N source). Invader growth was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Community productivity was defined as optical density (OD_{600}) of each community combination. Significant effects ($P < 0.05$) are highlighted in bold and the "up" and "down" arrows denote positive or negative effects, respectively.

	Invader growth			Community productivity		
	Df	F	*P*	Df	F	*P*
Model 1-" Richness-concentration"						
Nitrogen concentration (N)	1	16.95	**< 0.001** ↓	1	0.76	0.38
Phosphorus concentration (P)	1	0.42	0.52	1	14.02	**< 0.001** ↑
Richness	1	29.55	**< 0.001** ↓	1	15.77	**< 0.001** ↑
Richness *N	1	0.31	0.58	1	2.42	0.12
Richness * P	1	1.15	0.28	1	0.0095	0.92
Residuals	180			180		
Model summary	AIC: 363.61, R^2 = 0.21			AIC: 339.90, R^2 = 0.15		
Model 2-" Richness-ratio"						
NP ratio (NP)	1	10.31	**0.0016** ↓	1	2.22	0.14
CN ratio (CN)	1	7.44	**0.0070** ↓	1	20.36	**< 0.001** ↓
CP ratio (CP)	1	6.17	**0.014** ↑	1	93.10	**< 0.001** ↑
Richness	1	30.28	**< 0.001** ↓	1	24.54	**< 0.001** ↑
Richness * NP	1	0.0076	0.93	1	2.34	0.13
Richness * CN	1	1.49	0.22	1	1.35	0.25
Richness * CP	1	0.27	0.61	1	0.038	0.85
Residuals	178			178		
Model summary	AIC: 361.04, R^2 = 0.24			AIC: 135.62, R^2 = 0.45		

Table 3. ANOVA table summarizing the interactive effect of resource availability and species identity on invader growth and community productivity (NH_4^+ as sole N source). Invader growth was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Community productivity was defined as optical density (OD_{600}) of each community combination. Significant effects ($P < 0.05$) are highlighted in bold and the "up" and "down" arrows denote positive or negative effects, respectively.

	Invader growth			Community productivity		
	Df	F	*P*	Df	F	*P*
Model 3-"Identity-concentration"						
QL-A2	1	6.21	**0.014** ↑		Not retained	
QL-A3	1	5.85	**0.017** ↑		Not retained	
QL-A6	1	669.04	**< 0.001** ↓	1	2.55	0.11
QL-117	1	3.02	0.084		Not retained	
QL-140	1	30.73	**< 0.001** ↓	1	72.38	**< 0.001** ↑
Nitrogen concentration (N)	1	73.23	**< 0.001** ↓	1	1.06	0.30
Phosphorus concentration (P)	1	1.81	0.18	1	19.54	**< 0.001** ↑
QL-A6 * P	1	18.69	**< 0.001** ↓		Not retained	
QL-A6 * N	1	2.29	0.13	1	21.26	**< 0.001** ↑
Residuals	176			180		
Model summary	AIC: 95.22, R^2 = 0.82			AIC: 248.83, R^2 = 0.39		
Model 4-"Identity-ratio"						
QL-A2	1	10.10	**0.0018** ↑		Not retained	
QL-A3	1	9.52	**0.0024** ↑		Not retained	
QL-A6	1	1088.58	**< 0.001** ↑	1	5.24	**0.023** ↑
Ql-117	1	4.91	**0.028** ↑		Not retained	
QL-140	1	50.00	**< 0.001** ↓	1	148.91	**< 0.001** ↑
NP ratio (NP)	1	70.79	**< 0.001** ↓	1	4.20	**0.042** ↓
CN ratio (CN)	1	51.07	**< 0.001** ↓	1	38.61	**< 0.001** ↓
CP ratio (CP)	1	42.35	**< 0.001** ↑	1	178.35	**< 0.001** ↑
QL-A6 * NP	1	1.53	0.22		Not retained	
QL-A6 * CN	1	32.83	**< 0.001** ↑	1	43.75	**< 0.001** ↓
QL-A6 * CP	1	58.44	**< 0.001** ↓		Not retained	
QL-140 * NP	1	0.33	0.56	1	2.29	0.13
QL-140 * CN	1	1.84	0.18	1	0.12	0.73
QL-140 *CP	1	12.42	**< 0.001** ↑	1	13.11	**< 0.001** ↓
Residuals	171			176		
Model summary	AIC: 9.32, R^2 = 0.89			AIC: 18.46, R^2 = 0.72		

As there was no interactive effect between species richness and resource availability, we further investigated whether there are identity effects on community functions. We generated two additional models to examine whether some species contributed more than others to community productivity and invasion inhibition and whether these effects are alerted by resource availability.

First, we used a GLM model to analyze identity effects under the change of resource concentrations. Strain QL-140 had a main positive effect on community productivity (main effect of QL-140 on community productivity, Table 3, ***Model 3-"Identity-concentration"***). Phosphorus concentration still promoted the productivity of communities (main effect of P concentration on community productivity, Table 3, ***Model 3-"Identity-concentration"***). Although strain QL-A6 did not have a significant effect on productivity, its contribution to productivity promotion increased with high nitrogen (NH_4^+) concentration (interactive effect between QL-A6 and N concentration, Table 3, ***Model 3-"Identity-concentration"***). When invader growth was a function of resource concentration and identity effect, we observed that invader growth was promoted by QL-A2 and QL-A3, but reduced by QL-A6 and QL-140 (Table 3, ***Model 3-"Identity-concentration"***). The effect of QL-A6 on constraining invader growth became stronger with increasing phosphorus concentration (interactive effect between QL-A6 and P concentration on invader growth, Table 3, ***Model 3-"Identity-concentration"***).

Similarly, we also sought to investigate the effect of resource ratios on BEF. In this model, not only QL-140, but also QL-A6 positively affected community productivity (main effect of QL-A6 and QL-140 on community productivity separately, Table 3, ***Model 4-"Identity-ratio"***). QL-A6 had an interactive effect with C:N and QL-140 had an interactive effect with C:P on productivity (Table 3, ***Model 4-"Identity-ratio"***). Strains QL-A2, QL-A3, QL-A6 and QL-117 could facilitate growth of invader, and only QL-140 exhibited an ability to reduce invader growth (main effect of each strain on invader growth, Table 3, ***Model 4-"Identity-ratio"***). In this model, N:P, C:N and C:P had the same effect as the model shown above (Table 2, ***Model 2-"Richess-ratio"***). The effect of QL-A6 on invader growth increased as C:N increased. When C:P was high, the effect of QL-A6 was reduced (Table 3, ***Model 4-"Identity-ratio"***). QL-140 lost its power in inhibit invader success at high C:P (slope decreased, interactive effect between QL-140 and C:P, Table 3, ***Model 4-"Identity-ratio"***).

The different models were compared to find the mode that best predicted community productivity and invader growth. We found that ***Model 4-"Identity-ratio"*** performed best, with the lowest Akaike information criterion value and the highest explanatory power (R^2).

DISCUSSION

Biodiversity is an important determinant of ecosystem functioning and contributes to resistance to biological invasions (Loreau et al. 2001, van Elsas et al. 2012, Byrnes et al. 2014). Changes in resource availability impact both the performance of individual species as well as community biodiversity and resulting community functioning. Anthropogenic inputs of nutrients into the biosphere continue to increase, and biodiversity and ecosystem functioning are impacted by multiple resource inputs (Harpole and Tilman 2007, Hautier et al. 2009). Revealing the effect of resource stoichiometry on ecosystem functioning is therefore a priority. In this study, we investigated the effect of simple resource stoichiometry on BEF. Moreover, we also examined whether these relationships could be modulated and predicted by resource stoichiometry.

We observed that species richness positively correlated to community productivity, and negatively related to invader growth under NH_4^+ conditions. However, based on the explanatory power of the biodiversity effect, we found that identity effects may be more important than diversity effects in contributing to community functions. This suggests that species identity, rather than diversity *per se*, affected ecosystem functioning, regardless of resource availability. Previous studies have pointed the fact that identity effects can be primary drivers promoting community productivity (Loreau and Hector 2001, Hector et al. 2002) or enhancing invasion resistance (Eisenhauer et al. 2012b, Byun et al. 2013). This is because more diverse communities will more frequently contain certain species that perform well under the given environmental conditions, disproportionally contributing to community capabilities (Hector et al. 2002). Though the results showed relatively low importance of diversity effect on community functions, we still formulated models to test whether biodiversity-invasion relationships would be modulated by resource concentration or stoichiometry. However, we did not observe an interactive effect between species richness and resource availability on community functions. We therefore decided to focus on how the identity effect impacted community functions and whether it would be altered by resource concentration or stoichiometry.

We found that the GLM model based on resource stoichiometry (AIC = 248.83, R^2 = 0.39 for community productivity, AIC = 95.22, R^2 = 0.82 for invader growth) provided a better prediction of community functions than the one based on resource concentration (AIC = 18.46, R^2 = 0.72 for community productivity, AIC = 9.32, R^2 = 0.89 for invader growth). This indicates the importance of stoichiometry on BEF. Resource stoichiometry has been related to the modulation of BEF relationships in previous studies (Fridley 2002, González et al. 2010, Lewandowska et al. 2016), most of which attributed to impacts on community structure, growth capacity or tissue biochemical contents (Naddafi et al. 2009, Keiblinger et al. 2010, Moorthi et al. 2016). In our experiments, species richness

was artificially set the outset of the experiments. Furthermore, cell tissue contents are likely to be rather similar, as the strains used are very closely related. We therefore assumed that the environmental resources themselves determined the BEF.

In the Identity-ratio model, we found that QL-A6 and QL-140 promoted community productivity. Furthermore, the effects of these two strains on productivity was reduced as C:N and C:P increased. Because total carbon concentration was constant across different treatments, we could explain the interactive effects on the basis of nitrogen and phosphorus. We could conclude that the effect of QL-A6 on productivity was modulated by nitrogen and QL-140 modulated by phosphorus. Thus different species exhibited different relative responses to specific resources, thereby affecting community capabilities. QL-A6 appears to be more geared toward response to nitrogen amendment, whereas QL-140 shows the greatest response to phosphorus. When predicting invader growth, QL-140 constrained growth of invader, which was again modulated by phosphorus. However, QL-A6 facilitated the invader in response to both nitrogen and phosphorus. From previous studies, phosphorus limitation was reported to accelerates microbial transitions to stationary phase (Peterson et al. 2005), which constrained cell growth. Nitrogen is also a growth limitation factor, as it takes part in enzymes and proteins construction, which is essential for cell growth capacity (Harder and Dijkhuizen 1983). N and P limitations are known to impact microbial physiology in different ways, so it might be expected that they have different impacts in modulating BEF.

In addition to resource stoichiometry, the form in which elements are present may also impact community function. For instance, the form of inorganic N (nitrate (NO_3^-) or ammonium (NH_4^+)) may impact community function and response to N addition. Ammonium is typically the most abundant form of nitrogen in soil, while nitrate is chiefly present within plants (Boudsocq et al. 2012). We found that changing the source of nitrogen led to overall comparable trends (supplementary materials), although the invader grew in the present case better on nitrate than on ammonium. Therefore, invasion might also be attributable to resource complementarity in the nitrogen sources.

We conclude that resource stoichiometry can modulate biodiversity-invasion relationships. The underlying mechanism appears to be that resource stoichiometry alters identity effects within the community, due to different patterns of resource use efficiency across species. Resource stoichiometry could be a better predictor than resource concentration. As human activities continue to alter global elemental cycling (Vitousek et al. 1997), nitrogen leaching and eutrophication are growing problems that influence ecosystem functioning (Orenzen et al. 2003, Hautier et al. 2009). Our findings provide predictions on how biodiversity-invasion relationships may be impacted by changes in in elemental ratios. Furthermore, a better prediction of suitable

resource stoichiometry can lead to more efficient protection against invasion.

MATERIALS AND METHODS

Bacterial strains and plasmids

We used plant pathogen, *Ralstonia solanacearum* QL-Rs1115 (GenBank accession number GU390462) tagged with pYC12-mCherry plasmid as a model invader in our experiments (Yabuuchi et al. 1995). *R. solanacearum* is a soil-borne pathogen with wide host range (over 200 host species) causing bacterial wilt disease (Salanoubat et al. 2002, Wei et al. 2013).

Here we used five avirulent, but closely related, *Ralstonia spp.* isolates to construct our model resident communities. All the bacterial strains, as well as invader *R. solanacearum* QL-Rs1115, were all isolated from the tomato rhizosphere, and their 16S rRNA sequences are deposited under the GenBank accession numbers JN699058 (strain QL-A2, *Ralstonia mannitolilytica*), KJ780056 (strain QL-A3, *Ralstonia mannitolilytica*), HQ267096 (strain QL-A6, *Ralstonia pickettii*), KJ780054 (strain QL-117, *Cupriavidus taiwanensis*, formerly *Ralstonia sp*.), and KJ780055 (strain QL-140, *Cupriavidus sp*. , formerly *Ralstonia sp*.) (Wei et al. 2015). None of these avirulent bacteria showed antagonism against each other or the invader, indicating that resource competition should be the main means of interaction between each species. A detailed description of the bacteria and plasmid can be found in Supplementary Table 1. All bacteria were stored at -80 ℃ in 20 % glycerol prior to use.

Construction of resident communities

Communities were assembled with between one and five resident isolates, covering all possible combinations and richness levels (31 communities in total, Supplementary Table 2). Bacteria were pre-cultured from frozen stocks on Nutrient Agar (NA, glucose 10.0 g L^{-1}, trypton 5.0 g L^{-1}, beef extract 3.0 g L^{-1}, yeast extract 0.5 g L^{-1}, agar 15.0 g L^{-1}, pH 7.0). Prior to the experiments, single colonies were picked and grown in nutrient broth at 30 ℃ for 12 h with 170 r.p.m. agitation. Bacteria were washed three times in 0.85 % NaCl to remove nutrient residues and re-suspended to a density of 10^7 cells mL^{-1} in 0.85% NaCl. Resident bacterial communities were constructed on a substitutive design (the total initial bacterial biomass was constant in all treatments) as described in Supplementary Table 2.

