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# Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities

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## Summary

**1.** Rhizosphere bacteria antagonistic to fungal pathogens improve plant performance by preventing infection. In temperate grasslands, primary productivity often increases with plant diversity, and we hypothesized that this effect may in part rely on the interactions between plants and antagonistic bacteria.

**2.** We investigated the impact of plant diversity and functional group composition on soil bacteria producing the antifungal compounds 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) in a long-term grassland biodiversity experiment, as well as their impact on soil suppressiveness. Soil suppressiveness was investigated in a model infection assay with *Beta vulgaris* and the pathogen *Rhizoctonia solani*.

**3.** The abundance of DAPG and PRN producers increased with plant diversity and that of PRN also increased in the presence of grasses. Moreover, legume species richness and coverage decreased the abundance of DAPG and PRN producers, respectively, contrary to beneficial effects of legumes on soil microorganisms reported previously. In turn, soil suppressiveness was at maximum when DAPG and PRN producer abundance was high.

**4.** *Synthesis*. Our results suggest that plant diversity contributes to plant community resistance against pathogens by fostering beneficial bacterial communities. This indirect soil feedback mechanism may contribute to the positive relationship between plant diversity and productivity and could also help the development of more sustainable and environmentally friendly agricultural management strategies.

**Key-words:** 2,4-diacetylphloroglucinol, biodiversity–ecosystem functioning relationship, plant–microbe interactions, plant–soil (below-ground) interactions, *Pseudomonas fluorescens*, pyrrolnitrin, soil feedbacks, soil suppressiveness

## Introduction

Pathogen pressure is a major cause of yield loss in agricultural systems and an important factor affecting the structure and productivity of natural plant communities (Weller *et al*. 2002; Allan, van Ruijven & Crawley 2010). Plant roots are particularly threatened as the soil harbours myriads of infectious microorganisms. However, bacteria antagonistic to plant pathogens are known to reduce plant infection. These bacteria have been extensively studied in agricultural systems where they significantly contribute to soil suppressiveness, that is the natural potential of soils to inhibit plant pathogens (Weller *et al*. 2002; Haas & Defago 2005). Root-associated strains of ubiquitous *Pseudomonas fluorescens* form one of the best characterized bacteria suppressing plant pathogenic fungi, including genera responsible for some of the most devastating plant diseases, such as *Rhizoctonia*, *Fusarium*, *Thielaviopsis*, *Gaeumannomyces*, *Phomopsis*, *Aphanomyces* and *Pythium* (Baehler *et al*. 2006). Disease suppression is associated with the production of 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN; Raaijmakers, Vlami & De Souza 2002), two of the most common and potent bacterial toxins responsible for plant

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protection against fungal pathogens in agricultural soils (Garbeva, van Veen & van Elsas 2004; Haas & Defago 2005; de la Fuente *et al.* 2006). Suppression of pathogens by toxin-producing pseudomonads in natural ecosystems is also likely to be important (van der Heijden, Bardgett & van Straalen 2008). Recent grassland biodiversity experiments reported that soil pathogens are essential drivers of diversity–productivity relationships (Maron *et al.* 2011; Schnitzer *et al.* 2011), indicating that bacterial protection of plants against pathogens may play a crucial role in this context. However, knowledge on the role of such interactions for the structure and functioning of diverse plant communities is very limited (Garbeva, van Veen & van Elsas 2004; van der Heijden, Bardgett & van Straalen 2008).

The establishment of antagonistic bacteria in the rhizosphere is controlled by multiple factors. Plants have been suggested as the main factors determining soil microbial community structure (Garbeva, van Veen & van Elsas 2004). Plants provide carbon and energy sources to soil biota via root exudates and form a unique environment for bacteria colonizing the soil surrounding plant roots (Bais *et al.* 2006). Therefore, varying composition of root exudates occurring in diverse plant communities may alter the abundance, composition and activity of soil microorganisms (Garbeva, van Veen & van Elsas 2004; Chung *et al.* 2007; Liu *et al.* 2008). Plant diversity beneficially affects the abundance and activity of soil microorganisms (Liu *et al.* 2008; Eisenhauer *et al.* 2010), and in the long term, each plant species appears to contribute to ecosystem functioning and microbial activity in grasslands (Eisenhauer *et al.* 2010). Biodiversity also is a major driver of plant primary productivity: diverse grassland systems are often more sustainable and more productive (Balvanera *et al.* 2006; Tilman, Hill & Lehman 2006) than agricultural systems where monoculture may lead to continually declining yields (Zak *et al.* 2003; Garbeva *et al.* 2006). Hence, we hypothesize the existence of an indirect positive soil feedback effect in which diverse plant communities foster the abundance of soil bacterial populations with biocontrol potential. These bacteria reduce plant pathogen pressure, and thereby indirectly increase plant performance (van der Heijden, Bardgett & van Straalen 2008). It has been suggested that plants change soil community composition, which in turn affects plant performance (Bever, Westover & Antonovics 1997; Bever 2003; Bever *et al.* 2010). We add a further step into this feedback process by testing if plant community–specific soil bacterial communities indirectly influence plant performance by reducing plant pathogen pressure. We use the term indirect positive soil feedback for this additional mechanism.

