### Probiotic *Pseudomonas* communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning

**Running title:** Probiotic communities enhance plant growth

**Authors**

Jie Hu a, b, Zhong Wei a,Simone Weidner b,Ville-Petri Friman c,Yang-chun Xu a\*,Qi-rong Shen a,Alexandre Jousset a, b

**Affiliations**

Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Weigang 1, Nanjing, 210095, PR China a;Utrecht University, Institute for Environmental Biology, Ecology & Biodiversity, Padualaan 8, 3584CH Utrecht, the Netherlands b; University of York, Department of Biology, Wentworth Way, York, YO10 5DD, United Kingdom c

\* Corresponding author: Yangchun Xu (ycxu@njau.edu.cn)

**Contributions**

JH, ZW, SW, VF and AJ wrote the manuscript. YCX, JH, ZW, QRS and AJ developed the ideas and designed the experimental plans. JH, ZW performed the experiments. AJ, ZW and JH analysed the data.

#### Abstract:

Plant-associated microbes play an important role in plant growth and development. While the introduction of beneficial microbes into the soil could improve plant production in low-input agricultural systems, real-world applications are still held back by poor survival and activity of the probiotic microbes. In this study, we used a biodiversity-ecosystem functioning (BEF) framework to specifically test how *Pseudomonas* community richness shapes the bacterial inoculant survival and functioning in terms of plant growth. To this end, we manipulated the richness of a probiotic *Pseudomonas* spp. bacterial community inoculant (1, 2, 4 or 8 strains per community) and compared diversity and strain identity effects on plant biomass production and nutrient assimilation *in vivo* with tomato. We found that increasing the richness of the bacterial inoculant enhanced the survival and abundance of *Pseudomonas* communities leading to higher accumulation of plant biomass and more efficient assimilation of nutrients into the plant tissue. Diversity effects were clearly stronger than the *Pseudomonas* strain identity effects and diversity-mediated plant growth promotion could be linked with increased production of plant hormones, siderophores and solubilization of phosphorus *in vitro*. Together these results suggest that multi-strain microbial inoculants can promote plant growth more reliably and effectively compared to single-strain inoculants.

**Keywords:** Probiotic bacteria, Plant growth-promotion, Biodiversity-ecosystem functioning, Auxin, Gibberellin, Siderophores, Phosphate solubilization, *Pseudomonas* spp., Richness

#### Introduction

Rapid growth of the human population has created an increasing need for novel low-input and high-yield agricultural practices that do not harm natural ecosystems (Tilman, Cassman, Matson, Naylor, & Polasky, 2002). Application of beneficial microbes that promote plant growth is thought to hold potential for reducing the extensive use of chemical fertilizers and pesticides in modern agriculture (Vessey, 2003). Plant growth-promoting microbial communities can provide various services to the plant such as protection from pathogen invasion (Hu et al., 2016; Wei et al., 2015), production of plant growth hormones (Muhammad Arshad 1991) and mobilization of soil nutrients that would be otherwise inaccessible for plants (Lugtenberg & Kamilova, 2009). Unfortunately, current intensive agricultural practices that depend on high inputs of chemical fertilizers and pesticides are often detrimental to the plant-associated beneficial rhizosphere bacteria and microbial ecosystem functioning (Tsiafouli et al., 2015). While the loss of functionality can be restored by inoculating beneficial microbes into the microbe-poor rhizosphere (Wubs, van der Putten, Bosch, & Bezemer, 2016), this approach is often limited by poor survival of microbial inoculants. In particular, direct antagonism and competition for space and resources with the indigenous microbes can severely limit inoculant establishment and functioning in the rhizosphere (Kadam & Chuan, 2016). In this study, we applied the biodiversity-ecosystem functioning (BEF) framework to test how the richness of a probiotic bacterial community affects its survival and plant growth-promotion activity in the tomato rhizosphere *in vivo*.

 Biodiversity has consistently been shown to enhance the productivity and stability of ecosystems (Hautier et al., 2015; Hector et al., 1999; Reich et al., 2001). In the context of plant growth-promotion, high probiotic bacterial inoculant richness has been linked to increased pathogen suppression via enhanced resource and interference competition (e.g. antimicrobial activity) (Hu et al., 2016; Wei et al., 2015) and improved long-term probiotic survival in the rhizosphere (Hu et al., 2016). While the exact mechanisms are still unknown, these findings suggest that the performance of probiotic microbes could be enhanced by applying the strains as multi-species communities that survive and function better in the rhizosphere.