Invasion and community productivity in microcosms

In order to manipulate resource stoichiometry, we first set up a minimal medium without carbon, nitrogen or phosphorus. This medium contained MOPS 30mM, pH 7.0, $CaCl_2$ 0.1mM, $FeSO_4$ 3 mM, KCl 20mM, $MgCl_2$ 2mM, Na_2SO_4 14mM, NaCl 51mM and was supplemented with a mixture of sugars (fructose, glucose, sucrose, maltose, arabinose, galactose), which were added in equal concentrations to yield

a total concentration of 10 mM for all combined carbon resources, as described in

Table 1. Concentration of C (six sugars), N (NH_4Cl or $NaNO_3$) and P (NaH_2PO_4) resources as well as molecular C, N and P ratios in microcosms.

Resource environment	Sugar (mM)	$NH_4Cl/NaNO_3$ (mM)	NaH_2PO_4 (mM)	Compound C:N:P	Molar C:N:P (Absolute ratio)
1	10	1.5	0.09	106:16:1	8390:390:23
2	10	15	0.09	106:160:1	8390:3900:23
3	10	0.15	0.09	106:1.6:1	8390:39:23
4	10	1.5	0.9	106:16:10	8390:390:230
5	10	15	0.9	106:160:10	8390:3900:230
6	10	0.15	0.9	106:1.6:10	8390:39:230
7	10	1.5	0.09	106:16:1	8390:250:23
8	10	15	0.09	106:160:1	8390:2500:23
9	10	0.15	0.09	106:1.6:1	8390:25:23
10	10	1.5	0.9	106:16:10	8390:250:230
11	10	15	0.9	106:160:10	8390:2500:230
12	10	0.15	0.9	106:1.6:10	8390:25:230

a previous experiment (Wei et al. 2015). The concentration of total carbon resources (10 mM) held constant for all the treatments. We added NH_4Cl or $NaNO_3$ as two kinds of nitrogen resources and NaH_2PO_4 as phosphorus resource. A total of six kinds of CNP ratio combinations were set up, and the experiment was repeated with each of the two nitrogen sources (12 resource environments in total, Table 1, Redfield ratio: 106:16:1). Each kind of medium was then used to establish microcosms on 96-well microtiter plates in triplicate (36 microplates in total).

The different resident communities, as growth in these media, were subjected to inoculation with the pathogenic invader strain *R. solanacearum* QL-Rs1115 (10^5 cells mL^{-1}; 200 µL final volume) with medium addition without *R. solanacearum* QL-Rs1115 acting as a control. Microplates with bacteria were incubated for 72 h at 30 ℃ with 170 r.p.m. orbital agitation. Optical density (OD_{600}) of each well was measured as an indication of total bacterial growth. mCherry fluorescence signal (Excitation: 587 nm, Emission: 610 nm, gain: 60) were determined as a measure of invader abundance. The relative abundance of the invader was defined as the mCherry relative fluorescence unit (RFU, $mCherry/OD_{600}$) at the end of the experiment (after 72 h incubation). We used communities without invader to blank the fluorescence signal and control wells containing culture medium only to blank the OD_{600}.

Statistical analyses

Invader relative abundance (RFU, mCherry/OD_{600}) and resource absolute ratio (C:N, C:P and N:P) were log_{10}–transformed before further analyses. We defined invader growth as invader relative abundance (RFU) and productivity of uninvaded communities as the corresponding optical density (OD_{600}). We took mean value of each community treatment as final analyzed data to minimize the variation of raw data. Microcosms containing NH_4^+ and NO_3^- were analyzed in four separate general linear models (GLM).

First, we set up two separate GLMs investigating invader growth and community productivity as functions of resource stoichiometry, concentration and richness of resident species (diversity effect). In the first model (***Model 1-"Richness-concentration"***), we used community functions (community productivity and invader growth) as the function of the interactive effect of resource concentration (N and P concentration, continuous predictor) and richness (continuous predictor). The second model (***Model 2-"Richness-ratio"***) expressed community functions as the function of resource ratios (continuous predictor) and richness (continuous predictor).

The other two GLMs were designed to examine identity effects as a function of resource stoichiometry, concentration and richness of resident species, similar to the models were used for species richness. ***Model 3-"Identity-concentration"*** and ***Model 4-"Identity-ratio"*** were used to identify how species contributed disproportionately to community functions as resource concentration and stoichiometry changed.

For simplicity, we focus the main text on the NH_4^+ data. This resource is the main inorganic form of nitrogen in most soils and may thus be more relevant in the context of invasion of free-living microbial communities. Analysis of the data based on NO_3^- as nitrogen source, which may be relevant in the context of endophytic microbial communities, are provided in supplementary files (Supplementary Figure 1-2 and Supplementary Table 3-4).

All analyses were performed with R 3.3.1 (R Core Team 2013).

ACKNOWLEDGEMENTS

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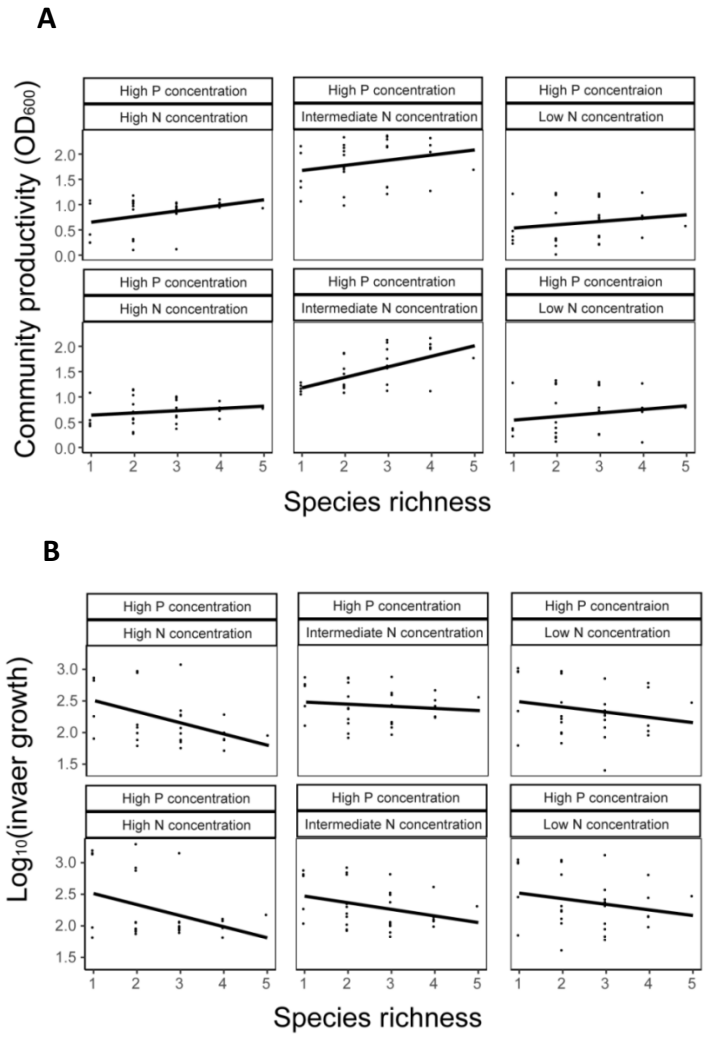
SUPPLEMENTARY INFORMATION

Supplementary Table 1. Information of the bacterial strains and plasmid used in this study.

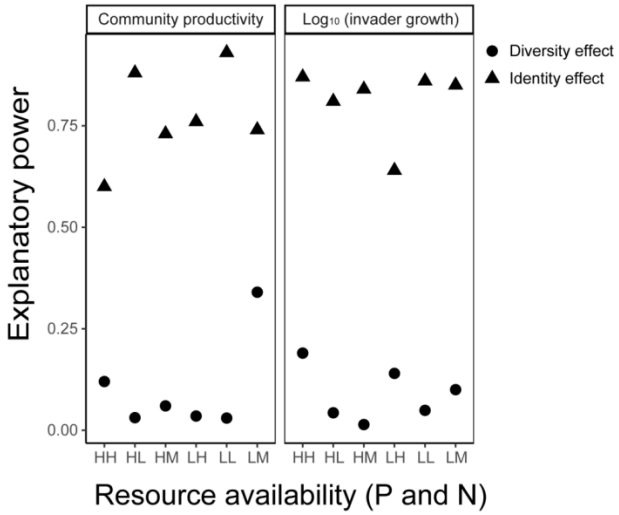
Strain		Close match	GenBank No.	Source
Resident species	QL-A2	*Ralstonia mannitolilytica*	JN699058	(Wei et al. 2013)
	QL-A3	*Ralstonia mannitolilytica*	KJ780056	(Wei et al. 2013)
	QL-A6	*Ralstonia pickettii*	HQ267096	(Wei et al. 2013)
	QL-117	*Ralstonia taiwanensis*	KJ780054	(Wei et al. 2015)
	QL-140	*Ralstonia sp.*	KJ780055	(Wei et al. 2015)
Invader	QL-Rs1115	*Ralstonia solanacearum*	GU390462	(Wei et al. 2011)
Plasmid	PYC12-M	mCherry inserted in the pYC12, under control of the constitutive Ptac promotor, gm^R		(Wei et al. 2015)

Supplementary Table 2. The composition of resident species communities used in the study. In the table, "1" denotes for the presence of the species in the community and "0" for the absence of species in the community. Richness denotes for species richness.

Community	QL-A2	QL-A3	QL-A6	QL-117	QL-140	Richness
1	1	0	0	0	0	1
2	0	1	0	0	0	1
3	0	0	1	0	0	1
4	0	0	0	1	0	1
5	0	0	0	0	1	1
6	1	1	0	0	0	2
7	1	0	1	0	0	2
8	1	0	0	1	0	2
9	1	0	0	0	1	2
10	0	1	1	0	0	2
11	0	1	0	1	0	2
12	0	1	0	0	1	2
13	0	0	1	1	0	2
14	0	0	1	0	1	2
15	0	0	0	1	1	2
16	1	1	1	0	0	3
17	1	1	0	1	0	3
18	1	1	0	0	1	3
19	0	1	1	1	0	3
20	0	1	1	0	1	3
21	0	0	1	1	1	3
22	1	0	1	1	0	3
23	1	0	0	1	1	3
24	0	1	0	1	1	3
25	1	0	1	0	1	3
26	1	1	1	1	0	4
27	1	0	1	1	1	4
28	0	1	1	1	1	4
29	1	1	0	1	1	4
30	1	1	1	0	1	4
31	1	1	1	1	1	5



Supplementary Figure 1. Relationship between resident community richness and productivity of uninvaded communities (panel A) and invader success (panel B) (for media containing NO_3^- as N source). Community productivity was defined as optical density (OD_{600}) of each community combination. Invader relative abundance was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Both panels present data based on microcosms containing ammonium as sole nitrogen source.



Supplementary Figure 2. The explanatory power of diversity (round points) and identity (triangular points) effects on community productivity and invader growth at each elemental resource availability level (for media containing NO_3^- as N source). Community productivity was defined as optical density (OD_{600}) of each community combination. Invader relative abundance was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Both panels present the data from microcosms containing ammonium as sole nitrogen source. Explanatory power stood for R^2 between biodiversity effect and community functions. HH: high P concentration and high N concentration, HL: high P concentration and low N concentration, HM: high P concentration and intermediate N concentration, LH: low P concentration and high N concentration, LL: low P concentration and low N concentration, LM: low P concentration and intermediate N concentration. Separate analyses were performed for each of the elemental resource levels. The explanatory power was defined as R^2 of the biodiversity effect (diversity effect versus identity effect) when fitted to community productivity and invader growth.

Supplementary Table 3. ANOVA table summarizing the interactive effect of resource availability and richness on invader growth and community productivity (for media containing NH_4^+ as N source). Invader growth was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Community productivity was defined as optical density (OD_{600}) of each community combination. Significant effects ($P < 0.05$) are highlighted in bold and the "up" and "down" arrows denote positive or negative effects, respectively.

	Invader growth			Community productivity		
	Df	F	*P*	Df	F	*P*
Model 1-" Richness-concentration"						
Nitrogen concentration (N)	1	4.00	**0.047** ↑	1	1.32	0.25
Phosphorus concentration (P)	1	0.27	0.60	1	2.82	0.095
Richness	1	16.23	**< 0.001** ↓	1	5.77	**0.017** ↑
Richness *N	1	1.81	0.18	1	0.0087	0.93
Richness * P	1	0.21	0.65	1	0.033	0.86
Residuals	180			180		
Model summary	AIC: 183.84, R^2 = 0.11			AIC: 339.90, R^2 = 0.052		
Model 2-" Richness-ratio"						
NP ratio (NP)	1	3.93	**0.049** ↑	1	0.028	0.87
CN ratio (CN)	1	0.37	0.55	1	11.06	**0.0011** ↓
CP ratio (CP)	1	1.31	0.25	1	287.07	**< 0.001** ↑
Richness	1	16.27	**< 0.001** ↓	1	16.03	**< 0.001** ↓
Richness * NP	1	1.93	0.17	1	0.047	0.83
Richness * CN	1	0.097	0.76	1	0.036	0.95
Richness * CP	1	1.13	0.29	1	2.58	0.11
Residuals	178			178		
Model summary	AIC: 185.31, R^2 = 0.12			AIC: 163.75, R^2 = 0.64		

Supplementary Table 4. ANOVA table summarizing the interactive effect of resource availability and species identity on invader growth and community productivity (NH_4^+ as sole N source). Invader growth was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD_{600}) after 72 h incubation. Community productivity was defined as optical density (OD_{600}) of each community combination. Significant effects ($P < 0.05$) are highlighted in bold and the "up" and "down" arrows denote positive or negative effects, respectively.