We investigated the impact of plant diversity and functional group composition on the protective function of root-associated bacteria in a long-term grassland biodiversity experiment 7 years after its establishment (Jena Experiment; Roscher *et al.* 2004). We monitored bacterial communities by quantitative PCR (qPCR) targeting the genes *phlD* and *prnD* responsible for the production of DAPG and PRN, respectively (Raaijmakers, Weller & Thomashow 1997; Garbeva, Voesenek & Van Elsas 2004). Given the plethora of different plant diversity indices (e.g. plant species richness, plant functional dissimilarity, functional group identity), which differ in their predictive power when modelling soil biotic communities (Eisenhauer *et al.* 2011), we used an explorative statistical approach to identify the best predictors for the abundance of antagonistic bacteria. Furthermore, we explored whether soil suppressiveness against fungal pathogens increases with an enhanced abundance of antagonistic bacteria using soil of the Jena Experiment and model plant and pathogen species.

## Materials and methods

### EXPERIMENTAL SETUP

The Jena Experiment was established in 2002 to investigate the role of biodiversity for element cycling and trophic interactions in grassland plant communities (Roscher *et al.* 2004). The study site is located in the floodplain of the river Saale north of the city of Jena (Thuringia, Germany). The soil is characterized as Eutric Fluvisol (Roscher *et al.* 2004). A gradient of plant species richness (1, 2, 4, 8, 16 and 60) was established from a pool of 60 grassland plant species. A total of 82 plots of 20 × 20 m varying in plant species, functional group richness and identity were set up. Plant species were ascribed to functional groups according to above- and below-ground morphological traits, phenological traits and the ability to fix $N_2$. Four functional groups were differentiated: grasses (16 species), small herbs (12 species), tall herbs (20 species) and legumes (12 species). The functional separation of graminoids, N-fixing and non-fixing dicot herbs is well established (e.g. Spehn *et al.* 2000; Stephan, Meyer & Schmid 2000; Roscher *et al.* 2004). In the Jena Experiment, non-fixing dicot herbs are further separated into small and tall herbs due to their functional dissimilarity (see Roscher *et al.* 2004 for details). Plots were arranged in four blocks following a gradient in soil texture. Plots were mown twice a year (June and September) as is typical for hay meadows in the region. To maintain the target species composition, plots were weeded twice a year (April and July). An area of 1 $m^2$ size was surveyed for species presence (0 and 1) and species-specific percentage of cover in spring 2009. We investigated soil samples from 78 well-established experimental plots (see Table S1 in Supporting Information).

### SAMPLING

Soil samples were taken in spring 2009. At each plot, five soil samples were taken to a depth of 5 cm using a metal corer (inner diameter 7 cm), pooled, homogenized and sieved (2 mm) to remove macrofauna, roots and stones. For DNA extraction, 10 g of the sieved soil was separated and stored at −20 °C, and the remaining soil material was stored at 5 °C. To prevent cross-contaminations, working tools were sterilized with 75% ethanol before and between the working steps.