Probiotic bacterial community richness could improve the survival and subsequent functioning of the inoculated strains in several ways. For example, diverse probiotic communities occupy a broader resource niche together versus alone (M. Loreau & Hector, 2001), which helps them compete more efficiently with already existing microbes in the rhizosphere (Richardson, 2014). Multispecies communities are also likely to harbor at least one species that can perform well under a given set of environmental conditions thereby stabilizing community functioning across different environments (S. Y. a. M. Loreau, 1999; Yang et al., 2017). Bacterial diversity also affects the level of microbial communication and cooperation in the rhizosphere. For example, diversity induces more intensive microbial communication and potentially trigger the expression of traits, such as secondary metabolite production (Garbeva, Silby, Raaijmakers, Levy, & Boer, 2011; Jousset et al., 2014; Tyc et al., 2014), which are not expressed in less diverse communities (Fujiwara et al., 2016). Diverse bacterial communities also improve plant growth by maintaining high community-level enzymatic activity, which could for example increase nitrogen mineralization (Weidner et al., 2015). Diversity also has negative effects on bacterial survival and functioning if it increases antagonism between the members of inoculant community (Mehrabi et al., 2016). However, as the level of antagonism often increases with increasing ecological similarity, the members of inoculant communities should be different enough to show complementary effects in terms of ecosystem functioning (Freilich et al., 2011).

Here we studied the impact of probiotic inoculant community richness on its survival and plant-growth promotion activity by using defined communities of eight fluorescent pseudomonads - a common bacterial taxon known for its plant growth promotion capability (Hol, Bezemer, & Biere, 2013). We have previously shown that these strains show synergistic antimicrobial activity against *R. solanacearum* (Hu et al., 2016) and could thus exert complementarity with respect to other plant growth-promoting traits. To study this, we created an inoculant richness gradient ranging from 1, 2, 4 or 8 *Pseudomonas* strains per community and tested the community survival and functioning in terms of plant biomass production and nutrient assimilation *in vivo* with tomato. The potential mechanisms underlying bacterial community functioning were also determined *in vitro* in terms of the production of plant hormones auxin (stimulates root growth) and gibberellin (affects elongation and expansion of stem and leaves) (Davies, 2010), iron-scavenging siderophores (positively affect iron availability) and phosphate rock solubilization (positively affect phosphorus availability). We hypothesized that increasing the richness of probiotic *Pseudomonas* communities has positive effects on plant growth via improved survival and expression of plant growth promoting traits in the rhizosphere.

#### Materials and methods

**2.1 Bacterial strains**

We included eight probiotic *Pseudomonas* strains (*P. fluorescens* 1m1-96, F113, mvp1-4, Phl1c2 and Q2-87; *P. protegens* Pf-5 and CHA0; *P. brassicacearum* Q8r1-96) in our study system. All strains have been extensively investigated in relation to their ability to promote plant growth (Loper et al., 2012) and to affect biodiversity-ecosystem functioning in microbial communities (Hu et al., 2016; Jousset, Schmid, Scheu, & Eisenhauer, 2011) (for more details see Table S1). All strains were routinely stored at -80 °C. Prior to experiments, one single colony of each strain was selected, grown overnight in lysogenic broth (LB), washed three times in 0.85% NaCl buffer and adjusted to an optical density of 0.5 (600 nm) before use.

**2.2 Establishing a probiotic community richness gradient**

A probiotic *Pseudomonas* community richness gradient was created by establishing four richness levels (1, 2, 4 and 8 strain communities in a total of 48 different combinations, Table S2) as described in a previous study (Becker, Eisenhauer, Scheu, & Jousset, 2012). Briefly, all *Pseudomonas* monocultures were replicated twice and 8-strain communities were replicated four times. For other richness levels, each probiotic strain was included equally often in the fully assembled communities, which allows to disentangle the effects of diversity and strain identity (Hu et al., 2016). Following assembly, communities were immediately used for subsequent experiments. We used a substitutive design where the total probiotic community biomass was kept constant at all richness level. The same experimental design was followed in both the greenhouse and *in vitro* experiments.