	Invader growth			Community productivity		
	Df	F	*P*	Df	F	*P*
Model 3-"Identity-concentration"						
QL-A2	1	16.65	**< 0.001** ↑		Not retained	
QL-A3	1	20.59	**< 0.001** ↑		Not retained	
QL-A6	1	221.19	**< 0.001** ↓	1	3.91	**0.050** ↑
QL-117	1	18.29	**< 0.001** ↑		Not retained	
QL-140	1	167.30	**< 0.001** ↓	1	16.96	**< 0.001** ↑
Nitrogen concentration (N)	1	12.78	**< 0.001** ↓	1	1.48	0.23
Phosphorus concentration (P)	1	0.87	0.35	1	3.16	0.078
QL-A6 * P		Not retained			Not retained	
QL-A6 * N	1	7.55	**0.0066** ↓	1	5.35	**0.022** ↑
QL-117 * N	1	2.58	0.11		Not retained	
QL-140 * N	1	2.08	0.15	1	2.69	0.10
QL-140 * P	1	2.69	0.10		Not retained	
Residuals	174			179		
Model summary	AIC: -26.37, R^2 = 0.73			AIC: 319.97, R^2 = 0.16		
Model 4-"Identity-ratio"						
QL-A2	1	20.48	**< 0.001** ↑	1	0.0005	0.98
QL-A3	1	25.32	**< 0.001** ↑	1	0.0079	0.93
QL-A6	1	272.08	**< 0.001** ↓	1	29.59	**< 0.001** ↓
Ql-117	1	22.49	**< 0.001** ↑		Not retained	
QL-140	1	205.79	**< 0.001** ↓	1	128.89	**< 0.001** ↑
NP ratio (NP)	1	15.38	**< 0.001** ↑	1	0.092	0.76
CN ratio (CN)	1	1.40	0.24	1	35.96	**< 0.001** ↓
CP ratio (CP)	1	5.13	**0.025** ↓	1	937.42	**< 0.001** ↑
QL-A2 * NP		Not retained		1	0.29	0.59
QL-A2 * CN	1	1.89	0.17	1	0.89	0.35
QL-A2 * CP		Not retained		1	1.73	0.19
QL-A3 *NP	1	2.38	0.12	1	0.0089	0.93
QL-A3 *CN		Not retained		1	0.23	0.63
QL-A3 *CP		Not retained		1	8.86	**0.0033** ↑
QL-A6 * NP		Not retained		1	13.62	**< 0.001** ↓
QL-A6 * CN	1	9.59	**0.0023** ↑	1	38.36	**< 0.001** ↓
QL-A6 * CP		Not retained		1	134.98	**< 0.001** ↑
QL-117 * NP	1	2.14	0.15		Not retained	
QL-140 * NP	1	0.14	0.71	1	8.12	**0.0049** ↑
QL-140 * CN	1	5.79	**0.017** ↓	1	15.99	**< 0.001** ↑
QL-140 *CP	1	35.32	**< 0.001** ↑	1	87.38	**< 0.001** ↓
Residuals	170			166		
Model summary	AIC: -61.21, R^2 = 0.79			AIC: -44.49, R^2 = 0.90		

Chapter 5

Genome reconstructions predict the functionality of microbial communities

Tianjie Yang, Zhong Wei, Ville-Petri Friman, George A. Kowalchuk, Yangchun Xu, Qirong Shen, Alexandre Jousset

ABSTRACT

Species diversity has been linked to ecosystem functioning in many contexts, including within microbial communities. However, it remains difficult to disentangle the mechanisms underlying diversity / function relationships. With the advent of high throughput sequencing technologies it has become possible to gain full insight into the genomic composition within microbial communities, thereby providing a meant to link genomic traits with community function. Here, we combined microbial genomics with a biodiversity-ecosystem relationship framework and to examine if community invasibility can be predicted from resident community resource consumption patterns as inferred from genomic sequences. A series of microcosm experiments were conducted with engineered microbial communities representing a biodiversity gradient. Community performance and susceptibility to invasion by a closely related plant pathogenic strain were examined as a function community metabolic patterns, as predicted by genomic analysis. Communities with a high inferred complementarity between species were shown to be more resistant to invasion. Our results demonstrate that functional metagenomics can be linked to theoretical community ecology to improve the prediction of microbial community function.

INTRODUCTION

Understanding the relationships between biodiversity and ecosystem functioning is a grand challenge in ecology (Tilman et al. 2014). Species-rich communities are often more productive and stable than species-poor counterparts (McCann 2000, Reich et al. 2012). Biodiversity-ecosystem functioning relationships also drive the productivity, functionality and invasibility of microbial communities (Bell et al. 2005, Becker et al. 2012). Microbial biodiversity-ecosystem functioning relationships are well described in model experimental communities, where the underlying mechanisms can be inferred (Jousset et al. 2011b). However, mechanistically analyzing the importance of biodiversity for the function of natural microbial communities remains a complicated process, especially for highly diverse natural communities where most community members are refractory to cultivation methods. Recent progress in metagenomics has, however, provided exciting windows into the genomic potential of microbial communities across a range of natural and engineered environments (Riesenfeld et al. 2004, Tringe and Rubin 2005). Nevertheless, it remains difficult to transfer these results into predictions of community function.

Summing up the abundance of functional genes within a community, as is typically done in current metagenomics surveys, is a very valuable first step. However, biodiversity-ecosystem functioning relationships studies across ecosystems have revealed that community function is more than a sum of the parts. The interactions between species are crucial to ultimate functioning. For example, a mixture of specialists each consuming a subset of the resources may perform better than a single generalist species harboring the same pool of metabolic genes (Venail et al. 2008, Kawecki et al. 2012). Similarly, the interplay between traits and species is important, and communities composed of functionally complementary species have, for instance, been shown to be more resistant to biological invasions (Symstad 2000, Byun et al. 2013). In this study, we combine genomic data with a biodiversity-ecosystem functioning framework. To validate this approach, we assess whether genomic data can predict the functioning of model constructed communities. Specifically, we focus on resource competition and complementarity in relation to the community's ability to resist invasion by a closely related plant pathogen.

Resource competition is an essential component of species interactions and is a good predictor of community productivity. The success of newly arriving potentially invasive species invasion largely depends on resource competition with the resident species. Resident species or assemblages with a high niche similarity with invaders may be more effected at inhibiting invader proliferation (Ji and

Wilson 2002, Dianese et al. 2003, Eisenhauer et al. 2012b). Moreover, the ratio of competitive interactions between residents and between residents and the invader is a strong predictor of community function and invader success. To this end, trophic networks of species or communities can be calculated to predict their resistance and stability (Thébault and Fontaine 2010, Baiser et al. 2010, Wei et al. 2015).

Resource use is comparatively easy to reconstruct from genome data. In the past two decades, new modelling methods have been developed to predict species metabolic characteristics and interactions based on genome-scale metabolic predictions (Mccloskey et al. 2013, Overbeek et al. 2014, Bordbar et al. 2014). In this study, we used flux-balance analysis (FBA) to reconstruct the ability of each species to consume a range of resources and to estimate community metabolic networks. FBA is a linear programming technique that predicts cellular phenotypes through the flux of biochemical reactions (Cuevas et al. 2016; Orth et al. 2010).

We assessed the ability of genome-based metabolic reconstructions to predict the invasibility of constructed microbial communities. We built a range of model communities composed of between 1 and 5 closely related microbial species, principally interacting via resource competition (*i.e.,* no directed antagonistic interactions). We used flux balance analysis to predict the resources that each species may potentially use. We then rebuilt a bipartite resource x consumer interaction matrix summarizing the ability of each species to consume various resources. In a previous study, we showed that trophic network structure can be a good predictor of invasibility in experimental microbial communities (Wei et al. 2015). In the present study, we sought to examine if similar or improved predictions should be made based upon the genomic content of the resident community members. We benchmarked our genome-based approach by comparing predictions based upon genome content to results obtained in a range of empirical biodiversity-invasibility functioning experiments performed in liquid medium and on host plants. If such genome-based predictions can be validated for relatively simple artificial communities, they might be extended to predicting functionality of more complex microbial communities.

RESULTS

Simulation of metabolic networks

We carried out FBA to simulate resource consumption and secretion by each strain. All the possible exchange fluxes are listed in the summary table of Model SEED, and the total resource exchange fluxes are summarized in Supplementary

(Supplementary Figure 2). The heatmap in Figure 1 represents the metabolic characteristics of each strain for a subset of 48 kinds of resources used in previous experiments (30 kinds of resources in predicted metabolic model, Figure 1).

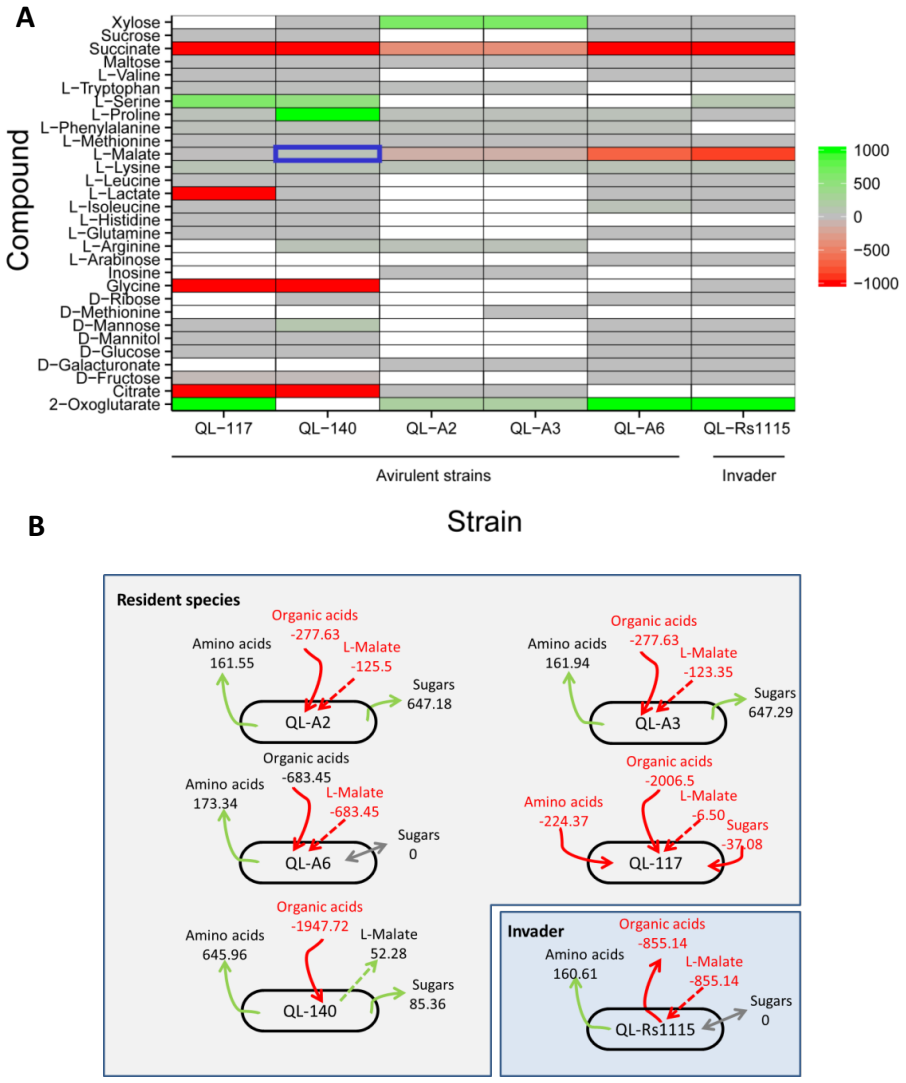


Figure 1. (panel A) Heatmap showing the exchange fluxes at equilibrium of the five resident species and the invader. Exchange fluxes represent a subset of 48 resources representative of rhizosphere environments. Red represents net resource consumption (exchange flux coefficient < 0). Green represents net resource production (exchange flux coefficient > 0). Grey represents an exchange flux coefficient = 0. White represents a lack of exchange flux. Blue-bordered squares show that the strain produces a resource that may benefit others. (panel B) Inferred scheme of resource use. Resource use of each strain is shown, including organic acids, amino acids, sugars. Green arrows represent coefficients of exchange flux greater than 0. Red arrows represent coefficients of exchange flux less than 0. Grey arrows represent coefficients of exchange flux near 0.

Flux balance analysis further revealed the potential for cross-feeding between strains. QL-140 had a positive value (more than 0) for L-Malate, suggesting that it generated a net efflux of this compound. In contrast, other strains (QL-A2, QL-A3, QL-A6 and QL-Rs1115) were probably acting as a metabolic sink for L-Malate. Although cross-feeding among the avirulent strains may contribute to community dynamics, we will not address cross-feeding further in this study in order to ensure comparability with the empirical data.

