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Research Article

RESEARCH ARTICLE

Conservation tillage and organic farming induce minor variations in *Pseudomonas* abundance, their antimicrobial function and soil disease resistance

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One sentence summary: The abundance and expression of *Pseudomonas* spp. genes required for the biosynthesis of antimicrobial metabolites is inconsistently influenced by conservation tillage and organic farming and is not indicative of variations in disease resistance of soils exposed to these practices.

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ABSTRACT

Conservation tillage and organic farming are strategies used worldwide to preserve the stability and fertility of soils. While positive effects on soil structure have been extensively reported, the effects on specific root- and soil-associated microorganisms are less known. The aim of this study was to investigate how conservation tillage and organic farming influence the frequency and activity of plant-beneficial pseudomonads. Amplicon sequencing using the 16S rRNA gene revealed that *Pseudomonas* is among the most abundant bacterial taxa in the root microbiome of field-grown wheat, independent of agronomical practices. However, pseudomonads carrying genes required for the biosynthesis of specific antimicrobial compounds were enriched in samples from conventionally farmed plots without tillage. In contrast, disease resistance tests indicated that soil from conventional no tillage plots is less resistant to the soilborne pathogen *Pythium ultimum* compared to soil from organic reduced tillage plots, which exhibited the highest resistance of all compared cropping systems. Reporter strain-based gene expression assays did not reveal any differences in *Pseudomonas*

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antimicrobial gene expression between soils from different cropping systems. Our results suggest that plant-beneficial pseudomonads can be favoured by certain soil cropping systems, but soil resistance against plant diseases is likely determined by a multitude of biotic factors in addition to *Pseudomonas*.

Keywords: *Pythium ultimum*; *Gaeumannomyces tritici*; phenazines; 2,4-diacetylphloroglucinol; cropping system; pyrrolnitrin; FAST

INTRODUCTION

Sustainable cropping systems and management practices, such as organic agriculture and conservation tillage are increasingly adopted by farmers worldwide to prevent soil erosion and nutrient losses as well as to increase soil organic matter content and water retention in the soil (Mäder *et al.* 2002; Pittelkow *et al.* 2014; Giller *et al.* 2015). Soil conservation cropping systems, where crops are sown directly in the field with no or minimal tillage, have been found to be advantageous particularly in non-irrigated cultivation systems in dry climates (Pittelkow *et al.* 2014). While in temperate climates with high rainfall, no tillage systems slightly decrease yields (Anken *et al.* 2004; Pittelkow *et al.* 2014), they nevertheless have a positive effect on soil structure and soil biota (Anken *et al.* 2004; Karlen *et al.* 2013; Verzeaux *et al.* 2016). No tillage leads to more stable soil aggregates and a higher soil organic matter content in the upper soil layers (Peigné *et al.* 2007). It has often been hypothesised that no tillage has positive effects on soil macrobiota and microbiota (Peigné *et al.* 2007; Navarro-Noya *et al.* 2013); however, results from field studies are so far not consistent. The abundance and diversity of individual taxonomical groups can be differentially influenced by tillage. Soil bacterial communities have been found to be different in tillage *versus* no tillage systems, with certain taxa being more frequent under no tillage compared to conventional tillage (Navarro-Noya *et al.* 2013; Carbonetto *et al.* 2014; Chávez-Romero *et al.* 2016; Degrune *et al.* 2016; Guo *et al.* 2016; Wang *et al.* 2016).

Organic agriculture becomes more and more common because it requires less external inputs and increases soil fertility (Mäder *et al.* 2002; Fließbach *et al.* 2007). Soils managed organically were found to harbour a greater diversity of soil microorganisms (Mäder *et al.* 2002; Li *et al.* 2012; Hartmann *et al.* 2015), but also to contain specific microbial communities, where certain taxa were more abundant than in conventionally managed soils (Li *et al.* 2012; Hartmann *et al.* 2015; Pershina *et al.* 2015; Bonanomi *et al.* 2016). In this context, it is of special interest how sustainable cropping systems impact on beneficial microorganisms, i.e. fungi and bacteria that improve plant growth and health.