### QUANTIFICATION OF ANTAGONISTIC BACTERIA

Soil DNA was extracted with the FastDNA SPIN Kit for Soil (Qbiogene Inc., Irvine, CA, USA), purified with the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany), quantified with the Quant-iT PicoGreen dsDNA reagent (Invitrogen, Karlsruhe, Germany) on a Stratagene Mx3005P instrument (FAM filter set; excitation 492 nm, emission 516 nm) and stored at −20 °C until analysis. 16S rRNA gene abundance was quantified via qPCR with the general eubacterial primers Eub338 and Eub518 as described elsewhere

(Fierer *et al.* 2005; Table S1) using a serial dilution ($10^8$–$10^0$ gene copies $\mu L^{-1}$) of the 16S rRNA gene of *P. fluorescens* Q2-87 as standard. Primers and probes were obtained from Eurofins MWG Synthesis GmbH (Ebersberg, Germany). The abundance of DAPG producers was analysed using a SYBR Green assay with the primers BPF2 and BPR4 (McSpadden Gardener *et al.* 2001; Table S2), targeting the biosynthetic gene *phlD*. A serial dilution from $10^8$ to $10^0$ gene copies $\mu L^{-1}$ of the *phlD* gene of *P. fluorescens* Q2-87 amplified with the more generic primer set Phl2a and Phl2b was used as standard (Raaijmakers, Weller & Thomashow 1997; Table S2; Drigo, Van Veen & Kowalchuk 2009). The efficiency of the primer set, resulting in an about 500-bp amplicon, was verified with a serial dilution of the *phlD* gene of *P. fluorescens* isolates from the BOX-PCR genotypes A, B, D, K, L, M and P (de la Fuente *et al.* 2006; Table S3). The abundance of PRN producers was quantified with an established TaqMan assay with the primers PrnD-F and PrnD-R, and PRN-probe targeting the *prnD* biosynthetic gene (Garbeva, Voesenek & Van Elsas 2004; Table S2) using a serial dilution from $10^8$ to $10^1$ gene copies $\mu L^{-1}$ of the *prnD* gene of *P. fluorescens* CHA0. qPCR reaction mixtures targeting 16S rRNA genes and the *phlD* gene contained 1 μL DNA template, 1× Brillant II SYBR Green qPCR master mix (Agilent Technologies, Stratagene Products Division, La Jolla, USA), 0.24 μM of each primer and 0.6 mg $mL^{-1}$ bovine serum albumin in 25-μL reaction volume. qPCR measures targeting *prnD* were carried out in 25-μL reaction volumes containing 1 μL of DNA template, 1× TaqMan Environmental MasterMix 2.0 (Applied Biosystems Inc., Foster City, CA, USA) and 0.3 μM primers and probe. The primers BPF2 and BPR4 (McSpadden Gardener *et al.* 2001; Table S2) allowed adequate amplification of the *phlD* gene in the tested genotypes (efficiency > 95%; detection limit of $10^2$ copies $\mu L^{-1}$).

## SOIL SUPPRESSIVENESS ASSAY

To examine the effect of DAPG and PRN on soil suppressiveness, we carried out a standardized infection assay with sugar beet seedlings (*Beta vulgaris* L.) and the model pathogen *Rhizoctonia solani* Kühn (AG 2–2 IIIB). This generalist pathogen can attack a broad range of host plants (Parmeter 1970; Sneh, Burpee & Ogoshi 1991) and is a convenient model to assess soil suppressiveness (Postma *et al.* 2008). Briefly, 200 g of sieved soil from each experimental plot was placed in two Magenta boxes (7.7 × 7.7 × 9.7 cm; Sigma-Aldrich, St. Louis, MO, USA). One half of a barley corn infested with *R. solani* was placed in the centre of the box, as opposed to the control treatments. Eight sugar beet seeds (germination rate 93%) were added to each box about 1 cm below soil surface. The jars were incubated at 21 °C and 12-h photoperiod (photon flux density: 120 μmol $m^{-2}$ $s^{-1}$) and randomized every 2–3 days over a total experimental time of 15 days. Infection of sugar beet seedlings by *R. solani* first resulted in brownish discolorations at the lower shoot, which then propagated to the cotyledons; with progressing infection, the plants snapped.

## DISSIMILARITY OF PLANT COMMUNITIES

In addition to plant species richness and plant functional group richness, we calculated the mean functional dissimilarity of the plant communities (Walker, Kinzig & Langridge 1999). This is important because soil biotic communities are likely to be significantly influenced by differences in plant functional traits, but the number of plant functional groups may not adequately mirror those differences (Eisenhauer *et al.* 2010, 2011). For this purpose, a trait matrix based on seven variables was assembled [*morphological traits* (*ordinal*): height of vegetative plant, depth of the root system, type of the root system; *phenological traits and longevity* (*ordinal*): life cycle, seasonality of foliage, duration of flowering period; *physiological trait* (*binary*): N fixing]. The data were compiled from the literature and normalized (range 0–1) as presented in Roscher *et al.* (2004). Ecological distance (ED) was determined as the euclidean distance between species (here plant species richness) in functional attribute space as:

$$\mathrm{ED}_{jk} = \left[\sum_{i=1}^{I} \left(A_{ij} - A_{ik}\right)^2\right]^{1/2} \quad \text{eqn 1}$$

with $A_{ij}$ and $A_{ik}$, the attribute values of species $j$, $k$ for attribute $i$ and $I$ the total number of attributes being considered. The mean functional dissimilarity, FD, was calculated as the sum of the pair-wise distances between plant species for all possible species–pair combinations divided by the number of interactions in the community ($\binom{n}{m}$) as:

$$\mathrm{FD} = \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \mathrm{ED}_{ij}\right) / \binom{n}{m} \quad \text{eqn 2}$$

with $n$ the number of species, $m$ the number of species selected from $n$ (pair-wise, $m = 2$) (Walker, Kinzig & Langridge 1999; Heemsbergen *et al.* 2004).

## STATISTICAL ANALYSES

Data were analysed with a linear model using the statistical software R 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria). If not stated otherwise, packages used refer to the standard installation of R. We estimated the effects of the explanatory variables block (factorial), plant species richness (continuous; $\log_{10}$-transformed), plant functional group richness (factorial), plant functional dissimilarity (continuous), grasses, small herbs, tall herbs, legumes as absence/presence coded (factorial), species richness ($\log_{10}(x + 1)$-transformed, continuous) and coverage of each functional group [$\log_{10}(x + 1)$-transformed, continuous] on the abundances of *prnD* and *phlD* genes (copies $g^{-1}_{soil}$, $\log_{10}$-transformed). As we had no *a priori* hypothesis on which variable is most influential, we used an exploratory statistical approach to identify the predictors explaining the most variance: After fitting the full model (model including all explanatory variables mentioned earlier) and the null model (model including only the intercept), we used the automated algorithm 'step' (mode of stepwise search: 'both'), which, in a stepwise way, deletes and respectively adds explanatory variables to explore the most parsimonious model using Akaike's information criterion (AIC). To avoid finding a local minimum of AIC, we started the 'step' algorithm from both the null model, yielding the bottom-up model, and the full model, yielding the top-down model, resulting in two models estimated via the 'step' algorithm for each DAPG- and PRN-producing bacteria. Results are supported by the most parsimonious models of the *phlD* and *prnD* analyses (i.e. models with lowest AIC are best, Bolker 2008). Note that as a rule of thumb, models with a ΔAIC lower than two are approximately equivalent, models with a ΔAIC between 4 and 7 are clearly distinguishable and those with a ΔAIC larger than 10 are totally different (Burnham & Anderson 2004; Bolker 2008).

We presented the data as partial residuals, $\varepsilon_p$:

$$\varepsilon_p = ax + b + \varepsilon \quad \text{eqn 3}$$

were $x$ is one of the explanatory variables, $a$ the respective slope, $b$ the intercept and $\varepsilon$ is the corresponding residual. Significances of slopes were determined via $t$-tests.

We estimated the effect of *phlD* and *prnD* gene abundances in soil ($\log_{10}$-transformed) on pathogen suppressiveness as follows: we excluded two replicates that showed high infection rates in the control

(5 and 6 infected plants) from the analyses and made sure that neither *phlD* nor *prnD* had an influence on the infection rates in the control treatments. As we found no correlation, we decided to continue further analyses with uncorrected data of the infection treatments. Data on soil suppressiveness showed a bimodal distribution where most treatments showed nearly 100% infection or none; thus, we analysed whether a treatment was infected (one or more infected seedlings in a pot) or not (no infected seedlings in a pot) by using a generalized linear model with a Bernoulli distribution (glm, family = binomial). Significances of slopes were determined via a *z*-test.

## Results

### EFFECTS OF PLANT COMMUNITY COMPOSITION ON ANTAGONISTIC BACTERIA

The most parsimonious model explaining the abundance of *phlD* comprised the explanatory variables species richness, functional dissimilarity and legume species richness (Fig. 1, Table 1). *PhlD* abundance increased with increasing plant species richness (Fig. 1a) and with increasing functional dissimilarity (Fig. 1b). Legume species richness was the only functional group identity measure remaining in the final model, and it decreased *phlD* abundance (Fig. 1c).