Validation of predicted variables with observed data

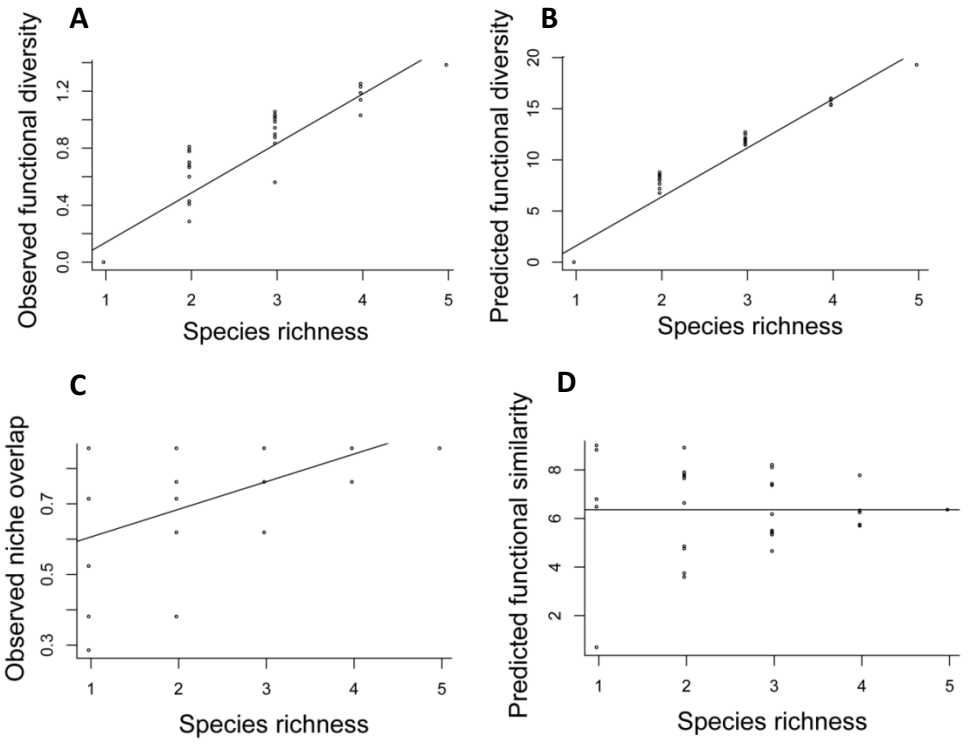


Figure 2. Validation of predicted functional diversity with species richness and observed functional diversity. Both observed (panel A) and predicted (panel B) functional diversity had positive correlations with species richness ($P < 0.001$, F = 300.48, $R^2 = 0.89$, panel A, $P < 0.001$, F = 144.33, $R^2 = 0.79$, panel B). The increase of species richness, resulted in an increase in observed niche overlap ($P < 0.001$, F = 20.09, $R^2 = 0.35$, panel C). Predicted functional similarity was not influenced by species richness (panel D).

We calculated predicted functional diversity and functional similarity and predicted these valued to empirical data. First, we validated predicted functional diversity by performing a linear regression with species richness. Observed functional diversity was positively correlated to species richness ($P < 0.001$, F = 300.48, $R^2 = 0.89$, Figure 2, panel A). As species richness increased, predicted

functional diversity also increased (P < 0.001, F = 808.98, df = 114, R^2 = 0.95, Figure 2, panel B). When compared to observed functional diversity, predicted functional diversity had a good linear correlation with observed data (P < 0.001, F = 572.86, df = 114, R^2 = 0.94).

Niche overlap increased when there were more species in the community (Figure 2, panel C). The more species that community contains, the more functional similarity between the community and invader (Figure 2, panel D). Since the concept of functional similarity indicating negative correlation with observed niche overlap, we also compared the predicted value with observed niche overlap. There was significant negative linear relationship between predicted functional similarity and observed niche overlap (P < 0.001, F = 28.22, R^2 = 0.43).

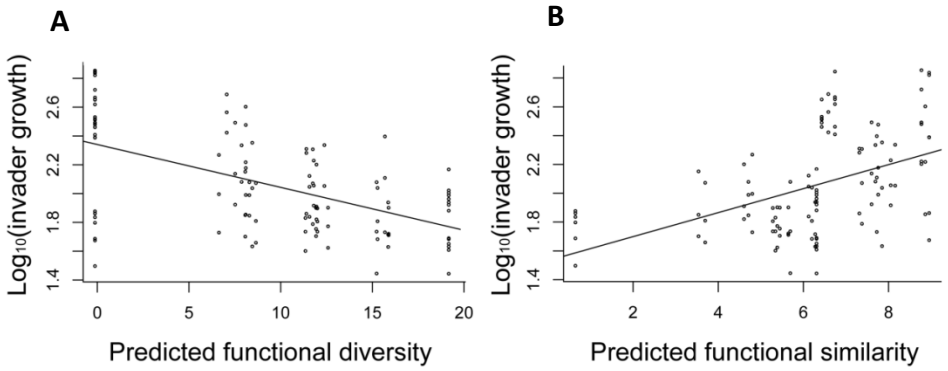


Figure 3. Validation of predicted functional diversity with *in vitro* invasion data. Predicted functional diversity could reduce invader growth significantly (P < 0.001, df = 116, R^2 = 0.33, panel A). Predicted functional similarity also had a negative effect on invader growth (P < 0.001, df = 116, R^2 = 0.21, panel B). Each point represents one individual replicate.

Subsequently, we validated predicted variables with *in vitro* invasion data. Predicted functional diversity showed a strong correlation with invader growth in microcosm experiments (P < 0.001, F = 51.13, R^2 = 0.31, Figure 3, panel A). Similarly, predicted functional similarity was also a good predictor of invasion success (P < 0.001, F = 30.84, R^2 = 0.21, Figure 3, panel B). The comparative empirical data is given in Supplementary Figure 3.

Table 1. ANOVA table summarizing interactive effects between inferred functional diversity and time (***Model 1-"FD"***) and between inferred functional similarity and time (***Model 2-"Distance to invader"***) on *in vivo* invasion success (proportion of wilted plants in each pot). The significant effects ($P < 0.05$) are highlighted in bold and the 'up' and 'down' arrows denote for positive or negative effects, respectively.

	Gnotobiotic system		Natural soil	
	Df	F	Df	F
Model 1 – FD				
Predicted functional diversity (FD)	1	**980.23 ↑**	1	3.80
Time	1	**3089.62 ↑**	1	**1022.41 ↑**
FD * Time	1	**520.48 ↓**	1	0.52
Residuals	696		1340	
Model summary	R^2 = 0.87		R^2 = 0.43	
Model 2 – Distance to invader				
Predicted functional similarity (Dis)	1	**14.93 ↓**	1	**6.83 ↑**
Time	1	**1008.64 ↑**	1	**1024.59 ↑**
Dis * Time	1	**6.21 ↑**	1	0.26
Residuals	696		1340	
Model summary	R^2 = 0.60		R^2 = 0.44	

We further used genome-inferred resource use patterns to predict disease incidence in plants preliminary coated with the different resident communities. As the invading pathogen, *R. solonacaearum,* must first invade root-associated microbial communities before infesting the host, the proportion of wilted plants is a good indicator of invasibility in such more natural systems (Wei et al. 2015). We analyzed two greenhouse experiments: One gnotobiotic experiment, in which we inoculated sterile plants with the resident communities (Wei et al. 2015), and one natural soil experiment in which the same bacteria were added to a natural, non-sterile soil. In sterile substrate, inferred functional diversity negatively affected invasion success (Table 1, FD main effect in sterile substrate, ***Model 1-"FD"***) and this effect increased with time (table 1, FD x Time interaction in sterile substrate, ***Model 1-"FD"***). In addition, invasion success was also predicted by the inferred community similarity to the invader (main effect of predicted functional

similarity on invasion success in sterile substrate, ***Model 2-"Distance to invader"***). During the later phase of invasion, the slope of the relationship between predicted functional similarity and invasion success increased (interactive effect Dis x Time in sterile substrate, ***Model 2-"Distance to invader"***). The same GLM made based on empirical data is presented in Supplementary Table 3.

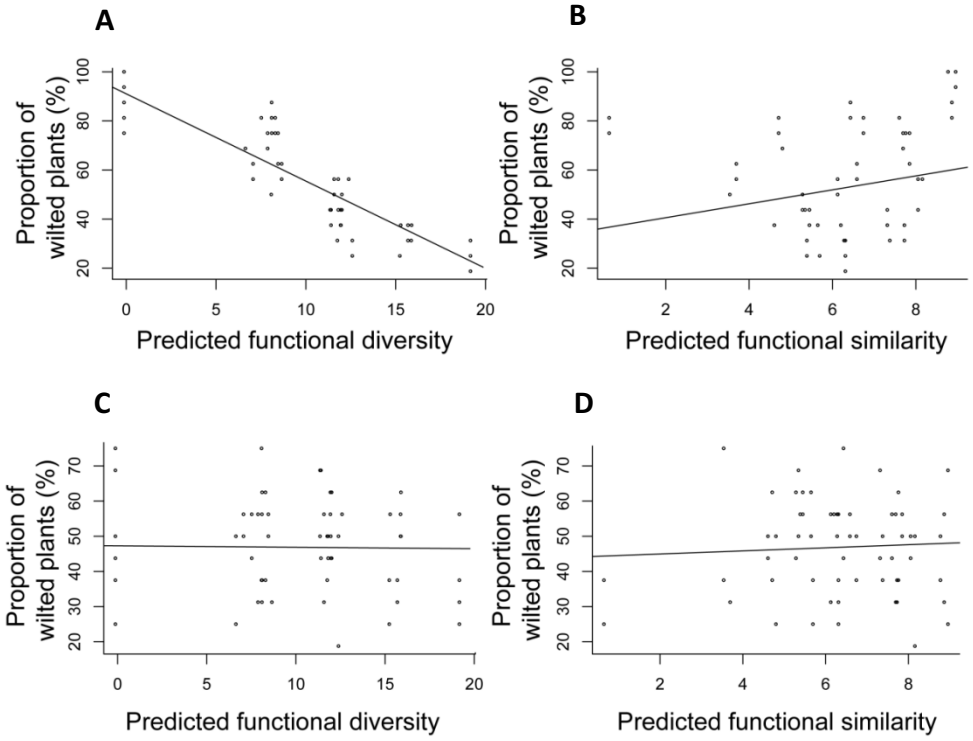


Figure 4. Validation of predicted functional diversity with *in vivo* invasion data. Predicted functional diversity could reduce invasion success significantly ($P < 0.001$, F = 169.04, df = 68, $R^2 = 0.71$, panel A). Predicted functional similarity had a negative, but not significant, effect on invader growth ($P = 0.086$, panel B). There was no significant effect of predicted functional diversity and functional similarity on invasion success in natural soil (both $P > 0.1$, panel C and panel D).

However, in natural soil, predicted functional diversity did not predict invasion success (Table 1, in natural soil, ***Model 2-"Distance to invader"***). Invasion success was only slightly promoted by predicted community distance (main effect of predicted functional similarity on invasion success in natural soil, ***Model 2-"Distance to invader"***). The same GLM based on empirical data is shown in Supplementary Table 3.

In order to find the main effect of predicted variables on invasion success, we selected data from the final day of the greenhouse experiment, where the proportion of wilted plants maintained stable. In sterile substrate, we found a

negative effect of predicted functional diversity on invasion success ($P < 0.001$, df = 68, F = 169.04, $R^2 = 0.71$, Figure 4, panel A). However, predicted functional similarity could not predict invasion success ($P = 0.086$, Figure 4, panel B). With reference to the natural soil, both predicted variables had no significant effect on invasion success in the greenhouse experiment ($P > 0.1$, Figure 4, panel C and panel D). The corresponding empirical data are presented in Supplementary Figure 4.

DISCUSSION

There have been numerous studies revealing biodiversity-ecosystem functioning relationships in microbial ecology (Hodgson et al. 2002, Jousset et al. 2011b, van Elsas et al. 2012), and such relationships have been reported for a range of soil processes. Despite the breakthroughs in metagenomics, it remains difficult to predict the actual microbial community function based upon genomic content of the resident community. In this study, we generated a genomic-scale metabolic model (Flux Balance Analysis, later FBA) to simulate resource-dependent metabolic patterns of individual species and constructed microbial communities. FBA can provide quantitative analysis on metabolic capacity and metabolites flow through biochemical pathways in or between cellular structures under specific resource constrains (Kauffman et al. 2003, Orth et al. 2010). We took resistance to invasion as a model community function to examine the correlation between genome-inferred resource use patterns and interspecific interactions. To this end, we chose five microorganisms interacting essentially via resource competition (thus allowing us to ignore antagonistic interactions at that stage) to build model resident communities and assessed their ability to resist invasion by an important plant pathogen. We found that predicted variables calculated from simulated metabolic models could predict invasion *in vivo* and *in vitro*, and these predictions were, in some cases, even better than those provide by observed resource use metrics.

We found a good match between inferred and observed trophic variables ($R^2 = 0.94$ for functional diversity and $R^2 = 0.43$ for functional similarity). This consistency suggests the reliability of FBA in simulating resource use profiles. FBA has been highly useful in predicting growth capacity (Edwards and Palsson 2000, Feist et al. 2007) and species trophic interactions (Mee et al. 2014). The robust prediction of phenotypes based on FBA indicates that inferred variables might be good predictors of specific ecosystem functions, such as resistance to invasion. By combining genome-inferred and experimental data, we demonstrated that inferred variables could be good predictors of community invasibility. Predicted functional diversity had a negative effect on pathogen invasion success in sterile conditions (microcosm and gnotobiotic system). FBA has proven to be a valuable

tool in other studies designed to predict species and community function (Jeffries 2006, Schwender 2008, Bordbar et al. 2014).

Compared to observed variables, predicted functional diversity (R^2 = 0.32 in microcosms, R^2 = 0.71 in gnotobiotic system) was a better predictor of invader growth than empirically determined functional capabilities (R^2 = 0.27 in microcosms, R^2 = 0.64 in gnotobiotic system). In contrast, observed niche overlap (R^2 = 0.37 in microcosms, R^2 = 0.51 in gnotobiotic system) had a higher explanatory power than inferred community similarity with respect to explaining the inhibition of invader growth (R^2 = 0.21 in microcosms, no significant effect in gnotobiotic system). Our results suggest highlight the importance of functional diversity. Community functional diversity had more explanatory power in predicting invasion success than niche overlap or functional similarity with invaders. It must however, we noted that FBA may have some inherent biases. The coefficients of exchange fluxes represent the fraction of metabolites flow through cells (Jeffries 2006), thus the exact amount of metabolites participating into cell growth and interactions is not known. Differences between prediction and observation may also be, for instance, due to ATP maintenance parameters or the production of by-products (Feist et al. 2007). Such analyses could be further improved by using simulated biomass reactions, which show the actual amount of reactants that are contributing to species biomass production (Henry et al. 2010).

For model validation with natural soil data, none of the predicted and observed variables could explain invasion success. This may be because other indigenous species also function as effective inhibitors of invasion (Lugtenberg and Kamilova 2009, Van Nevel et al. 2013) or that the diverse microbial community buffers against the effects of inoculated species (Lugtenberg and Kamilova 2009). Although the inoculated communities are composed of soil microbes, the introduced species may still be uncompetitive after introduction into the natural soil community, thereby reducing the impact on pathogen invasion (Compant et al. 2005).