Bacteria of the genera *Pseudomonas* and *Bacillus*, for example, are considered among the important taxa for soil health, in particular for their ability to suppress soilborne fungal pathogens (Weller *et al.* 2002; McSpadden Gardener 2004; Haas and Defago 2005). The genus *Pseudomonas* comprises species ranging from human- and plant-pathogenic to plant-beneficial organisms. Similarly, within the genus *Bacillus*, only some species are considered to be plant beneficial (McSpadden Gardener 2004). A limitation of most studies investigating the diversity of soil bacteria was that the taxonomic resolution was not detailed enough to distinguish between beneficial and non-beneficial bacteria at the species and subspecies level. Many species in the *Pseudomonas fluorescens* group (Gomila *et al.* 2015), but not all of them, exhibit multiple plant-beneficial properties, i.e. induction of systemic resistance (Bakker *et al.* 2013), competition with pathogens on the root surface (Haas and Defago 2005; Lemanceau, Maurhofer and Defago 2006) and production of metabolites with broad-spectrum antimicrobial activity (Haas and Keel 2003; Haas and Defago 2005; Weller *et al.* 2007). Certain *Pseudomonas* spp. strains with antimicrobial activity have been commercialised as biocontrol agents against a variety of plant diseases (Berg 2009; Mosimann *et al.* 2016). Among the most important antimicrobial metabolites that have an effect against fungal pathogens are 2,4-diacetylphloroglucinol (DAPG) (Haas and Keel 2003; Weller *et al.* 2007), phenazines (PHZ) (Thomashow and Weller 1988; Mavrodi, Blankenfeldt and Thomashow 2006) and pyrrolnitrin (PRN) (Hwang *et al.* 2002). These metabolites are effective against the pathogens *Pythium ultimum* and *Gaeumannomyces tritici*, among other pathogens (Thomashow and Weller 1988; de Souza *et al.* 2003). Antimicrobial metabolite-producing pseudomonads have been found in high abundances in suppressive soils, where specific pathogens are present but plants show little or no disease symptoms (Weller *et al.* 2002; Lemanceau, Maurhofer and Defago 2006). However, their presence cannot be used as sole indicator of disease suppressiveness since these bacteria are also present in disease conducive soils (Frapolli, Défago and Moënne-Loccoz 2010; Almario *et al.* 2013a; Kyselkova *et al.* 2014).

The effect of cropping systems on the abundance of antimicrobial pseudomonads is not well known. PRN producing bacteria were found to be more abundant in grassland compared to arable land (Garbeva, Voesenek and Elsas 2004). DAPG producing pseudomonads were more abundant in conventionally managed than in organically managed soils (Hiddink *et al.* 2005), but there is no study assessing, in the same field experiment, the effect of different cropping systems on abundance of different groups of antimicrobial pseudomonads. Moreover, there is little knowledge on the resistance of soils to soilborne pathogens under different cropping systems. In studies by Van Bruggen (1995) and by Hiddink *et al.* (2005), soils from organic systems were more resistant to soilborne pathogens than soils from conventional systems. However, also here, to date there is no study comparing the influence of tillage and organic management on soil resistance to root pathogens in the same year and the same field site.