The most parsimonious model explaining the abundance of *prnD* comprised the explanatory variables species richness, grass and tall herb species richness and coverage of legumes, as well as presence/absence of grasses (Fig. 2, Table 1). *PrnD* abundance increased with increasing plant species richness (Fig. 2a), whereas it decreased with increasing grass and tall herb species richness (Fig. 2b,c). Furthermore, *prnD* abundance decreased with the coverage of legumes (Fig. 2d) and increased in presence of grasses (Fig. 2).

Overall, the respective other models determined via the step function provided useful additional information (ΔAIC lower 4, Burnham & Anderson 2004; Bolker 2008; see Table S4 and S5). In the case of *phlD* abundance, functional dissimilarity was the only predictor occurring in both models identified via the step function, highlighting the importance of this diversity measure. The species richness of legumes was replaced by the cover of legumes, which acted as surrogates ($r = 0.85$, $P < 0.001$). In the case of *prnD* abundance, the positive effect of grass presence and the negative effect of legumes as well as tall herbs occurred in both models.

Additional analyses with the sown plant community data generated virtually the same results (Figs S1 and S2), underlining the strong correlation between sown and realized plant community properties in the Jena Experiment (Marquard *et al.* 2009).

### EFFECTS OF ANTAGONISTIC BACTERIA ON SOIL SUPPRESSIVENESS

P*hlD* and *prnD* abundance interactively affected suppressiveness against *R. solani* (Fig. 3). Soil suppressiveness was at maximum when both *phlD* and *prnD* abundances were high (Fig. 3, upper right), it was at minimum in samples with high abundance of only one gene (Fig. 3, lower right and upper left). When both gene abundances were low, the model predicted an additional increase in suppressiveness (Fig. 3, lower left).

## Discussion

### EFFECTS OF PLANT COMMUNITY COMPOSITION ON ANTAGONISTIC BACTERIA

Bacteria antagonistic to plant pathogens contribute to the prevention of plant diseases (Haas & Keel 2003), and soil suppressiveness can be induced by crop monoculture as in the case of take-all disease (Raaijmakers & Weller 1998). On the other hand, crop monocultures often lack soil suppressive effects even after more than 20 years (Rodriguez-Kabana & Canullo 1992), and increasing plant diversity often decreases the severity of plant diseases in agricultural (Mundt 2002; Li *et al.* 2009) as well as grassland systems (Knops *et al.* 1999; Maron *et al.* 2011; Schnitzer *et al.* 2011). Here, we demonstrate that high abundances of bacteria antagonistic to plant pathogens increase soil suppressiveness and that their beneficial effects are tightly linked to the diversity and functional composition of plant communities.

Plant species richness increased the abundance of DAPG- and PRN-producing bacteria (Figs 1a and 2a), supporting the

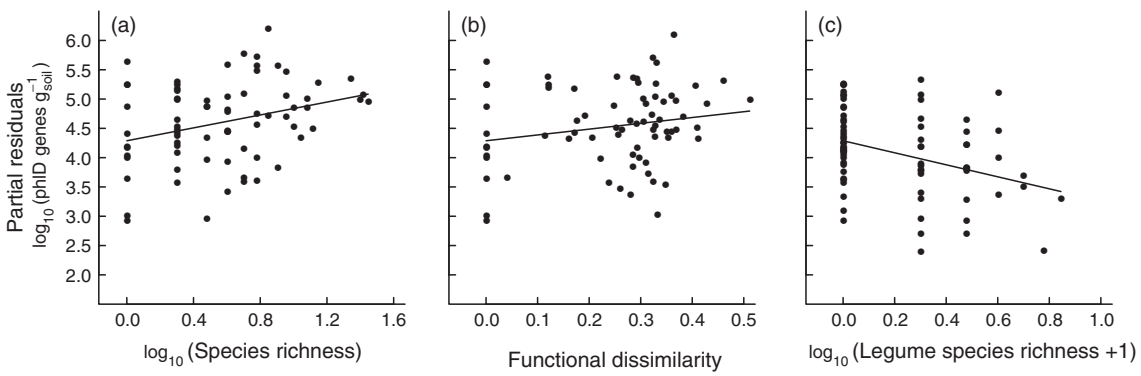


**Fig. 1.** Partial residual plots of variables that remained in the most parsimonious model (intercept 4.29). Displayed is the $\log_{10}$-transformed *phlD* gene abundance as a function of (a) $\log_{10}$-transformed plant species richness (slope 0.55, $P = 0.035$), (b) plant functional dissimilarity (slope 0.99, $P = 0.159$) and (c) $\log_{10}(x + 1)$-transformed legume species richness (slope −1.03, $P = 0.007$).