Our study demonstrates that genomic-scale metabolic model can be used to predict community-wide species interactions that determine important ecosystem properties such as susceptibility to invasion. This opens new venues to interpret metagenomic data by means of genomic reconstruction, inferring interactions and predicting community function under specific environmental conditions. We conclude that reliable genomic-metabolic analyses can predict metabolic capacity, species interactions and community function. Our findings may provide framework for linking genomic-based prediction with community trophic networks in the real world. Rapid advances in metagenomics are providing ever-increasingly detailed catalogs of community structure and genetic potential

(Tyson et al. 2004). We suggest that metabolic predictions, as afforded by FBA, may prove to be a fruitful approach in predicting microbial community function.

MATERIALS AND METHODS

Bacterial strains and growth conditions

We used wildtype *Ralstonia solanacearum* QL-Rs1115 (GenBank accession GU390462) as the invading pathogen (Wei et al. 2011). Five avirulent, but closely related *Ralstonia* species, were chosen to construct the model communities. Their 16S rRNA sequences are deposited under the accession numbers JN699058 (strain QL-A2, *Ralstonia mannitolilytica*), KJ780056 (strain QL-A3, *Ralstonia mannitolilytica*), HQ267096 (strain QL-A6, *Ralstonia pickettii*), KJ780054 (strain QL-117, *Cupriavidus taiwanensis*, formerly *Ralstonia sp*.), and KJ780055 (strain QL-140, *Cupriavidus sp*. , formerly *Ralstonia sp*.) (Wei et al. 2015). None of the bacteria showed antagonism towards each other or *R. solanacearum* QL-Rs1115 based upon assays conducted on nutrient agar plates, suggesting that resource competition was probably the main means of competitive interactions between each other and with the pathogenic strain. All information on the bacterial strains can be found in Table_1_SuppInfo.

In order to obtain a single colony of each strain, bacterial stocks were recovered from -80 ℃ 20 % glycerol stocks and streaked out on nutrient broth agar plates (NB, glucose 10.0 g L^{-1}, tryptone 5.0 g L^{-1}, beef extract 3.0 g L^{-1}, yeast extract 0.5 g L^{-1}, agar 15 g, pH 7.0) for 48 h at 30 ℃

Genome sequencing and assembly

Single colonies of each strain were inoculated into NB medium and grown for 12 h at 30 °C with 170 r.p.m. orbital agitation. Genome DNA was extracted using the QIAGEN blood and cell culture DNA Kit (catalog No. QIAGEN Genomic-tip 100/G, Midi 13343). All the strains were sequenced using the Miseq platform (2*300 bp) at the Utrecht Sequencing Facilities, Utrecht, the Netherlands. In order to obtain more accurate genome assembly, reads were pre-processed as follows: We first trimmed adapter sequences, low-quality nucleotides occurring at 5’ and 3’ ends with a Phred quality score < 20 and reads containing N more than 10 %. We then excluded reads shorter than 50 bp. Assembly was carried out in two steps. First, we used SOAPdenovo v2.04 to assemble optimized oligonucleotide into contigs and scaffolds based on K-mer size (available at http://soap.genomics.org.cn/). Second, GapCloser v1.12 was used to close gaps merging during the scaffold process by SOAPdenovo (Li et al. 2010).

Genome-scale metabolic model reconstruction

We used the Model SEED pipeline to generate metabolic phenotypes of all the strains used in our study (Henry et al. 2010). Genome assembly was conducted by Biozeron (http://www.biozeron.com/). First, assembled genomes were uploaded to the RAST server (http://rast.nmpdr.org/), which was used for automated genome annotation (Aziz et al. 2008). The RAST server is connected to biochemical reactions encoded by Model SEED (Overbeek et al. 2014) and can be used to import annotations into Model SEED. On the basis of annotations given, preliminary genome reconstructions could be generated via Model SEED (http://www.theseed.org/models/). Finally, Flux Balance Analysis (FBA) was used to generate metabolic models based on transportable nutrients to simulate biomass production in the growth medium.

We performed FBA based on the exchange fluxes of each strain as provided in the standard Model SEED settings. Coefficients of exchange fluxes describe the net flow of metabolites entering or leaving the cell (Jeffries 2006). This net flux represents metabolic flow through metabolic pathways and the rates derived from total rates of nutrient consumption, growth and substrate production. Coefficients of exchange fluxes were used as a proxy of resource use capacity. An exchange flux coefficient greater than 0 indicates a net secretion of a given compound, and a value below 0 indicates that the species consumes more of this resource than it produces.

Simulation of metabolic networks

In order to compare predicted resource use profiles to observed ones, we selected the FBA coefficients for a subset of 48 carbon sources that are representative of the rhizosphere environment (see Chapter 2 with reference to mimic the rhizosphere environment, Supplementary Table 2). We used the FBA coefficient matrix (later: inferred indices) and the empirical data from a previous experiment, including Chapter 2 “Greenhouse experiment”, Chapter 3 “microcosms experiment” (at low resource availability, 1.85, 0.925 and 0.37 g L^{-1} NB medium, catabolic similarity as the main effect inhibiting invasion) and an additional greenhouse experiment with natural soil (later: empirical indices) to compute three indices. We first calculated a distance matrix between all six tested isolates, using the dist() function in R. We then used this matrix to compute the inferred functional similarity between the resident community and the invader, defined as the averaged ecological distance between resident species and the invader. This index is a good estimator of the intensity of resource competition. In order to ensure comparability with previous experiments, we used niche overlap as an empirical niche distance measurement. Observed niche overlap was defined as

the fraction of resources consumed by the invader that are also consumed by at least one of the resident species (Ji and Wilson 2002), based on the observed resource use patterns of all the species (Supplementary Table 2). We then calculated inferred and empirical functional similarity using the pd() function in the R package vegan. Functional similarity is a good proxy for niche differentiation within the species composing the resident community and a good predictor of invasibility (Fargione and Tilman 2005).

Validation of predicted variables with observed data

To validate the genome-inferred metabolic model, we used the experimental data generated in Chapter 2 “Greenhouse experiment”, Chapter 3 “microcosms experiment” and an additional greenhouse experiment with natural soil (data shown in Supplementary Figure 1). For these experiments, bacterial resource use was obtained by growing each strain separately in a minimal medium supplemented with an array of 48 carbon sources representative for the rhizosphere environment (Wei et al. 2015, Yang et al. 2017).

We constructed a general linear model (GLM) expressing invasion success as a function of interactive effects of trophic variables and time. The first model (***Model1-“FD”***) expressed invasion success as a function of the interactive effect of functional diversity and time. The second model (***Model2-“Dis”***) expressed invasion success as a function of the interactive effect of functional similarity and time. All analyses were performed with R 3.3.1.

ACKNOWLEDGEMENTS
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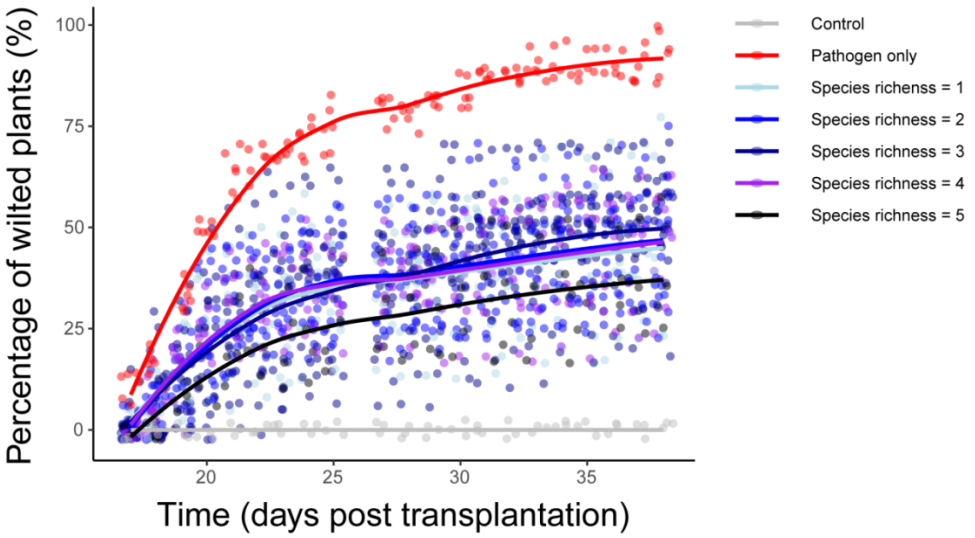
SUPPLEMENTARY INFORMATION

Supplementary Table 1. Based on the total length of assembled sequencing, scaffold numbers and scaffold N50 *etc.*, we chose K-mer size as 25. The final assembly results as following.

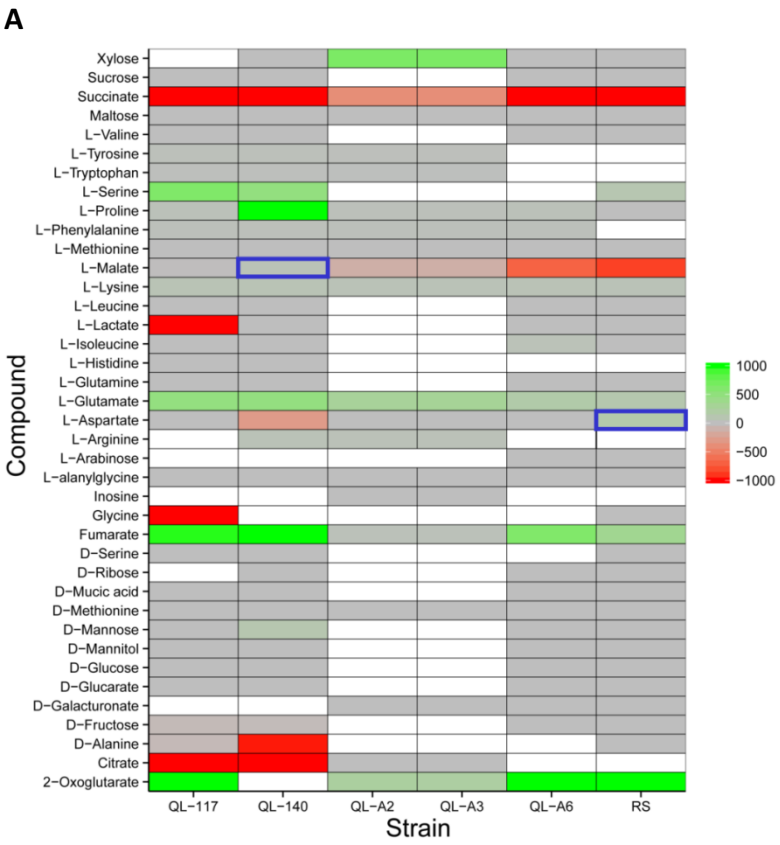
Strain	QL-A2	QL-A3	QL-A6	QL-117	QL-140	QL-Rs1115
No. of all scaffolds	27	29	18	65	348	116
Bases in all scaffolds	5184487	5185010	5299298	6609321	9935933	5640215
No. of large scaffolds (> 1000 bp)	25	24	17	53	272	89
Bases in large scaffolds	5183049	5181859	5298540	6600794	9881773	5619816
Largest length	822522	822522	890039	721446	398784	483985
Scaffold N50	556165	556017	743349	386286	103713	152534
Scaffold N90	157094	157094	175769	113920	21482	50123
G+C content	36.708	36.708	63.827	67.488	65.129	67.049
N rate	0.001	0.002	0	0	0	0

Supplementary Table 2. The resource use patterns of each resident species and the pathogen on 48 different resources. The plus sign denotes for species ability to grow on specific resources.

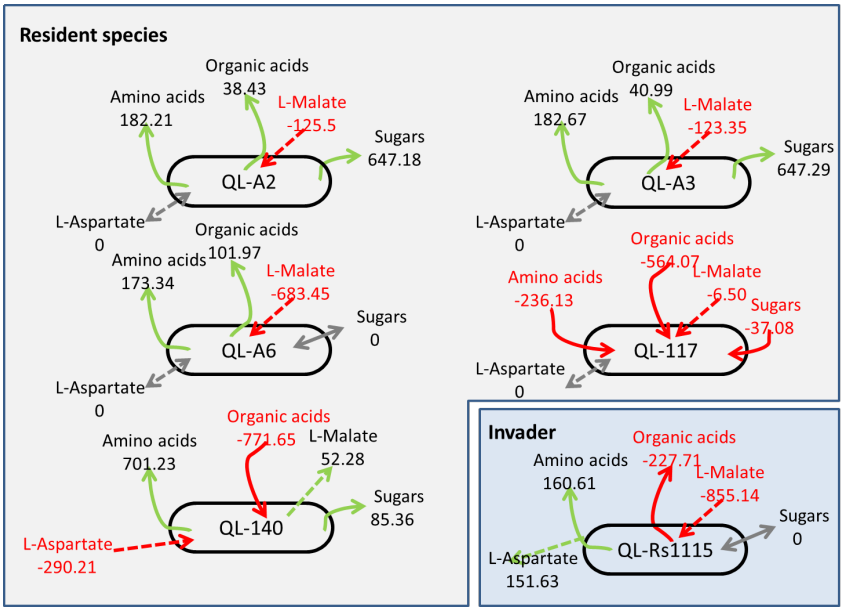
No.	Resources	QL-A2	QL-A3	QL-A6	QL-117	QL-140	QL-Rs1115
1	Acetic aicd(Ace)			+		+	+
2	L-Alanine(Ala)			+	+	+	+
3	β-Alanine(Ala)		+	+	+	+	
4	L-Arginine(Arg)						
5	Ascorbic acid(Asc)					+	
6	L-Asparagine(Asn)	+	+	+	+	+	+
7	γ-Aminobutyric acid(Ami)	+	+	+		+	+
8	Citric acid(Cit)			+		+	+
9	Citrulline(Cin)			+	+	+	+
10	Ethanolamine(Eth)						
11	Formic acid(For)						
12	Fructose(Fruc)			+		+	
13	Galacturonic acid(Galac)			+			+
14	Glucose(Glu)	+	+	+			+
15	L-Glutamine(Gln)	+	+	+	+	+	+
16	Glutaric acid(Glut)					+	
17	L-Glycine(Gly)			+		+	
18	Glycolic acid(Glyc)			+	+	+	
19	L-Histidine(His)		+	+	+	+	+
20	Isoleucine(Iso)	+			+	+	
21	Lactic acid(Lac)	+	+	+	+	+	+
22	L-Lysine(Lys)						
23	L-Leucine(Leu)				+	+	
24	Maleic acid(Male)					+	
25	Malic acid(Mal)			+	+	+	+
26	Malonic acid(Malon)			+	+	+	
27	L-Methionine(Met)						
28	Myoinositol(Ino)						+
29	2-Oxoglutaric(Oxo)			+		+	+
30	L-Phenyalanine(Phe)			+	+	+	+
31	L-Proline(Pro)	+	+	+	+	+	+
32	Pyruvic acid(Pyr)		+	+	+	+	+
33	L-Serine(Ser)			+			+
34	Succinic acid(Succ)			+	+	+	+
35	Sucrose(Sucs)						+
36	Tartaric acid(Tar)						+
37	L-Threonine(Thr)			+	+	+	
38	L-Tryptophan(Try)			+	+	+	
39	L-Valine(Val)		+	+	+	+	
40	Maltose(Mal)			+		+	
41	L-Arabinose(Ara)			+		+	
42	D-Galactose(Gal)		+	+			
43	D-Mannose(Man)		+	+			
44	D-Xylose(Xyl)						
45	D-Ribose(Rib)						
46	D-Mannitol		+				
47	Inosine(I)		+				
48	Oxalic acid(Oxal)						