In this study, we made use of the Swiss farming systems and tillage (FAST) experiment that compares conventional and organic farming, each with intensive and with conservation tillage (Wittwer *et al.* 2017) to address the above mentioned gaps. We examined the impact of different cropping systems on (i) the abundance of *Pseudomonas* spp. within the microbiomes of the wheat roots and of bulk soil, (ii) the abundance of specific groups of beneficial pseudomonads harbouring antimicrobial genes, (iii) the ability of the soil to support the expression of antimicrobial genes in *Pseudomonas* reporter strains, (iv) the abundance in soil of the two important soilborne pathogens *P. ultimum* and *G. tritici* and (v) the soil resistance to these two pathogens. We define the soil resistance as the capability of a soil and its properties (including its microflora) to influence the health of crop plants after introduction of a pathogen. We evaluated this soil resistance in different cropping systems by measuring the difference in shoot biomass between plants grown in soil inoculated with

the above mentioned pathogens and plants grown in uninoculated soil. *Pythium ultimum* causes damping-off and root rot on various crop plants, and *G. tritici*, formerly named *G. graminis* var. *tritici* (Hernández-Restrepo *et al.* 2016), causes take-all of wheat. The overall aim of this study was to better understand the relationships between the abundance, diversity and activity of *Pseudomonas* spp. and natural resistance to root pathogens in soils in response to different cropping systems. This knowledge will be important for the development of new strategies for the reduction of soilborne diseases.

MATERIAL AND METHODS

FAST experiment

The FAST experiment was established in 2009 on a field site at the Agroscope research station Reckenholz in Zurich, Switzerland (latitude 47°26'N, longitude 8°31'E). The FAST experiment compares organic and conventional farming in combination with two levels of tillage intensity based on the following four cropping systems: organic reduced tillage (O-RT), organic intensive tillage (O-IT), conventional no tillage (C-NT) and conventional intensive tillage (C-IT). The conventional systems are managed according to the 'Proof of Ecological Performance' guidelines of the Swiss Federal Office for Agriculture. The organic systems are managed according to the guidelines of Bio Suisse, the governing body for organic producers in Switzerland. All systems are cultivated with a crop rotation of six years and the present study was performed during the fourth year of the experiment (Table 1). The FAST experiment consists of two replicate experiments (FAST I and II) that are located side by side on the same field but with the crop rotation staggered by one year. Each replicate experiment comprises four replicate blocks with the cropping systems as main plots. The main plots are further subdivided into four subplots of 3 m x 15 m, three of which were sown with different cover crops (non-legume, legume and mixture) between main crops and one subplot was a control without cover crop. The factor cover crop was not included in this study and all assessments were performed in the legume cover crop treatment. The conventional treatments were fertilised with mineral fertiliser according to the quantities allowed in Swiss agriculture (Flisch *et al.* 2009); while crops in organic systems were fertilised with cattle slurry (1.4 livestock units ha^{-1}). The treatments are summarised in Table 1. The experiment is described in depth in the study by Wittwer *et al.* (2017).

Sampling and DNA extraction

Both FAST replicated experiments where sampled, FAST I in 2013 and FAST II in 2014, in the fourth year of the crop rotation. Winter wheat roots and bulk soil were sampled from all 16 main plots (four plots each: O-RT, O-IT, C-NT and C-IT). The wheat variety in both years was 'Titlis'. For each sampled plot, root systems from five plants were collected and pooled. Sampling was performed when the wheat plants were at flowering stage. The bulk soil samples were collected at 0–20 cm depth between wheat rows. Five soil cores were collected per plot and pooled. To collect bacteria from the root surface, the root systems were rinsed with tap water to remove bulk soil, incubated overnight at 3°C in sterile Erlenmeyer flasks in 50 mL 0.9% NaCl solution and subsequently shaken on an orbital shaker at 350 rpm for 30 min. Roots were then separated from the suspension and dried for 2 days at 100°C to determine dry weight. The suspensions were centrifuged at 3500 rpm for 20 min and 0.5 g of the obtained pellet was used for DNA extraction with the FastDNA Spin kit for soil (MP Biologicals, Illkirch, France). Bulk soil samples were thoroughly mixed and 0.5 g were used for DNA extraction with the same kit as used for the roots. DNA concentrations were measured with the Qubit fluorometer broad range dsDNA assay (Thermo Fisher Scientific, Waltham, USA).