**2.3 Setting up the greenhouse experiment**

We assessed the effect of the inoculated *Pseudomonas* communities on plant growth in a 50-day long greenhouse experiment. We used natural soil that was collected from a tomato field in Qilin (118°57'E, 32°03'N): a town near the city of Nanjing, China. Soil was first sieved through 5 mm mesh to remove stones and roots and then homogenized thoroughly. Tomato seeds (*Lycopersicon esculentum,* cultivar “*Jiangshu*”) were surface-sterilized by soaking in 70% ethyl alcohol for 1 min, washed with sterile water, immersed in 3% NaClO for 5 mins, and finally rinsed six times with sterile water. Surface-sterilized seeds were then germinated on water-agar plates (three days) before sowing into seedling plates containing Cobalt60-sterilized seedling substrate (Huainong, Huaian soil and fertilizer Institute, Huaian, China). At the three-leaf stage (12 days after sowing), tomato plants were transplanted to seedling trays that contained natural, non-sterile soil. The tray dimensions were 370 mm × 272 mm × 83 mm, each tray contained 8 separated sub boxes and each sub box contained 600 grams of soil and two tomato plants. After 10 days of transplantation, sub boxes were inoculated with probiotic *Pseudomonas* communities using a root drenching method (Wei et al., 2013). Briefly, 5 mL of *Pseudomonas* probiotic communities were pipetted on to the surface soil at an initial concentration of 5×107 inoculated *Pseudomonas* cells g-1 soil. In total, we used 52 seeding trays that contained all 48 different probiotic *Pseudomonas* communities (Table S2) and 4 control treatments that contained only the naturally occurring *Pseudomonas* species present in the non-sterile soil. All tomato plants were watered daily with sterile water. Seedling trays were arranged in arbitrary order and rearranged randomly every two days.

**2.4 Extracting rhizosphere soil DNA and quantifying *Pseudomonas* densities with real-time PCR**

After 20 and 40 days of *Pseudomonas* probiotic community inoculation, two plants per each *Pseudomonas* community (52 in total including the control treatment) were harvested. Rhizosphere soil was collected by gently removing plants from the sub boxes, shaking off the excess soil then the soil attached to the root system was collected (the whole root system or randomly selected part of a larger root system; the total size of the root sample was kept the same for all the plants). To homogenize and store the soil samples in smaller volume, samples were suspended in 30 mL sterile H2O and centrifuged (5000 g for 30 min at 4 °C) before transferring the soil pellets into 2 mL tubes for storage at -80 °C. We used Power Soil DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA) to extract the rhizosphere soil DNA from 0.3 g of soil per each sample by following the manufacturer's protocol. The DNA concentration and purity were determined by using NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

We used quantitative PCR (qPCR) to measure the densities of *Pseudomonas* bacteria in the rhizosphere soil based on the abundance of *Pseudomonas*-specific *phlD* gene copy number per gram of soil (extracted from the original 0.3 g soil samples). This gene is shared by all the probiotic *Pseudomonas* strains and could be also used to quantify the naturally occurring *Pseudomonas* densities in the control treatment (shown as red dashed lines in Fig. 1). Two primers were used: B2BF (5'-ACC CAC CGC AGC ATC GTT TAT GAG C-3') and B2BR3 (5'-AGC AGA GCG ACG AGA ACT CCA GGG A-3') (Almario, Moënne-Loccoz, & Muller, 2013). The qPCR analyses were carried out with Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, CA, USA) by using SYBR Green I fluorescent dye. Each reaction (20 μL volume) contained 10 μL of SYBR Premix Ex Taq (TaKaRa Biotech. Co., Japan), 2 μL of template, and 0.4 μL of both forward and reverse primers. The PCR was performed by initially denaturing at 95 °C for 30 s and cycling 40 times with a 5 s denaturizing step at 95 °C. This was followed by a 34 s elongation step at 60 °C and melt curve analysis at 95 °C for 15 s, at 60 °C for 1 min and at 95 °C for 15 s. Each rhizosphere sample was replicated three times.