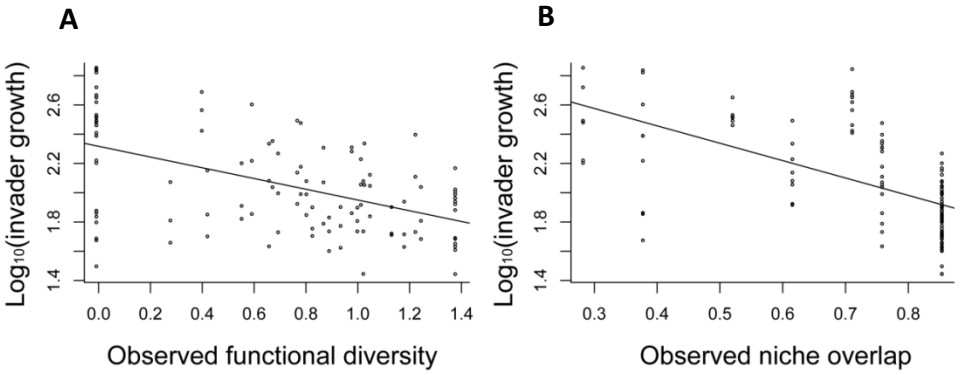
Supplementary Figure 1. Spread of bacterial wilt plant disease in the absence and presence of resident communities (control denotes for treatment without any bacteria).



B



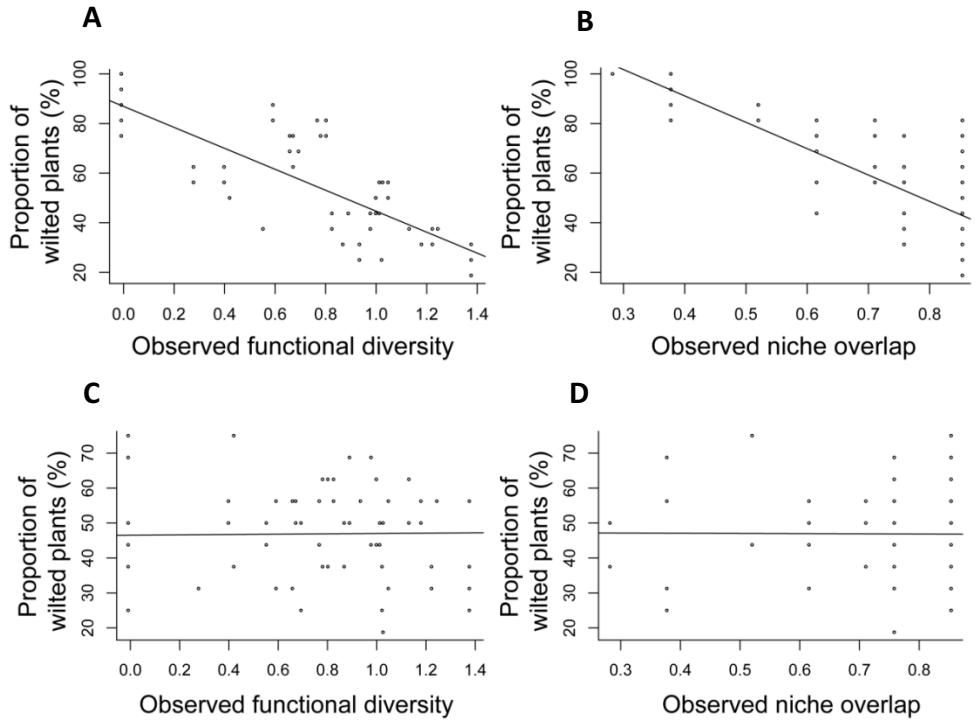
Supplementary Figure 2. Heatmap showing the exchange fluxes at equilibrium of the five resident species and the invader. Exchange fluxes represented resources representative for rhizosphere conditions. Red represented net resource consumption (exchange flux coefficient < 0). Green represented net resource production (exchange flux coefficient > 0). Grey represented exchange flux coefficient = 0. White represents there was no exchange flux. Blue bordered square represented the strain produced resource that may benefit others (panel a). Inferred scheme of resource use. Resource use of each strain was shown, including organic acids, amino acids, sugars. Green arrow represented coefficient of exchange flux more than 0. Red arrow represented coefficient of exchange flux less than 0. Grey arrow represents coefficient of exchange flux was 0 (panel b).



Supplementary Figure 3. Predicted functional diversity could reduce invader growth significantly ($P < 0.001$, $F = 43.27$, $R^2 = 0.27$, panel a). Predicted community distance to invader had negative effect on invader growth ($P < 0.001$, $F = 68.48$, $R^2 = 0.37$, panel b).

Supplementary Table 3. ANOVA table summarizing interactive effect between empirical functional diversity and time (***Model 1-"FD"***) and between empirical functional similarity and time (***Model 2-"Distance to invader"***) on *in vivo* invasion success (proportion of wilted plants in each pot). The significant effects ($P < 0.05$) are highlighted in bold and the 'up' and 'down' arrows denote for positive or negative effects, respectively.

	Gnotobiotic system		Natural soil	
	Df	F	Df	F
Model 1 – FD				
Predicted functional diversity (FD)	1	**512.19 ↑**	1	0.27
Time	1	**2108.51 ↑**	1	**1020.64 ↑**
FD * Time	1	**290.96 ↓**	1	1.63
Residuals	696		1340	
Model summary	$R^2 = 0.81$		$R^2 = 0.43$	
Model 2 – Distance to invader				
Predicted functional similarity (Dis)	1	**277.94 ↑**	1	**8.49 ↓**
Time	1	**1606.59 ↑**	1	**1026.35 ↑**
Dis * Time	1	**168.34 ↑**	1	0.90
Residuals	696		1340	
Model summary	$R^2 = 0.75$		$R^2 = 0.44$	



Supplementary Figure 4. Validation of predicted functional diversity with *in vivo* invasion data. Predicted functional diversity could reduce invasion success significantly ($P < 0.001$, $F = 122.13$, $R^2 = 0.64$, panel a). Predicted community distance to invader had negative effect but not significant on invader growth ($P < 0.001$, $F = 69.44$, $R^2 = 0.51$, panel b). There was no significant effect of predicted functional diversity and community distance to invader on invasion success in natural soil (both $P > 0.1$, panel c and panel d).

Chapter 6

Summarizing discussion

Tianjie Yang

Understanding biodiversity-invasion relationships

Species diversity is an important determinant of community invasibility (Tilman et al. 2014). Species-rich communities are often more resistant to invasion if they include more functionally divergent species (Fargione and Tilman 2005). Biodiversity has consistently been found to reduce invasion in terrestrial (Knops et al. 1999, Naeem et al. 2000), aquatic (Stachowicz et al. 1999) and microbial communities (Jousset et al. 2011b, van Elsas et al. 2012). Nonetheless, biodiversity-invasion relationships are far from straightforward. Variation between species and environmental conditions (Eisenhauer et al. 2012b) may affect the competitive interactions and as a consequence the fate of invasions.

In this thesis, I sought to examine the relationship between biodiversity and invasion under changing resource conditions. To this end, I established a model system composed of six bacterial strains: Five microbial strains belonging to the closely related genera *Ralstonia* and *Cupriavidus* as model residents, and the plant pathogen *Ralstonia solanacearum*, as a model invader. This pathogen is a severe threat to agricultural production and causes wilt disease in more than 50 plant families (Hayward 1994). A series of related experiments was conducted in which microbial community composition was systematically manipulated to examine the influence of diversity and functional capabilities of the resident community on community performance and resistance to pathogen invasion. The ultimate goal was to determine the community functional parameters that best prevent pathogen invasion, thereby providing insight into the potential strategies to improve plant health and protection from disease.

In **Chapter 2**, I investigated the importance of resource competition patterns for community invasibility. Different rhizosphere situations were mimicked by randomly selecting resources representative of those found it plant root surroundings. The results showed a negative relationship between species richness and community invasibility in microcosms. As there is no antagonism among all the strains comprising the community, I assumed this relationship could be linked to resource competition. I therefore focused on patterns of resource use and overlap. In order to verify our findings, greenhouse experiments were carried out using sterile soil as a growth substrate basis and tomato plants as hosts. The proportion of wilted plants of each pot (the total plants in each pot is the same) was

used as a proxy of pathogen invasion success in host plants. In the late stage of pathogen invasion, treatments with higher species richness had lower numbers of wilted plants, indicating a negative biodiversity-invasion relationship *in vivo*. These results are in line with other studies have that demonstrated that biodiversity-invasion relationships suggested under artificial conditions are retained when examined *in vivo* (Raupach and Kloepper 1998, Hu et al. 2016). The results also demonstrated that simplified laboratory systems can predict the function of natural communities and pinpoint the underlying mechanisms. Furthermore, this study highlights that changes in resource composition may alter the type of competitive interactions between species.

In order to extend our predictive framework, the impact of changing resource availability on biodiversity-invasion relationships was tested. The same communities as used in chapter 1 were grown in a dilution series of culture medium. In **Chapter 3**, the results showed that resource availability modulated biodiversity-invasion relationships by altering the contributions of specific species to invader growth. In **Chapter 4**, the study linked biodiversity research to ecological stoichiometry, thereby showing that different ratios of elemental nutrients (carbon, nitrogen and phosphorus) modulate identity effects on community productivity and invader growth by altering contributions of out-leading species.

In **Chapter 5** I attempt to further generalize these results and test whether invasibility can be inferred from the genome sequence of the resident community members. I inferred the metabolic potential of the resident bacteria from their genomic sequence and demonstrated that genomic information can robustly predict the functioning of microbial communities. These results offer new perspectives into how to combine experimental communities with soil metagenomics.

Competitive interactions mechanistically explain biodiversity-invasion relationships

Competitive interactions between resident community and an invader

Most reported negative biodiversity-invasion relationships have been attributed to competition between the resident community and the invader (Fargione and Tilman 2005, Jousset et al. 2011b, Byun et al. 2013). Mechanistically, two types of competition may play a role, resource competition and antagonism. In order to focus on resource competition, I selected a model system in which no antagonism among resident species and between resident species and the invader could be detected. In this way, I could focus specifically on resource competition. Moreover, resource competition between a given resident community and a potential invader have been shown to play important role in invasion suppression

(Eisenhauer et al. 2012b, Mallon et al. 2015). If a community with high diversity consumes nearly all resources, few resources remain unconsumed. The invader is thus inhibited by low resource availability due to the strong resource competition with resident community.

In order to quantify the resource competition between the resident community and the invader, the concept of niche overlap has proven useful for reflecting the strength of resource competition. I found that high niche overlap between the resident community and the invader could profoundly reduce the density of the invasive pathogen in rhizosphere-like microcosms and greenhouse experiments. I also demonstrated that pathogen invasion was suppressed by high niche overlap in the latter stages of the invasion process (**Chapter 2**). This suggests that even after an invader is initially established a new environment, competing for resources remains vital for further proliferation. In **Chapter 3**, I highlighted that the importance of resource complementarity for invasion was mainly visible at low resource availability. At the high resource availability, invasion was generally driven by the inclusion of particular, fast-growing species in the resident community.

Competitive interactions within the community

In **Chapter 2**, connectance and nestedness were good predictors of invasion inhibition. Interestingly, the relative importance of niche overlap appeared to be lower than the impacts of nestedness and connectance in the rhizosphere. Taken together, these results suggest that competitive interactions among species within the community are also a determinant factor for invasion success *in vivo* and *in vitro*, together with niche overlap.

Communities with a high connectance were more resistant to invasion, likely by using a greater proportion of all available resources (**Chapter 2**). However, highly connected communities may also promote invasion if interactions lead to strong competition within the resident community (Baiser et al. 2010, Lurgi et al. 2014). The impact of connectance on invasion therefore depends on the experimental scale. At small special scales, resources may be distributed homogeneously, which allows high resource use efficiency of highly connected community and less intra-specific resource competition (Amarasekare 2003). This may explain why connected communities are more robust to invasion (Case 1990, Pimm 1991, Romanuk et al. 2009). However, at relatively larger scales such as plant roots, where resources may be distributed in more heterogeneously, invasion is facilitated by communities with high connectance. For example, in the greenhouse experiment presented in **Chapter 2**, connectance sped up the development of disease symptoms. In this case, competition within the community is quite intense, leading to a destabilized community structure. In a spatially heterogeneous environment, species that are susceptible to the invader will assemble in small regions without the invader. The high density of susceptible

species then leads to high intra-specific competition (Amarasekare 2003).