Twenty-five litres of soil per plot were collected in 2014 for the disease resistance and gene expression experiments. Soil cores (0–25 cm) were collected randomly through the plots, sieved with a 1-cm-mesh sieve to remove stones and large plant debris, and thoroughly mixed. The soil samples were stored at 15°C.

Bacteria community analysis using 16S rRNA gene amplicon sequencing

To study the relative abundance of *Pseudomonas* spp. and other bacterial taxa on the roots and in bulk soil, the V5–V7 regions of the 16S rRNA gene were sequenced using the DNA samples from the FAST II experiment (collected in 2014). We used the methodology described in Hartman *et al.* (2017). Briefly, PCR primers used were 799F (5′-AACMGGATTAGATACCCKG-3′ (Chelius and Triplett 2001) and 1193R (5′- ACGTCATCCCCACCTTCC-3′ (Bodenhausen, Horton and Bergelson 2013). Universal amplification of the primers was tested in-silico with the TestPrime tool on the Silva database (Klindworth *et al.* 2013). Primers were fused at the 5′ end to an 8 bp barcode (Faircloth and Glenn 2012) and a 5 bp padding sequence [5′-padding- $barcode_{xy}$-primer-3′]. PCR reactions consisted of 1 × 5Prime Hot Mastermix (5Prime, Boulder, USA), 0.3% Bovine Serum Albumin (New England Biolabs, Ipswich MA, USA), 400 nM of each tagged primer (Microsynth, Balgach, Switzerland) and 10 ng template DNA in a total reaction volume of 20 μL. Samples containing the PCR mastermix and water were used as negative controls. PCRs were performed on an iCycler instrument (BioRad, Hercules, CA, USA) with cycling conditions consisted of an initial denaturation of 3 min at 94°C, 30 cycles of 45 s at 94°C, 30 s at 55°C and 1 min 30 s at 65°C, followed by a final elongation of 10 min at 65°C. Band size of the PCR products was verified by gel electrophoresis before purification with the NucleoSpin Gel and PCR cleanup kit (Macherey-Nagel, Oensingen, Switzerland). PCR product concentrations were measured with a Varian fluorescence plate reader (Varian, Palo Alto, USA) using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, USA) and Herring Sperm DNA (Invitrogen, Carlsbad, USA) as the standard solution. The samples were equimolarly pooled to a library containing 50 ng PCR products per sample. The library was purified with Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, USA) and the concentration of the library was measured after purification with a Varian fluorescence plate reader (Varian, Palo Alto, USA). Sequencing adapters were ligated to the library by the Functional Genomics Center Zurich (Zurich, Switzerland, http://www.fgcz.ch/) followed by sequencing on the Illumina MiSeq instrument in paired-end 2 × 300 bp mode (Illumina, San Diego, USA).

Sequence processing was conducted according to Hartman *et al.* (2017). Briefly, the raw sequencing read data (available at European Nucleotide Archive database, accession no. PRJEB20139) were quality filtered using PRINSEQ v0.20.4 (Schmieder and Edwards 2011), merged with FLASH v.1.2.9 (Magoč and Salzberg 2011) and de-multiplexed (barcode-to-sample assignments are documented in Table S4 in Supplementary Data D2, Supporting Information) employing Cutadapt v1.4.2 (Martin 2011). The high-quality 16S rRNA gene sequences

Table 1. The Farming Systems and Tillage experiment (FAST, Wittwer *et al.* 2017).