**2.5 Quantifying plant growth and nutrient content**

Two plants per each *Pseudomonas* community were harvested 50 days after transplanting; the plants were at the initiation of flowering stage. The aboveground biomass of all plant samples was first dried at 105 °C for 30 mins and then at 70 °C for 5 days. Plant growth promotion by each *Pseudomonas* community was calculated as the percent change of plant aboveground dry weight (see more details about plant preparation below) relative to the control treatment (no inoculation of probiotic *Pseudomonas* community). To this end, dried material from duplicate plants per each *Pseudomonas* community were pooled, ground and mixed thoroughly. Subsequently, 0.5 g of ground plant powder was digested with concentrated HNO3-H2O2 following the protocol previously described by (Huang, Bell, Dell, & Woodward, 2004). The digested plant material was then used to determine Phosphate (P), Potassium (K) and iron (Fe) content by using inductively coupled plasma atomic emission spectroscopy (710 ICP-OES, Agilent Technologies, California, USA). The nitrogen (N) content was analyzed with the Vario EL elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

**2.6 Measuring the link between probiotic *Pseudomonas* community diversity and plant growth-promotion traits *in vitro***

We assessed four probiotic bacterial community traits potentially important for plant growth promotion: auxin production (a plant hormone that stimulates root growth), gibberellin production (a plant hormone important for stem elongation and leaf expansion), siderophore production (iron scavenging molecules that improve iron availability in the rhizosphere) and phosphate solubilization (an ability to enhance the mobilization of soil phosphorus).

***2.6.1 Quantifying auxin and gibberellin production***

To measure the auxin production, we grew all *Pseudomonas* communities in Landy’s medium (Landy, Warren, & et al., 1948): [glucose 20 g·L-1, L-glutamic acid 5 g·L-1, KH2PO4 1 g·L-1, yeast extract 1 g·L-1, MgSO4·7H2O 0.5 g·L-1, KCl 0.5 g·L-1, MnSO4·4H2O 5 mg·L-1, CuSO4·7H2O 0.16 mg·L-1, FeSO4·7H2O 0.15 mg·L-1, L-phenylalanine 2 mg·L-1, L-tryptophan 1 g·L-1, pH 7.0] for 72 h at 22 °C in the dark with shaking (90 rpm). Bacterial cultures were then centrifuged (at 10 000 g for 5 mins) and the auxin concentration of the supernatants (ng/ml) was measured with the IAA ELISA Kit (R&D, Shanghai, China) following the manufacturer's protocol.

To measure the gibberellin production, we grew all *Pseudomonas* communities in nutrient broth [tryptone 10 g·L-1, yeast extract 3 g·L-1, NaCl 5 g·L-1] for 72 h at 30 °C in the dark with shaking (200 rpm). After centrifugation (10 000 g for 5 mins), the concentration of gibberellin was measured from the bacterial supernatant (pmol/L) using the ELISA Kit (R&D, Shanghai, China) according to the manufacturer's protocol. Measurements were replicated three times for each probiotic community, auxin and gibberellin production was measured once per replicate culture.

***2.6.2 Quantifying siderophore production***

To measure siderophore production, we grew all probiotic *Pseudomonas* communities in MKB medium (Neilands, 1987) [K2HPO4 2.5 g·L-1; MgSO4·7H2O 2.5 g·L-1, glycerin 15 mL·L-1, casamino acids 5.0 g·L-1, pH 7.2] for 48 h at 30 °C with shaking (170 rpm). After centrifugation (10 000 g for 5 min), the siderophore concentration of the supernatant was measured with a CAS-shuttle assay (Swapan kumar ghosh, 2015). Briefly, 0.5 mL of culture supernatant were mixed with 0.5 mL of CAS assay solution. After reaching equilibrium, absorbance of the mixture was measured at an optical density of 630 nm with a spectrophotometer (Spectra Max M5, Molecular Devices, Sunnyvale, CA, USA). Control reference was prepared by mixing 0.5 mL sterile MKB medium with 0.5 mL CAS assay solution. Measurements were replicated three times for each probiotic community and siderophore production was measured once per replicate culture. Siderophore concentration of the supernatant was calculated using following formula (Swapan kumar ghosh, 2015):



Ar = Absorbance of control reference at 630 nm (CAS reagent)

As = Absorbance of the community sample at 630 nm.