**Table 1.** Akaike's information criterions (AIC's) of each of the four models investigated. The ΔAIC of 0 shows the model with the best fit; the higher the ΔAIC-value, the greater is the deviation from the best model

| | phlD | | prnD | |
|---|---|---|---|---|
| | d.f. | ΔAIC | d.f. | ΔAIC |
| Null model* | 2 | 7.00 | 2 | 13.22 |
| Full model† | 21 | 19.96 | 21 | 18.38 |
| Bottom-up model‡ | 5 | 0 | 5 | 3.42 |
| Top-down model§ | 10 | 0.91 | 7 | 0 |

*Full model (model including all explanatory variables).
†Null model (model including only the intercept).
‡Bottom-up model [model selected by the step function (R 2.12.1) beginning at the null model].
§Top-down model [model selected by the step function (R 2.12.1) beginning at the full model].

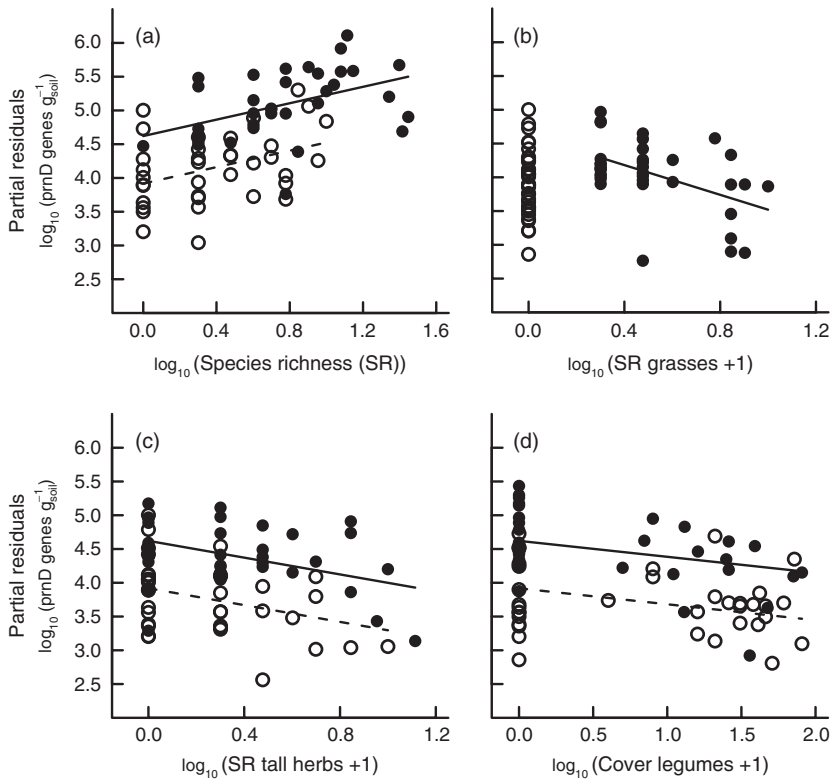


**Fig. 2.** Partial residual plots of variables that remained in the most parsimonious model. Displayed is the $\log_{10}$-transformed *prnD* gene abundance as a function of (a) $\log_{10}$-transformed plant species richness (slope 0.61, $P = 0.026$), (b) $\log_{10}(x + 1)$-transformed grass species richness (slope −1.10, $P = 0.018$), (c) $\log_{10}(x + 1)$-transformed tall herb species richness (slope −0.62, $P = 0.010$) and (d) $\log_{10}(x + 1)$-transformed cover of legume species (slope −0.24, $P = 0.009$). Data related to the presence/absence of grasses are integrated in each figure ($\text{intercept}_{\text{without grasses}}$ 3.92, $\text{intercept}_{\text{with grasses}}$ 4.62, $P = 0.003$). Plots with grasses are denoted by filled dots (continuous regression line) and those without grasses by open dots (dashed regression line).

role of plant community structure as a major driver of the functionality of soil microbial communities (Stephan, Meyer & Schmid 2000; Zak *et al.* 2003; Eisenhauer *et al.* 2010). Positive effects of species diversity on ecosystem processes have been explained by functional niche complementarity (Loreau 2000). Although only marginally significant, functional dissimilarity, accounting for complementarity between species (Jousset *et al.* 2011), remained in the most parsimonious model of the *phlD* analyses. Notably, the calculation of functional dissimilarity does not consider the number of species in a plot; thus, functional dissimilarity can be high even if only two very different species are present.