Treatment name	Treatment description	Tillage depth (cm)	Fertilisation (ha^{-1})[a]	Crop rotation
C-NT	Conventional, no tillage	No tillage	120 kg N, 88 kg P, 128 kg K	Cover crop, wheat, cover crop, maize, field bean, wheat[b], grass-clover, grass-clover
C-IT	Conventional, intensive tillage	20–25	120 kg N, 88 kg P, 128 kg K	Cover crop, wheat, cover crop, maize, field bean, wheat[b], grass-clover, grass-clover
O-RT	Organic, reduced tillage	5	Slurry 1.4 livestock units	Cover crop, wheat, cover crop, maize, field bean, wheat[b], grass-clover, grass-clover
O-IT	Organic, intensive tillage	20–25	Slurry 1.4 livestock units	Cover crop, wheat, cover crop, maize, field bean, wheat[b], grass-clover, grass-clover

[a]Average fertilisation for winter wheat in 2013 and 2014.
[b]Sampling time point in the crop rotation.

were trimmed to a fixed length of 360 bp, sorted by abundance, de-replicated and clustered to operational taxonomic units (OTUs, ≥ 97% sequence similarity) with UPARSE v8.1.1812 (Edgar 2013). Only OTUs with a minimal coverage of five sequences were included. Chimeric OTU sequences were removed after identification with UCHIME (Edgar *et al.* 2011) against the GOLD database (Reddy *et al.* 2014). Taxonomy assignment was performed using the SILVA 16S v119 database (Quast *et al.* 2013) with the RDP classifier implemented in QIIME v1.8 (Caporaso *et al.* 2010). Microbiome profiles were filtered to exclude OTUs classified as Cyanobacteria or assigned to mitochondria. The bioinformatics script including all individual parameters used is provided as Supplementary Data D1 and Data D2 (Supporting Information).

Quantitative real-time PCR

To quantify *Pseudomonas* spp. producing antimicrobial metabolites on roots and in bulk soil, quantitative real-time PCR (qPCR) was used, targeting the genes *phlD* (biosynthesis pathway of 2,4-diacetylophloroglucinol) and *phzF* (biosynthesis pathway of PHZ) according to Imperiali *et al.* (2017) and *prnD* (biosynthesis pathway of PRN) as described by Garbeva, Voesenek and Elsas (2004). Primers and cycling conditions of the qPCR assays are described in Table S1 and Table S2 (Supporting Information). The assays targeting *phlD* and *phzF* are specific for *Pseudomonas* of the *P. fluorescens* lineage (Imperiali *et al.* 2017), while the assay targeting *prnD* additionally detects *Burkholderia* and *Serratia* (Garbeva, Voesenek and Elsas 2004). The functions of the genes mentioned above are summarised in Table S3 (Supporting Information). To quantify the plant pathogenic oomycete *P. ultimum*, a qPCR assay targeting the internal transcribed spacer region was used (Cullen *et al.* 2007). Additionally, the pathogenic ascomycetes *G. tritici* and *Gaeumannomyces avenae* were quantified with a qPCR assay targeting the ITS region (Bithell *et al.* 2012b). All qPCR assays and preparation of standard curves for quantification of fungal ITS regions and of *Pseudomonas* harbouring antimicrobial genes on roots are described in detail by Imperiali *et al.* (2017). Briefly, pseudomonads carrying antimicrobial genes were quantified with *in-vivo* standard curves prepared by adding defined numbers of cells to sterile wheat roots. This allows us to directly relating the cycle threshold (Ct) values of the qPCR assays to cell numbers of *Pseudomonas* carrying antimicrobial genes. Moreover, since *Pseudomonas* carry only one copy per genome of antimicrobial biosynthesis genes *phlD*, *phzF* and *prnD*, cell numbers per gram of root are comparable to gene copies per gram of root (Imperiali *et al.* 2017). For quantification of *Pseudomonas* carrying antimicrobial genes in bulk soil, *in-vitro* standard curves with genomic DNA from strains *P. protegens* CHA0 (*phlD*, *prnD*) and *Pseudomonas synxantha* 2-79 (*phzF*) were performed, ranging from 2×10^6 to 2 genome copies $reaction^{-1}$ in six 10-fold dilutions. Three technical replicates were performed for each of the 16 main plots (four replicate plots for each treatment, i.e. O-RT, O-IT, C-NT and C-IT).