***2.6.3 Quantifying phosphate solubilization***

To measure phosphate solubilization, all probiotic *Pseudomonas* communities were grown in NBRIP medium (Nautiyal, 1999) [ glucose 10 g·L-1, Ca3 (PO4)2 5 g·L-1, MgCl2·6H2O 5 g·L-1, MgSO4·7H2O 0.25 g·L-1, KCl 0.2 g·L-1, (NH4)2SO4 0.1 g·L-1, pH 7.0] for 7 days at 30 °C with shaking (170 rpm). After centrifugation (10 000 g for 10 mins) the soluble phosphate concentration (μg/ml) of the supernatant was measured using the molybdenum antimony colorimetric method (Tsang, Phu, Baum, & Poskrebyshev, 2007). Measurements were replicated three times for each probiotic community and phosphate solubilization was measured once per replicate culture.

**2.7 Statistical analysis**

We used generalized linear models (GLM) to analyze our data. The *Pseudomonas* community survival in the rhizosphere was measured as the log10-transformed abundance of *Pseudomonas*-specific *phlD* gene copy number per gram of rhizosphere soil. Plant growth promotion by each *Pseudomonas* community was calculated as the percent change of plant aboveground dry weight relative to the control treatment (no inoculation of probiotic *Pseudomonas* community). We used various analyses to explore how plant growth-promotion observed *in vivo* (greenhouse experiment) could be explained with *Pseudomonas* community density, richness, *Pseudomonas* strain identity and *in vitro* expression of plant growth-promoting traits (auxin, gibberellin and siderophore production and phosphate solubilization). *Pseudomonas* strain identity effect was expressed the *Pseudomonas* community density in rhizosphere, plant growth-promotion observed *in vivo*, and plant growth-promoting traits expression *in vitro* as functions of the presence of each strains (based on binary predictors). Due to potential correlations between different explanatory variables, and to differentiate between diversity and strain identity effects, sequential analyses were performed to uncover the most parsimonious GLMs. To achieve this, we used stepwise model selection based on Akaike information criteria (AIC) and chose the model with the best explanatory power [step () function in R]. We used both backward elimination starting with the full model and a forward-selection model (from simple to full model) to avoid selecting a local AIC minimum (Latz et al., 2012). All analyses were performed using R 3.2.2 (R Core Development Team, Vienna, Austria).

#### Results

**3.1 The effect of probiotic community richness for *Pseudomonas* survival and abundance in the rhizosphere**

The abundance of the *Pseudomonas-*specific *phlD* gene copy number was generally higher in the inoculated compared to non-inoculated control rhizosphere soils and varied both within and between richness levels (Fig. 1). Increasing community richness had a positive effect on *Pseudomonas* probiotic survival and abundance at both 20 days (F1, 46 = 45.5, P < 0.0001, Fig. 1A) and 40 days (F1, 46 = 36.4, P < 0.0001, Fig. 1B) post inoculation (dpi). *Pseudomonas* densities were generally higher at 20 dpi compared with 40 dpi regardless of the probiotic community richness level (non-significant interaction). The effects of *Pseudomonas* strain identity on *phlD* gene abundance in the rhizosphere at 20 dpi and 40 dpi were non-significant (Table S3). However, some single strains and combinations did equally well relative to 8-strain combination at 20 dpi: single strain Q2-87, single strain mvp1-4, combination of strains Q2-87 and Pf-5 and combination of strains mvp1-4 and Q2-87 (Figure 1A). Similarly, single strain Q2-87, combination of strains Pf-5 and 1m1-96, combination of strains mvp1-4 and Q8r1-96 and combination of strains F113 and Q8r1-96 did equally well relative to 8-strain combination at 40 dpi (Figure 1B).