The abundances of DAPG- and PRN-producing bacteria were negatively correlated with both legume species richness and legume coverage. This negative relationship is surprising as legumes are assumed to have beneficial effects on soil microorganisms (Spehn *et al.* 2000; Milcu *et al.* 2008), for example by improving N availability (Eisenhauer *et al.* 2010). Interestingly, legumes produce steroid saponins, an effective class of antifungal and antibacterial compounds (Mahato, Ganguly &

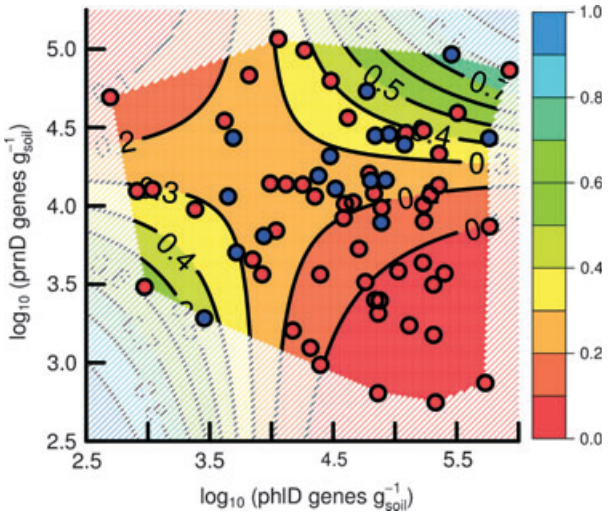


**Fig. 3.** Effects of $\log_{10}$-transformed *phlD* and *prnD* abundances on soil suppressiveness (intercept 30.04, $\text{slope}_{\text{phlD}}$ −7.94, $P_{\text{phlD}}$ = 0.036; $\text{slope}_{\text{prnD}}$ −7.35, $P_{\text{prnD}}$ = 0.069; $\text{slope}_{\text{interaction}}$ 1.88, $P_{\text{interaction}}$ = 0.038). Pots with one or more seedlings infected are denoted by red dots, those with healthy plants by blue dots. The colour code denotes the prediction of soil suppressiveness; red denotes 100% likelihood that one or more seedlings in a pot are infected, and blue denotes 0% likelihood that one or more seedlings in a pot are infected.

P. 1982). Saponin-producing plants, such as oats and some lilies, have previously been shown to negatively affect DAPG-producing pseudomonads (Raaijmakers & Weller 1998; Bergsma-Vlami, Prins & Raaijmakers 2005). Plants producing antifungal compounds of their own may get little advantage from bacteria antagonistic to fungal pathogens, while still suffering the phytotoxic side effects of these compounds (Brazelton *et al.* 2008). Further studies are needed to unravel the role of plant secondary metabolites in interactions between plants and rhizobacteria beneficial to plants (Bais *et al.* 2006).

In contrast to the negative effect of legumes, the presence of grasses enhanced the abundance of PRN producers considerably, but this trend was dampened by the negative effect of grass species richness. Fungal pathogens reduce the abundance of grasses, thereby increasing the abundance of legumes in perennial grassland (Allan, van Ruijven & Crawley 2010), indicating that grasses may be more prone to fungal pathogen pressure. In gnotobiotic systems, *Pseudomonas* spp. reach densities of up to ten times higher on the roots of monocots than on those of dicots (Lugtenberg, Dekkers & Bloemberg 2001), suggesting that grasses may rely on the activity of bacteria antagonistic to fungal pathogens, whereas legumes may have different strategies to suppress pathogens.

The final statistical model predicting *prnD* abundance included species richness as well as species richness within several functional groups. Adding a species to a system had a positive effect due to the overall species richness effect, as well as a negative effect due to functional group identity (i.e. grasses, legumes or tall herbs). Interestingly, the only functional group that had no negative effect on *prnD* abundance was small herbs. Thus, a community consisting of small herbs and some grasses may be most beneficial to *prnD* abundance. Furthermore, grasses and legumes function as key plant functional groups for soil microbial communities (Spehn *et al.* 2000; Milcu *et al.* 2008; Eisenhauer *et al.* 2010), and our results suggest that this also applies for DAPG- and PRN-producing bacteria. The detrimental effect of legumes and tall herbs and the beneficial effect of grass presence suggest that – in addition to plant diversity – the identity of plant functional groups fundamentally affects pathogen-antagonistic microbial communities.