For all qPCR assays, quantitative PCR cycle threshold (Ct) values were normalised for variation in DNA extraction efficiency by adding a specified quantity of APA9 plasmid as internal standard prior to DNA extraction as described in Imperiali *et al.* (2017) and in Von Felten, Defago and Maurhofer (2010). Briefly, a fixed number of copies of a cassava mosaic virus sequence were mixed to each sample prior to DNA extraction. Each sample was then analysed by two qPCR runs, one quantifying the target gene and the other the internal APA9 standard. The proportion added/quantified standard allowed us to determine DNA extraction efficacy for each sample.

Detection limits of the antimicrobial metabolite qPCR assays were 2 cells per reaction (*phzF* in-vivo standard curve), 20 cells per reaction (*phlD* and *prnD* in-vivo standard curves), 2 genome copies per reaction (*phzF* and *prnD* in-vitro standard curves) and 20 genome copies per reaction (*phlD* in-vitro standard curve). Detection limits of qPCR assays targeting the ITS regions of pathogens were 200 attograms DNA per reaction (*P. ultimum*) and 10^3 attogram DNA per reaction (*G. tritici* and *G. avenae*).

In situ reporter strain assay for quantification of antimicrobial gene expression

The reporter assays were conducted as detailed by Imperiali *et al.* (2017). Briefly, the expression of antimicrobial genes on the roots of wheat plants was quantified with GFP-marked variants of *P. protegens* CHA0 (CHA0::*att*Tn7-*gfp*; Péchy-Tarr *et al.* (2013)) and *P. chlororaphis* PCL1391 (PCL1391::*att*Tn7-*gfp*; Imperiali *et al.* (2017)), harbouring mCherry-based reporter plasmids pME9012 (*phlA-mcherry*; Rochat *et al.* (2010)), pME11011 and pME11017 (*prnA-mcherry* and *phzA-mcherry*, respectively; Imperiali *et al.* (2017)). The expression of the reporter fusions *phlA-mcherry* and *prnA-mcherry* (genes involved in the biosynthesis

of 2,4-diacetylphloroglucinol and the biosynthesis of pyrrolnitrin, respectively) was measured in strain *P. protegens* CHA0, whereas the expression of the reporter fusion *phzA-mcherry* (gene involved in the biosynthesis pathway of PHZ) was monitored in *P. chlororaphis* PCL1391. Reporter strains were extracted from wheat roots and soil after five days of incubation, because after this time, the difference between gene expressions was more pronounced and easy to observe (data not shown). Spring wheat seeds of the variety 'Rubli' (Delley Seeds, Delley, Switzerland) were surface disinfested for 12 min in 4% v/v NaClO, washed with distilled water and germinated on soft agar (Agar, Agar SERVA, 9 g L^{-1}) by incubating for 48 h at room temperature in the dark. The germinated wheat seedlings were transferred to 200 mL Erlenmeyer flaks containing 60 g of soil. Soil sampled in 2014 as described above was used. Three seedlings per flask were planted. The *Pseudomonas* reporter strains were grown overnight in 8 mL of NYB supplemented with gentamycin (10 μg mL^{-1}) and kanamycin (25 μg mL^{-1}), at 30°C and 180 rpm. Each wheat seedling was inoculated with 1 mL suspension of washed bacteria cells corresponding to 3–4 × 10^8 CFU. Control treatments were performed with wild type *P. protegens* CHA0 and *P. chlororaphis* PCL1391 and with GFP-tagged *P. protegens* CHA0-*gfp* and *P. chlororaphis* PCL1391-*gfp* with or without empty vector control (Imperiali *et al.* 2017). Flasks were incubated for 5 days in a growth chamber at 60% relative humidity with a 16 h light period at 176 μE m^{-2} s^{-1} and 25°C and an 8 h dark period at 20°C. Wheat roots were harvested and cell suspensions from root washes prepared as described above. The suspensions were filtered using a 5.0 μm sterile syringe single-use filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany), transferred on ice and immediately analysed by FACS with a BD LSRFortessa flow cytometer (Becton-Dickinson, San Jose, USA). Gating and settings for detecting GFP and mCherry fluorescence emitted by reporter strains were the same as described previously (Imperiali *et al.* 2017). Fresh and dry weight of wheat roots were recorded and the number of GFP-marked *Pseudomonas* cells present in root wash was determined by FACS and expressed as CFU g $root^{-1}$. The experiment was performed twice. Three technical replicates were performed for each of the 16 investigated main plots (four replicate plots for each treatment, i.e. O-RT, O-IT, C-NT and C-IT) and for each of the control treatments.