**Figure 1. Densities of introduced probiotic *Pseudomonas* communities in the tomato rhizosphere. D**ensities of introduced *Pseudomonas* communities were estimated as the abundance of *Pseudomonas*-specific *phlD* gene copy number per gram of soil. Panels A and B denote *Pseudomonas* densities at 20 and 40 days post probiotic inoculation (dpi), respectively. The red dashed lines show *Pseudomonas* densities in the control treatments (natural soil without *Pseudomonas* inoculation). The data on the y-axes are presented on log10-scale and the data on x-axes are on a log2-scale for better readability. In all panels, each point corresponds to different *Pseudomonas* community (monocultures were duplicated and eight-genotype communities were replicated four times). Shaded area shows 95% confidence interval of the fitted curves.

**3.2 The effect of *Pseudomonas* community richness on plant growth and nutrient assimilation**

We found that increasing *Pseudomonas* community richness had a positive effect on plant growth (plant aboveground dry weight) measured at 40 dpi (Fig. 2A). While inoculating plant rhizosphere with a single *Pseudomonas* strain increased the plant dry weight by 22.16%5.36% (mean standard error), this effect became stronger with increasing *Pseudomonas* community richness: up to 48.34% 0.69% increase in plant dry weight was observed in eight-strain probiotic communities (main effect of richness: F1, 46 = 42.2, P < 0.0001, Fig. 2A). We further explored the effect of *Pseudomonas* community richness for the assimilation of nutrients into the plant tissue. We found that increasing community richness had a positive effect on the concentration of phosphate (F1, 46 = 26.9, P < 0.0001, Fig. 2C), potassium (F1, 46 = 9.9, P < 0.0001, Fig. 2D) and iron (F1, 46 = 71.8, P < 0.0001, Fig. 2E), while no effect was found for nitrogen content (Fig. 2B). In the case of both plant dry weight and nutrient assimilation, all probiotic strain identity effects were non-significant (Table S4). Together these results suggest that increasing *Pseudomonas* community richness had a positive effect on both the plant biomass and nutrient content in the tomato plant tissue.

**Figure 2. The effect of probiotic *Pseudomonas* community richness on plant growth and nutrient assimilation.** Plant growth promotion (panel A) was calculated as the change in the percentage of aboveground plant dry weight of probiotic versus control treatments. Panels B-E denote the relationship between *Pseudomonas* community richness and the assimilation of nitrogen (B), phosphate (C), potassium (D) and iron (E) per gram of dried plant tissue. The data on x-axes are presented on a log2-scale for better readability. In all panels, each point corresponds to different *Pseudomonas* communities (monocultures were duplicated and eight-genotype communities were replicated four times). Shaded area shows 95% confidence interval of the fitted curves.

**3.3 The effect of *Pseudomonas* community richness on *in vitro* expression of plant growth-promoting traits**

All probiotic *Pseudomonas* communities increased the expression of plant growth-promoting traits with increasing community richness *in vitro* (Fig. 2): auxin (F1, 46 = 25.9, P < 0.0001, Fig. 3A), gibberellin (F1, 46 = 22.1, P < 0.0001, Fig. 3B), siderophores (F1, 46 = 40.4, P < 0.0001, Fig. 3C) and phosphorus solubilization (F1, 46 = 8.8, P < 0.0001, Fig. 3D). Only Pf-5 and CHA0 *Pseudomonas* strains had significant identity effects on siderophore production and phosphate solubilization, respectively (Table S5). Together, these results show that increasing community richness had positive effects on *in vitro* expression of several plant growth-promoting traits and that probiotic community richness had a relatively larger effect than strain identity.

**Figure 3. The effect of *Pseudomonas* community richness on *in vitro* expression of plant growth-promoting traits.** Thepanels denote auxin production (A), gibberellin production (B), siderophore production (C), and phosphorus solubilization (D). The data on x-axes are presented on a log2-scale for better readability. In all panels, each point corresponds to a different *Pseudomonas* community (monocultures were duplicated and eight-genotype communities were replicated four times). Shaded areas show 95% confidence interval of the fitted curves.