On average, monocultures had a negative impact on the abundance of antagonistic bacteria, which is consistent with the observed decline of yields occurring in monocultures in agricultural systems (Zak *et al.* 2003; Garbeva *et al.* 2006). However, this varies with the identity of the monocultures, suggesting that plant–bacteria interactions vary between plant species. Further studies are needed to identify keystone plant species for antagonistic soil bacteria.

### EFFECTS OF ANTAGONISTIC BACTERIA ON SOIL SUPPRESSIVENESS

Soil suppressiveness peaked when both *phlD* and *prnD* gene abundances were high (Fig. 3). Both DAPG and PRN are well-known antifungal compounds suppressing a wide range of plant pathogens (Haas & Keel 2003; Haas & Defago 2005), and our results suggest that both compounds are required for efficient control of plant diseases. This supports previous observations showing that antifungal activity against *R. solani* increases when both genes for DAPG and PRN synthesis are expressed (Vincent *et al.* 1991). Such a toxin cocktail was also suggested to be more efficient in the defence of *P. fluorescens* against predators (Jousset *et al.* 2010).

In a recent study, diverse plant communities have been shown to be more resistant against attack by fungal pathogens (Maron *et al.* 2011), and our results suggest that this effect may rely – at least in part – on the promotion of plant-protecting bacteria at high plant diversity. The increase of soil suppressiveness in diverse plant communities is also in agreement with results of Garbeva *et al.* (2006), showing that soils planted with permanent (species-rich) grassland showed higher levels of suppressiveness of *R. solani* AG3 compared with long-term arable land. Increased plant protection against root pathogens at high plant diversity represents a novel indirect positive soil feedback effect, which is likely to contribute to the positive diversity–productivity relationship in grassland plant communities (Balvanera *et al.* 2006; Schmid *et al.* 2009).

In addition, soil suppressiveness was negatively affected when one gene dominated. The relationship between *phlD* and *prnD* abundance and soil suppressiveness supports the idea that a threshold population density is required for effective disease suppression (Duffy & Defago 2000). When both gene abundances were low, the model predicted an additional increase in suppressiveness. We cannot explain this pattern on a biological basis and attribute it to a statistical artefact. Healthy pots are distributed from intermediate to high values of *phlD* and *prnD*, and the negative effect of either *prnD* or *phlD* on suppressiveness is mainly supported by infected pots at high abundances. Due to the missing data points on the left

side of the figure, the apparent increase in suppressiveness at low *prnD* and *phlD* abundances is likely to result from the extrapolation of these trends to an empirically poorly supported region. Additionally, the presence of uninfected pots in this region supports the predicted increase of suppressiveness. Further studies are needed to focus on additional antibiotic producers, accompanying antibiotics tested at different concentrations and additional plant–pathogen model infection assays to unravel the role of antagonistic bacteria on soil suppressiveness and their dependence on plant diversity.

## Conclusions

We demonstrate a significant positive relationship between plant diversity and the abundance of bacteria antagonistic to soil-borne plant pathogens. In turn, high abundance of both DAPG- and PRN-producing bacteria resulted in increased pathogen suppression, highlighting the importance of plant community structure for soil microbial community functioning. Interestingly, the key plant functional groups, grasses and legumes, had opposing effects on the bacteria suppressing fungal pathogens, with legumes having a detrimental effect, whereas grass presence was beneficial. Our results suggest that diverse plant communities with a low proportion of legumes may be most beneficial for DAPG producers, and plant communities dominated by small herbs and some grasses should be most beneficial for PRN producers. The results further suggest that the loss of biodiversity is likely to reduce the resistance of plant communities to soil-borne diseases and highlight that the interrelationships between plants and beneficial microorganisms need closer consideration to understand the functioning of ecosystems and to manage agricultural systems in an environmentally friendly and sustainable way.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Experimental setup.

**Table S2.** Primers and probe for qPCR assays.

**Table S3.** Reference bacterial strains of DAPG-producing *Pseudomonas fluorescens*.

**Table S4.** Summary table of the bottom-up and top-down model phlD analyses.

**Table S5** Summary table of the bottom-up and top-down model prnD analyses.

**Figure S1.** Partial residual plots for trends in phlD abundance as functions of variables according to sown data.

**Figure S2.** Partial residual plots for trends in prnD abundance as functions of variables according to sown data.

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