Assessment of soil resistance to root pathogens

The effect of the different cropping systems of the FAST experiment on the resistance of the soil to the two common soilborne plant pathogens *P. ultimum* and *G. tritici* was tested in a greenhouse experiment as described in detail by Imperiali *et al.* (2017). As all cropping systems in the FAST experiment include crop rotation, it appears legitimate to assess soil disease resistance to a pathogen that is not specific to wheat. We have chosen *P. ultimum* owing to its broad host range covering many monocot (including wheat) and dicot crops. *Pythium ultimum* causes damping-off and root rot on many host plants in conventionally used crop rotations in Switzerland. The *P. ultimum*-cucumber pathosystem has been frequently used to assess soil disease resistance and antifungal activity of plant beneficial bacteria (Paulitz and Loper 1991; Notz *et al.* 2001; Carisse, Bernier and Benhamou 2003; Scheuerell, Sullivan and Mahaffee 2005; Flury *et al.* 2016). The *P. ultimum*-cucumber system allows us to assess damping-off symptoms more precisely and with a smaller inoculum quantity compared to the *P. ultimum*-wheat system (our unpublished data, Notz *et al.* 2001). Briefly, pathogen inoculum was prepared by growing *P. ultimum* on autoclaved millet seeds and *G. tritici* on autoclaved oat seeds. Soil (200 g per pot) sampled from the 16 main plots (four replicate plots for each treatment, i.e. O-RT, O-IT, C-NT and C-IT) was filled in pots, amended with increasing quantities of pathogen inoculum and planted with three sterile-germinated cucumber seedlings (*Cucumis sativa* var. 'Chinese Snake') in the *P. ultimum* system or three sterile-grown spring wheat seedlings (*Triticum aestivum* var. 'Rubli') in the *G. tritici* system. Six replicate pots were prepared per plot and pathogen concentration (four pathogen concentrations and one control treatment without inoculum). Plants were grown for 10 days (cucumber) or 21 days (wheat) in the greenhouse with a 16-h-day period (210 μmol $m^{-2}s^{-1}$) at 22°C (cucumber) or 18°C (wheat) and an 8-h-night period at 18°C (cucumber) or 15°C (wheat) with an air moisture of 70%. At the end of the experiment, fresh shoot weights per pot were determined as a measure to assess the disease resistance of the soils.

Data analysis

All data were analysed with the R software version 3.2.3 (RCoreTeam 2015).

The OTU and taxonomy tables were imported in R for further analysis. We followed Weiss *et al.* (2015) and tested for differences between the number of reads from different sample groups and treatments was tested with non-parametric Kruskal–Wallis test (package 'coin'). No significant difference was found; therefore, the data were not rarefied but normalised by the sampling depth. Relative abundances of OTUs were obtained by normalising the OTU count data with the centred log-ratio transformation (Data D2, Supporting Information). OTUs assigned to the genus *Pseudomonas* with a relative abundance greater than 0.1% were selected for further analysis. The differences between the relative abundances of *Pseudomonas* OTUs in the different treatments was calculated with Kruskal–Wallis test followed by Dunn's post-hoc test (R package 'dunn.test').