**3.4 Linking plant growth promotion observed *in vivo* with *Pseudomonas* community abundance and *in vitro* expression of plant growth-promoting traits**

Finally, we assessed if plant growth promotion observed *in vivo* (plant aboveground dry weight and nutrient assimilation) could be explained by *Pseudomonas* community abundance in the rhizosphere and *in vitro* expression of plant growth-promoting traits. We found that *Pseudomonas* abundances (at 40 dpi) positively correlated with the plant biomass (aboveground dry weight) and the assimilation of nutrients into plant tissues (except for the potassium, Table 1, Fig. S1A-B). While the *in vitro* expression of auxin and gibberellin positively correlated with the plant biomass measured *in vivo* (Table 1, Fig. S1A-B), it seems that different plant growth-promoting traits might have different effects on the assimilation of specific nutrients into plant tissues. Only gibberellin production had a positive correlation with plant nitrogen concentration, while auxin production and phosphate solubilization had a positive correlation with the assimilation of phosphate (Table 1). Siderophore production has a positive correlation with the assimilation of potassium and iron, while gibberellin production had a positive correlation with assimilated iron concentrations in plant tissue (Table 1). Furthermore, we found that phosphorus solubilization (Table 1, Fig. S1C) and siderophore production (Table 1, Fig. S1D) positively correlated with the phosphorus and iron concentrations in the plant tissues. Taken together, these results suggest that the *Pseudomonas* community richness could have increased the plant growth and nutrient assimilation via upregulation of important plant-growth promoting traits.

Table 1. Linking the plant growth promotion (dry weight of aboveground plant biomass and nutrient assimilation (Nitrogen (N), Phosphate (P), Potassium (K) and Iron (Fe) concentration in plant tissue) observed *in vivo* with *Pseudomonas* community abundance and *in vitro* expression of plant growth-promoting traits. Upward arrows denote for positive effects of retained explanatory variables in all models.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Plant dry weight | Plant N concentration | Plant P concentration | Plant K concentration | Plant Fe concentration |
|  | Df | F | P | Df | F | P | Df | F | P | Df | F | P | Df | F | P |
| *In vitro* auxin production  | 1 | 18.56↑ | 0.0001  |  | NR | NR | 1 | 12.75↑  | 0.0009  |  | NR | NR |  | NR | NR |
| *In vitro* gibberellin production | 1 | 10.55↑ | 0.0022  | 1 | 4.39↑  | 0.0418  |  | NR | NR |  | NR | NR | 1 | 23.46↑  | <0.0001 |
| *In vitro* siderophore production |  | NR | NR |  | NR | NR |  | NR | NR | 1 | 1.97↑  | 0.1667  | 1 | 24.07↑  | <0.0001 |
| *In vitro* P solubilization | NR | NR |  | NR | NR | 1 | 4.60↑  | 0.0376  |  | NR | NR |  | NR | NR |
| *Pseudomonas abundance* (phlD gene) | 1 | 5.95↑ | 0.0189  | 1 | 2.59↑  | 0.1143  | 1 | 9.23↑  | 0.0040  |  | NR | NR | 1 | 14.75↑  | 0.0004  |
| No.of residuals | 44 |  |  | 45 |  |  | 44 |  |  | 46 |  |  | 44 |  |  |
| Model summary |  | R2 = 0.41 AIC= -72.7 |  | R2 = 0.10 AIC=896.8 |  | R2 = 0.33 AIC=75.6 |  | R2=0.02 AIC=290.7 |  | R2=0.56 AIC= -183.3 |

#### 4. Discussion

Here we studied how probiotic *Pseudomonas* community richness affects community survival and subsequent expression of plant growth-promoting traits in the tomato rhizosphere. We found that high inoculant richness increased survival and abundance of *Pseudomonas* bacteria in the rhizosphere (Fig. 1) resulting in an increase in plant biomass and assimilation of nutrients into plant tissue (Fig. 2). *Pseudomonas* strain identity had only a small impact on plant growth (Table S4), which suggests that the observed positive effects were mainly mediated by community richness. Plant growth promotion could be mechanistically linked to solubilization of phosphorus and enhanced production of plant hormones and siderophores by the *Pseudomonas* communities. Together these results suggest that multi-strain microbial inoculants can improve plant growth more reliably and effectively compared to single-strain inoculants via enhanced rhizosphere ecosystem functioning.