I also found that nestedness had positive correlations with pathogen invasion both *in vivo* and *in vitro* in **Chapter 2**. Nestedness represents the resources that specialists use as a subset of generalists and the total community. Highly nested communities have high niche overlap between specialists and generalists, suggesting low resource use complementarity. This leads to strong competition between specialists and generalists and low community stability (Okuyama and Holland 2008, Thébault and Fontaine 2010), which results in an increased susceptible to invasion.

Thus, communities with high connectance and low nestedness are more resistant to invasion. Our results showed that connectance and nestedness had interactive effects on invasion, which emphasizes the importance of considering both of these trophic variables when predicting invasion success. In conclusion, community trophic structures can be robust predictors of invasion both *in vivo* and *in vitro*.

Resource availability drives biodiversity-invasion relationships

Many abiotic factors impact biodiversity-invasion relationships (Kennedy et al. 2002, Theoharides and Dukes 2007) and resource availability may be a key parameter (Lennon et al. 2003, Li and Stevens 2012). Theoretical models, such as the fluctuate resource hypothesis, predict that increasing resources reduce the competition between the resident community and the invader, thereby allowing for greater invader success (Davis et al. 2000). Some studies have shown an interactive effect between species richness and resource availability with respect to invasiblity (Romanuk and Kolasa 2005, Liu et al. 2012).

In relation to this thesis, I found a negative correlation between biodiversity and invasion in **Chapter 2**, but it is still not known whether this relationship could be maintained or shifted with resource availability alternation. Resources in the soil differ spatially and temporally. The rhizosphere contains higher resource concentrations than bulk soil, due to large amounts of root exudates in rhizosphere (Walker et al. 2003). Furthermore, resource availability changes during the plant growth period (Aulakh et al. 2001). However, it must also be considered that invader species may also profit from plant-derived resources. Within this complex ecological landscape, numerous interactions may impact the ultimate success of invasion, and previous data do not provide a clear prediction of the impact of changing nutrient conditions (Berendsen et al. 2012, Carvalhais et al. 2015). Therefore, in **Chapter 3 and Chapter 4**, resource availability was manipulated to investigate the impact of such nutrient shifts on biodiversity-invasion relationships.

In **Chapter 3**, a dilution gradient of nutrient broth was used in liquid

microcosms to simulate different resource availability levels. Results showed that there was no interactive effect between resource availability and species richness on invader growth, indicating that the community constrained invasion by high species richness in a resource-independent way. However, the effect of individual species in the community on invasion was modulated by resource availability. The community could reduce the growth of invader when containing species with certain traits that could out-compete with the invader across all resource availabilities. When resource availability is low, species with high catabolic similarity had a stronger negative effect on invasion. The total breadth of resource use becomes more important to inhibition of pathogen growth. This result is in line with the finding that resource competition is more intensive in resource-limited environments. It has commonly been found that specific species have a disproportional contribution to inhibiting the progress of species invasion (Hodgson et al. 2002, Jiang et al. 2011). This may be due to resource competition if particular species are better equipped than the potential invader to consume available resources. This identity effect is more likely to occur at low resource availability because of stochastic match (Eisenhauer et al. 2012b). As resource availability increases, fast-growing species had the largest effect on invasion. There is clear a tradeoff between the rapid use of available resources and actual resource use efficiency. Thus, it is not surprising that resource availability modulates biodiversity-invasion relationships by shifting the effect of individual species with disparate resource use strategies.

The effect of resource stoichiometry on the relationship between biodiversity and ecosystem functioning (community productivity and invasibility) was further investigated in **Chapter 4**. We found that diversity effects had a relatively small impact on promoting community productivity and constraining invasibility. Species richness had no interactive effect with resource stoichiometry on both community productivity and invasibility. Given the poor predictive capacity of species richness on ecosystem functioning through all the resource conditions, we further investigated the effect of species identity. Identity effects were found to be the main effects influencing ecosystem functioning upon changes in resource stoichiometry. This suggests that communities contain certain species that contribute disproportionately to ultimate community function. Resident species responded in different way to the different resource ratios examined. The ultimate impact of such difference could be further investigated by examining the resource use patterns of each strain, such as growth rate, under various nutrient limitations.

Together, the results summarized from **Chapter 3** and **Chapter 4** suggest that species richness had significant effects on community productivity and invasibility, but without the interaction with resource availability. This demonstrates that diversity effects on ecosystem functioning are relatively stable.

The contributions of individual species, however, can be affected by changes in resource availability. The importance of identity effects highlights the importance of also considering the performance of individual species in the community, when studying biodiversity-ecosystem functioning relationships. This may provide a guideline on making optimal selection of species to promote ecosystem functioning.

Prediction of community function by genomic-scale metabolic model

In **Chapter 5**, I generated genome reconstructions and computed metabolic models to simulate metabolic patterns under specific resource conditions. According to simulated metabolisms, predicted trophic variables were calculated and validated using experimental data. Predicted variables showed strong correlations with empirical data. This suggests that genomic-scale metabolic models are consistent with actual observed activity patterns. Moreover, predicted functional diversity and functional similarity were highly robust indicator of invasion success in microcosms. This further indicates the reliability of genomic-scale metabolic modeling for summarizing community trophic characteristics and demonstrates its feasibility for predicting the metabolic outcomes in constructed microbial communities.

When verified with gnotobiotic data, inferred functional diversity even offered a better explanatory power than observed metabolic patterns, but the inferred functional similarity was not a good predictor. One reason for this result may be uncertainty of resource use patterns as predicted by the metabolic models. The coefficients of exchange fluxes show metabolite exchanges, given as the value of resource production minus consumption through the whole metabolic pathways (Jeffries 2006). Absolute quantities of produced and consumed resources are hard to determine. Another way to improve predicted metabolic models would be examine the amount of substance during biomass reactions, for instance by extrapolating to what is known to form one gram of biomass (Raghunathan et al. 2009, Henry et al. 2010). The reactants can represent as exponential growth rate of the species (Orth et al. 2010). As only the microcosm data at low resource availability (Chapter 3) was used to validate the inferred variables in **Chapter 5**, it is necessary to verify empirical data at high resource availability (fast-growing species had main effect on inhibiting invader growth). By knowing the amount of reactants contributing to biomass production, it is possible that inferred resource use patterns of each species can be predicted, and also the fast-growing species in the community can be decided. Microcosm invasion data thus can be fully validated by inferred metabolic variables. This may give a complete example about making prediction across all resource availability.

When applied to a natural soil system, none of the inferred trophic variables was a good predictor of pathogen invasion success. This may because of

the indigenous species function as a buffer or as competitors with inoculated avirulent species. In order to better understand interactions among indigenous species, inoculants and invasive pathogen, it would be interesting to first measure resource use patterns of diluted soil samples.

Genomic-scale metabolic models were demonstrated to be good indicators of community functions under certain conditions. It remains to be seen if such approaches can be predictive for more complex communities and in field applications.

Conclusions

Species migration is not necessarily a directed process. In some cases, pathogen invasion for instance, can bring undesired impacts to a local ecosystem. Biological invasions can be a threat to local biodiversity, and they alter ecosystem functioning. Although it is clear that invasions, pathogen invasions in particular, need to be addressed, appropriate measures cannot be determined without an understanding of the underlying mechanisms that determine invader success. Given the impact of plant pathogens on crop production, I focused on inhibition of a plant pathogen as my primary target ecosystem function. Invasion success was examined across a range of constructed resident communities representing a gradient of diversity and function and impacts were examined in relation to nutrient availability and stoichiometry. Results showed that biodiversity can reduce invasion by consuming a greater proportion of resources and thereby minimizing the possibility for an invader establishment both *in vivo* and *in vitro*. Community trophic networks, which indicate competition within the resident community as well as between the resident community and the invader, were also strong predictors of invasion success. Mechanistically, resource availability modulated the relationship between biodiversity and invasion. Contributions of species identity to invasion reduction were also altered by increases in resource availability. Specifically, species with different trophic traits out-lead in the resident community at different resource availability levels. In addition, not only total resource availability, but also resource stoichiometry shifts biodiversity-invasion relationships. In order to infer ecosystem functioning under given resource conditions, genomic-scale metabolic models can be generated to simulate species interactions and community functions.

Application and future prospective

In terms of biological invasion, one of the most aspects related to human beings is invasion by plant pathogens. Soil-borne plant pathogens reduce plant productivity by causing irreversible and sometimes devastating plant diseases. World food productivity is under threat by plant pathogens (Strange and Scott 2005). Therefore, it is necessary to develop novel ways to control soil-borne diseases by

reducing pathogen abundance in the soil across different agronomic environments. This work potentially sets the stage for the development of new strategies for promoting plant health by engineering microbial community trophic networks. Species richness has a relatively clear negative effect on invasion success through different resource availability levels. Furthermore identity effects within a community can be shifted due to changes in resource availability and stoichiometry. Indeed, invader growth can be inhibited by species with different trophic traits as they responded to changes in resource availability. In total, this research provides new insights into how to better select for resident communities with desired attributes with respect to resource availability response and interaction with plant-derived substrates. Given the fact that environmental conditions sometimes are difficult to reproduce in the laboratory, the possible using genomic-scale metabolic models provides new possibilities to simulate trophic networks and predict ecosystem function. It is hoped that the proof-of-principle demonstrated here for simple engineered communities can be extrapolated to help infer the functioning of diverse microbial communities via metabolic interpretation of metagenomic data.

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SUMMARY

Species migration is increased by anthropogenic activities, in further bringing new functions to local environment. It can be regarded as beneficial, if new species facilitate the abundance or functions of resident species. However, new species may result in unexpected consequences, such as reducing available niches or altering species interactions. This undesired species migration can be called biological invasion. Biological invasion brings disturbance to the local environment. In order to reduce the undesired consequences caused by invasion, the determinant factors suppressing invasion success become important.

The success of invasion depends on resident species and resource availability. Many theoretical and empirical studies pointed out that resident community with higher diversity could reduce the success of invasion, via more intensive competition between resident community and the invader. The contribution of resident species to the competition with the invader has been summarized into complementary effect and identity effect. To be specific, the successful suppression on invasion can be realized by resident species in the community consuming resources complementarily due to functional diversity or outstanding resource consumption ability of certain species. Therefore, the resources left for the invader are not enough for its establishment in the environment. These biodiversity-invasion relationships are reflections of competition between resident and the invasive species, which may shift according to different environments.

Resource availability is another factor determining the success of invasion. Additional resources in the environment increase invasion success even with a high diverse community, as the invader can be favored by released extra resources. This suggests that the impact of resource availability on the invasion is more significant than community richness. There are few explanations that demonstrate why community richness became less important as resource increase in the system. Moreover, the underlying mechanism that explains how resource availability modulates competition between resident species and the invader is still required to be investigated.

Especially in the rhizosphere, biological invasion can be referred to plant pathogen. While plant pathogens invade, resident microbial community suppresses the pathogen by competing for resources in the rhizosphere. Root exudates are the key resources that support the growth of resident species in the rhizosphere, and create the opportunity of pathogen invasion. Using the resident community to reduce pathogen invasion has been verified by many studies, which show that invasion decreases as community diversity increases. However, the mechanism that explains how high diversity can suppress pathogen invasion in the rhizosphere is still not well studied. In other words, it is necessary to know which

traits of the community are related to pathogen invasion. Furthermore, resource availability in the rhizosphere changes during the plant growth period. Competitive interactions between resident species and the pathogen may be shifted under different resource availability conditions. By knowing the mechanism behind, the optimal microbial community would be selected based on different plant growth period.

In this thesis, three main questions were studied: (1) how is the biodiversity-invasion relationship in the rhizosphere; (2) does resource availability affect biodiversity-invasion relationship; (3) how to improve the prediction on biodiversity-invasion relationship. In order to investigate these questions, one pathogenic bacterial (*Ralstonia solanacearum*) and five avirulent congeneric species were used in the study as the model invader and resident species. Five levels of community richness (one to five) were constructed by combining five avirulent congeneric species (ranging from monoculture to all species combined). In Chapter 2, biodiversity-invasion relationship was studied *in vivo* and *in vitro*. The impact of resource availability on biodiversity-invasion relationship was shown in Chapter 3 and 4. Based on genomic metabolic models, inferred trophic traits were used to simulate biodiversity-invasion relationship under given resource conditions in Chapter 5.

The relationship between the resident community and the invader shows a negative effect, suggesting that invasion success can be reduced by high community richness. As the main interaction between resident species and the invader is resource competition, trophic traits (niche overlap, nestedness, connectance) can well predict invasion success both *in vivo* and *in vitro*. Niche overlap between resident community and the invader is one of the determing factors for invasion success. Moreover, invasion success also depends on trophic networks within the resident community, *ie*. nestedness and connectance. Community with species which are less competitive on resources among each other (low nestedness) can better enhance their invasion resistance. Resources can be consumed complementarily by different species in the community (high connectance), therefore less vacant resources are left for the invader. A negative effect of community richness on invasion success was also found in the *in vivo* experiment.

As the experiment was only carried out using one resource concentration, it is hard to conclude whether high community richness maintains its strong negative effect on invasion with changing resource availability. Therefore, an invasion experiment was also done at different resource availability levels using the same resident community construction. The results show that high community richness can reduce invasion through all resource availability levels, but without any interactive effect with resource availability. Instead, single species in the

community with certain trophic traits have relatively important impact on invasion suppression. In a nutrient-complex condition, species that have high catabolic similarity with the invader can better reduce the invasion at low resource availability, while fast-growing species become more important in the community as resources increase. Biodiversity-invasion relationships were also investigated under more simple resource conditions with modulation of carbon (C), nitrogen (N) and phosphorus (P) ratios. The results demonstrate that CNP ratios can shift biodiversity-invasion relationships also by changing the identity effect.