Similar to a previous study (Hu et al., 2016), we found that increasing the richness of the probiotic *Pseudomonas* community increased its survival and abundance in the tomato rhizosphere (Fig. 1). However, in the current study we could link the positive richness-survival relationship with the expression of various bacterial traits that could have directly or indirectly improved the probiotics survival in the rhizosphere. For example, we found that more phosphorus was solubilized and higher concentrations of siderophores were produced with increasing *Pseudomonas* community richness (Fig. 3). These changes could have increased *Pseudomonas* survival directly by increasing their competitive ability relative to the indigenous bacterial flora present in the soil at the time of probiotic inoculation (Hu et al., 2016). Alternatively, phosphorus solubilization and siderophore production could have boosted tomato root exudation having indirect positive effects on *Pseudomonas* survival via enhanced plant-mediated resource availability. Chemical signaling (e.g. quorum sensing) between the members of the *Pseudomonas* community could be a potential mechanism affecting the expression of secondary metabolites and cooperative behavior in the rhizosphere (Becker et al., 2012; Fujiwara et al., 2016; Jousset et al., 2014).

*Pseudomonas* community survival positively correlated with increased plant biomass production and assimilation of nutrients into the plant tissue (Table 1). Community richness had a positive effect on the concentration of phosphate, potassium and iron, while no effect was found on the concentration of nitrogen in the plant tissue (Fig. 2). To provide plant growth promoting activities, microbes need to first survive and establish viable populations in the rhizosphere (Adam et al., 2016; Dekkers, 1999; Hu et al., 2016; Weller, 1988). In supporting for this, *Pseudomonas* community densities positively correlated with the assimilation of nutrients into the plant tissues (Table 1). To explain these results more mechanistically, we conducted *in vitro* laboratory assays to measure the plant growth promoting activities along the *Pseudomonas* community richness gradient. We found that in addition to having positive effects on phosphorus solubilization and siderophore production, community richness increased the production of two important plant hormones, auxin and gibberellin. This suggests that more diverse *Pseudomonas* communities could have had a direct positive effect on the plant growth via hormone production. However, we measured the plant growth promoting activity of *Pseudomonas* communities only in simplified laboratory conditions *in vitro* and it is still unclear how well these measurements reflect *Pseudomonas* gene expression in the rhizosphere. As a result, more future work is needed to quantify the expression of *Pseudomonas* plant growth promoting traits in the rhizosphere environment.

The effect of *Pseudomonas* strain identity played only a small role in our experiments (Table S3, Table S4 and Table S5) compared to the *Pseudomonas* community richness effect. This suggests that *Pseudomonas* mediated plant growth promotion was an emergent, community-level ecological property, and that specific species did not have consistent and predictable effects on *Pseudomonas* growth and functioning. Even though *Pseudomonas* strain identity effects played a minor role in our experiment, it would be interesting to quantify changes in the relative abundances of different *Pseudomonas* strains in the probiotic rhizosphere communities. This would potentially help to unravel the mechanistic basis of plant growth promotion and to construct more specific microbial inoculant communities with desired plant growth promoting functions. For example, some of the mono and two-strain *Pseudomonas* communities could grow and express equally high levels of plant growth promoting activity as the 8-strain communities (Figs. 1-2). This raises a question: how many species are needed to construct an effective probiotic community? Using less diverse communities would be more feasible in terms of manufacturing microbial inoculants. However, less diverse communities are likely to be more inconsistent as their effectiveness will be considerably reduced by a random loss of one of the constituent strains. Moreover, even though we conducted our experiment in natural soil containing the indigenous microbial flora, we did not specifically determine the effects of inoculation in the wider rhizosphere microbial community context. For example, it is possible that probiotic *Pseudomonas* species interacted with the indigenous soil bacteria having synergistic effects on the plant growth promotion (Mendes et al., 2011). One way to study these questions in the future is to use next generation sequencing techniques to unravel how probiotic inoculation affects the indigenous microbiome structure, and to which degree the efficacy of probiotic inoculants depends on the diversity and composition of the background microbial community.

In conclusion, our results show that increasing the richness of a *Pseudomonas* inoculant community clearly increased the plant biomass and assimilation of various important nutrients into the plant tissue. Manipulating bacterial community richness could thus be an efficient way to promote the establishment and activity of probiotic plant growth promoting microbes. We hope that better understanding of plant-microbe interactions and biodiversity-ecosystem functioning will pave the way for environmentally friendly agriculture and crop production in the future.

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