Based on the data collected from empirical studies in the chapters before, observed trophic traits can predict invasion success very well *in vivo* and *in vitro*. However, the natural environment is far more difficult to simulate in the laboratory. The construction of biological control community depends on the resource availability, based on the results showed in former chapters. Thus, it is necessary to generate a model that can better inferring invasion success under a given resource condition. Genome analyses have been becoming a reliable way of knowing metabolic characteristics of each microbial strain in these two decades. In the last chapter, genomic metabolic models were used to calculate inferred trophic traits of resident species and the invader. Furthermore, a good prediction on invasion was found by using the inferred trophic traits. Not only *in vitro* invasion, but also invasion *in vivo* can be well predicted.

In conclusion, a negative relationship between biodiversity and invasion is consistently found under different resource conditions. Trophic networks within the resident community emphasize the importance of community structure when constructing a biological community. Moreover, the relative importance of single species in the community can be modulated by the change of resource availability. This suggests that the strategy of selecting better-performance species in the community may be adjusted according to resource availability in the rhizosphere. In order to simulate biodiversity-invasion relationships under given resource conditions without laboratory work, genomic metabolic models can be a good tool to predict community invasibility. The prediction based on genomic metabolisms allows people to make efficient and optimal selections without mimicking natural system in the lab.

NEDERLANDSE SAMENVATTING

Migratie van soorten wordt verhoogd door menselijke activiteiten, door nieuwe functies aan te brengen in het lokale milieu. Het kan als een voordeel worden beschouwd als nieuwe soorten de abundantie en functies van al aanwezige organismen faciliteren. Nieuwe soorten kunnen echter leiden tot onverwachte gevolgen, zoals het verminderen van beschikbare niches of het veranderen van interacties tussen soorten. Deze ongewenste migratie van soorten kan worden omschreven als biologische invasie. Biologische invasie brengt verstoring van het lokale milieu. Om de ongewenste gevolgen door invasie te verminderen worden de determinant factoren belangrijk voor het onderdrukken van het invasiesucces.

Het succes van de invasie is afhankelijk van al aanwezige soorten en beschikbaarheid van hulpbronnen. Veel theoretische en empirische studies wijzen erop dat een al bestaande microbiologische gemeenschap met een hogere diversiteit beter in staat is om het succes van de invasie te verminderen door intensievere concurrentie met de indringer. De bijdrage van de al aanwezige soorten in de competitie met de indringer is beschreven als complementair en “identity effect”. Specifiek; de succesvolle onderdrukking van een biologische invasie kan worden gerealiseerd door al aanwezige soorten, door verbruiken van dezelfde hulpbronnen. Dit is mogelijk door functionele diversiteit of door het vermogen om hulpbronnen te consumeren door bepaalde organismen. Daardoor zijn er voor de indringer te weinig hulpbronnen over om zich te vestigen. Deze biodiversiteit-invasie relaties zijn een afspiegeling van de competitie tussen al aanwezige en invasieve soorten, die kunnen verschuiven onder invloed van verschillende milieus.

Beschikbaarheid van hulpbronnen is nog een factor die het succes van een biologische invasie bepaalt. Additionele hulpbronnen in het milieu verhogen het invasiesucces zelfs met een zeer diverse microbiologische gemeenschap, omdat de indringer succesvol kan worden door beschikbare extra hulpmiddelen. Dit suggereert dat de impact van de beschikbaarheid van hulpmiddelen op de invasie belangrijker is dan de mate van diversiteit. Er zijn weinig verklaringen waarom rijkdom in diversiteit minder belangrijk is dan een toename van hulpmiddelen in het ecosysteem. Bovendien is het nodig om het onderliggende mechanisme dat de manier waarop de beschikbaarheid van hulpbronnen concurrentie tussen de al aanwezige soorten en de indringer moduleert verder te onderzoeken.
Vooral in de rhizosfeer kan biologische invasie worden verbonden aan plant-pathogenen. Terwijl de plant-pathogenen binnendringen, onderdrukt de al aanwezige microbiële gemeenschap de ziekteverwekker door competitie over hulpmiddelen in de rhizosfeer. Wortel exudaten zijn de belangrijkste hulpmiddelen die de groei van de al bestaande soorten in de rhizosfeer ondersteunen, en de

mogelijkheid scheppen voor een pathogeen invasie. In een groot aantal studies is al beschreven hoe een al aanwezige microbiologische gemeenschap kan worden gebruikt om een pathogeen invasie te verminderen. Hieruit blijkt dat de invasie afneemt als diversiteit in de gemeenschap toeneemt. Het mechanisme dat verklaart waarom een hoge diversiteit een pathogeen invasie in de rhizosfeer kan onderdrukken is nog niet goed bestudeerd. Met andere woorden, is het noodzakelijk te weten welke eigenschappen van een gemeenschap zijn gerelateerd aan een pathogeen invasie.

De beschikbaarheid van hulpbronnen in de rhizosfeer verandert bovendien tijdens de groeiperiode van planten. Competitieve interacties tussen al aanwezige soorten en het pathogene organisme kunnen verschuiven onder verschillende condities van beschikbaarheid van hulpbronnen. Door het mechanisme ontrafelen, zou een optimale microbiële gemeenschap kunnen worden geselecteerd op basis van de verschillende plantengroei perioden.

In dit proefschrift werden drie hoofdvragen onderzocht: (1) hoe is de biodiversiteit-invasie relatie in de rhizosfeer; (2) heeft de beschikbaarheid van hulpbronnen invloed op de biodiversiteit-invasie relatie; (3) hoe kan een betere voorspelling van de biodiversiteit-invasie relatie worden gemaakt. Om deze vragen te onderzoeken, werden het bacteriëel pathogeen (Ralstonia solanacearum) en vijf avirulente bacterie soorten gebruikt in de studie als model indringer en al aanwezige soorten. Vijf niveaus van diversiteit (1-5) werden geconstrueerd door het combineren van de vijf avirulente species (van monocultuur tot alle soorten samen). In hoofdstuk 2 werd de biodiversiteit invasie relatie bestudeerd in vivo en in vitro. De impact van de beschikbare hulpbronnen op de biodiversiteit-invasie relatie werd aangetoond in hoofdstuk 3 en 4. In hoofdstuk 5 werden afgeleide trofische eigenschappen gebruikt, gebaseerd genomische metabole modellen, om de biodiversiteit-invasie relatie onder bepaalde hulpbron omstandigheden te simuleren.

De relatie tussen de al aanwezige microbiologische gemeenschap en de indringer laat een negatief effect zien. Dit suggereert dat invasiesucces kan worden verminderd door een hoge diversiteit. Aangezien de belangrijkste interactie tussen al aanwezige soorten en de indringer concurrentie is om hulpbronnen kunnen trofische eigenschappen (niche overlap, nestedness, connectance) goed voorspellen of de invasie succesvol wordt (zowel *in vivo* en *in vitro*). Niche overlap tussen de al aanwezige gemeenschap en de indringer is een van de determinante factoren voor invasiesucces. Bovendien hangt invasiesucces ook af van trofische netwerken binnen de al aanwezige biologische gemeenschap (nestedness en connectance). Een biologische gemeenschap met soorten die onderling minder concurrerend zijn om hulpbronnen (laag nestedness) zijn beter in het versterken van weerstand tegen invasie. Hulpbronnen kunnen

complementair worden geconsumeerd door verschillende soorten in de gemeenschap (hoog connectance), waardoor minder beschikbare hulpbronnen overblijven voor de indringer. In een vivo experimen werd ook een negatief effect van de diversiteit rijkdom op invasiesucces gevonden:
Omdat het experiment alleen bij één hulpbron concentratie werd uitgevoerd, is het moeilijk om te concluderen of een hoge diversiteit zijn sterk negatief effect op de invasie handhaaft als hulpbron beschikbaarheid verandert. Daarom werd het invasie experiment ook gedaan met verschillende niveaus van beschikbaarheid van hulpbronnen met dezelfde al bestaande microbiologische gemeenschap. De resultaten tonen aan dat hoge diversiteit een invasie kan bestrijden op elk beschikbare hulpbron niveau, maar zonder interactief effect met de beschikbaarheid van de hulpbronnen. In plaats daarvan hebben afzonderlijke soorten, in de gemeenschap met bepaalde trofische karaktertrekken , een relatief belangrijke invloed op onderdrukking van een invasie.

Bij een complexe voedsel situatie , kunnen soorten die een hoge katabole gelijkenis hebben met de indringer, bij een lage beschikbaarheid van hulpbronnen, een invasie beter onderdrukken , terwijl de snelgroeiende soorten belangrijker worden in een gemeenschap als de hulpbronnen toenemen. Biodiversiteit-invasie relaties werden ook onderzocht onder meer eenvoudige hulpbron condities met modulatie van koolstof (C), stikstof (N) en fosfor (P) ratio's. De resultaten tonen aan dat CNP verhoudingen ook biodiversiteit-invasie relaties kunnen verschuiven door verandering in het “identity effect” in de gemeenschap waardoor sommige soorten meer bijdragen dan anderen.

Gebaseerd op data vanuit empirische studies in de voorgaande hoofdstukken, kunnen waargenomen trofische eigenschappen invasiesucces uitstekend voorspellen in zowel vivo en in vitro. De natuurlijke condities zijn echter veel moeilijker te simuleren in het laboratorium. De constructie van een bio control gemeenschap is afhankelijk van de beschikbare hulpmiddelen, die gebaseerd zijn op de resultaten beschreven in vorige hoofdstukken. Derhalve is het noodzakelijk om een goed model te produceren dat invasiesucces kan voorspellen onder bepaalde hulpbron conditie.

Genoom analyses zijn een betrouwbare manier geworden om metabole eigenschappen te onderzoeken van elke microbiële stam die beschreven is in de afgelopen twee decennia. In het laatste hoofdstuk, werden genomische metabole modellen gebruikt om afgeleide trofische kenmerken van al aanwezige soorten en de indringer te berekenen. Verder bleek het gebruik van afgeleide trofische kenmerken een goede voorspeller voor invasie. Niet alleen in vitro invasie, maar ook invasie in vivo kan goed worden voorspeld.

Tot slot, is er consequent een negatieve relatie gevonden tussen biodiversiteit en invasie onder verschillende hulpbron omstandigheden. Trofische

netwerken binnen de al aanwezige gemeenschap benadrukken het belang van de gemeenschap structuur bij constructie van een biologische gemeenschap. Bovendien kan het relatieve belang van een enkele soort in de gemeenschap gemoduleerd worden door de verandering van de beschikbaarheid van hulpbronnen. Dit suggereert dat de strategie van het selecteren van betere presterende soorten in de gemeenschap kan worden aangepast op basis van de beschikbaarheid van hulpbronnen in de rhizosfeer. Om biodiversiteit-invasie relaties bij bepaalde hulpbron condities, zonder laboratoriumwerk, te simuleren, kunnen genomische metabole modellen een goed hulpmiddel zijn om de gevoeligheid voor invasie in een gemeenschap te voorspellen. Met de voorspelling op basis van genomische stofwisseling kan men efficiënte en optimale selecties maken zonder het natuurlijke systeem te hoeven nabootsen in het lab.

Mad River Canoe
ADVENTURE 16

ORIGINEEL CHINEES RESTAURANT

Team Name
The Invaders
Time Left
01:03
We succeed!

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I have been thinking how I will feel when I write the acknowledgement in my thesis already one or two years ago. Thankful and grateful of course, but also unforgettable.

When I decided to do PhD at first, I didn't think too much of it, just because I like doing experiment and wanted to continue. I didn't realize that doing PhD is not an easy job and there are a lot of problems, questions and difficulties waiting for me. I am glad that I am a kind of innocent that are willing to do PhD without any hesitation, which also helped me overcome many difficulties in the next years. This innocence encourages me doing science with passion, even there were many times I was frustrated by many necessary and unnecessary things. But the best thing of science is once you try, you will absolutely get some results, including the useless ones. This makes me feel myself valuable.

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PUBLICATION LIST

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Hu J, Wei Z, Friman V P, Gu S, Wang X, Eisenhauer N, **Yang T**, Ma J, Shen Q, Xu Y, Jousset A. Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression[J]. mBio, 2016, 7(6): e01790-16.

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IN PROGRESS

Yang, Tianjie, et al. “Competition between avirulent and pathogenic species.” Mini Review

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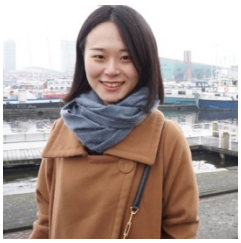
Yang, Tianjie, et al. “Genome reconstructions predict the functionality of microbial communities.”

CURRICULUM VITAE



Tianjie Yang was born on 01 July 1990, in Nanjing, a beautiful and historical city in southeast of China. She developed a strong interest in biology from childhood by reading children's scientific books. In the second year of high school, she chose biology as one of the major study.

In 2008, she started with the study Biology in Life Science College at Nanjing Agricultural University. She was as a lab assistant in plant stress lab, helping with the study of maize cold resistance mechanism for half a year. After that, she joined the student research group as part of the Students Research Training (SRT) project to investigate the salinity stress mechanisms of soya beans during the third year of bachelor. After finishing this project, she chose to do master study in Plant Nutrition group of Resources and Environmental Science College in 2012. In the second year of Master, she decided to continue doing research as a PhD in the same group. Her topic focuses on microbial ecology, under the supervision of Prof. Qirong Shen, Prof. Yangchun Xu and Dr. Wei. In 2014, she got the scholarship from China Scholarship Council, which can support her visit in the Netherland for two years. She came to the Ecology and Biodiversity group in Utrecht University continuing her project, which was supervised by Prof. G.A.Kowalchuk and Dr. A.L.C.Jousset.