Gene expression per gram of roots was calculated by multiplying the relative red fluorescence per cell with the number of detected events (cells) per gram of roots (dry weight). Data from the two experiments were pooled, since no significant difference was found between the results of experiment 1 and experiment 2 (linear mixed effect model with 'experiment' as a fixed effect, function 'lmer' from package 'lme4').

Pathogen resistance in the different treatments was calculated by expressing the fresh shoot weight of the plants from inoculated pots as a percentage of the mean fresh shoot weight from control plants of the same treatment.

Significant differences between treatments were determined with a linear mixed effect model (function 'lmer' from package 'lme4') with 'cropping system' and 'block' as fixed effects and 'plot' as a random effect. Technical replicates were nested within biological replicates (i.e. plots). For qPCR assays, three technical replicates per plot were performed, while for greenhouse assays, six technical replicates per plot were performed. A post-hoc test was performed for 'cropping system' (Tukey's HSD, function 'glht' from package 'multcomp').

RESULTS

Pseudomonas spp. in the root and soil microbiome

We determined the relative abundance of the genus *Pseudomonas* on the wheat root surface and in soil in FAST II (2014), and whether they differ between cropping systems, using 16S rRNA

gene amplicon sequencing. We sequenced 32 samples and generated a total of 1 398 161 high-quality sequences, of which 1856 different OTUs were detected. On average, 43 717 high-quality filtered reads per sample were obtained. The highest numbers of OTUs were assigned to the phyla Proteobacteria (740 OTUs, 52% relative abundance on roots and 38% in bulk soil), Actinobacteria (271 OTUs, 18% relative abundance on roots and 32% in bulk soil) and Bacteroidetes (173 OTUs, 18% relative abundance on roots and 13% in bulk soil). We found three OTUs with a relative abundance >0.1% that were assigned to the genus *Pseudomonas*. The most abundant was OTU1, being the second most abundant OTU in the entire dataset (Fig. S1, Supporting Information), with an average relative abundance of 7.6% on roots and 2.6% in bulk soil (Fig. 1A and B). The second *Pseudomonas* OTU152 had an average relative abundance of 0.9% on roots and 0.3% in bulk soil (Fig. 1C and D). The third, OTU140 had an average relative abundance of 0.18% on roots and 0.13% in bulk soil (Fig. 1E and F). OTU1 and OTU152 were significantly more abundant on roots compared to bulk soil, while for OTU140 this was only the case for the organic treatment with reduced tillage. The cropping system had no significant effect on relative abundances of *Pseudomonas* OTUs (Fig. 1). Overall, *Pseudomonas*, together with *Flavobacterium* and *Variovorax*, were found to be among the most abundant taxa on wheat roots and in the soil of the FAST field experiment (Fig. S1, Supporting Information). No difference was found between cropping systems for the relative abundance of the above mentioned taxa (data not shown).

Pseudomonas spp. harbouring biosynthesis genes of antimicrobial metabolites

In a second step, we quantified pseudomonads carrying the well-known antimicrobial genes *phlD*, *phzF* and *prnD* (for description of genes and their function see Table S3, Supporting Information). While OTUs belonging to the genus *Pseudomonas* were not significantly influenced by cropping system at the taxonomic level, we found significant differences in the abundance of *Pseudomonas* harbouring antimicrobial genes between the different cropping systems. Pseudomonads carrying the antimicrobial genes *phlD* and *phzF* were quantified with a qPCR assay specific for the *P. fluorescens* lineage (Imperiali *et al.* 2017), while *prnD* carrying bacteria were quantified with a qPCR assay that detects *prnD*+ *Pseudomonas*, *Burkholderia* and *Serratia* (Garbeva, Voesenek and Elsas 2004). Pseudomonads harbouring the gene *phlD* (2,4-diacetylphloroglucinol biosynthesis) were significantly more abundant on roots in conventional farming with no tillage, compared to organic farming with reduced tillage in both investigated years (Figs 2A and 3A). The bulk soil of C-NT harboured more *phlD*+ pseudomonads compared to the O-RT, O-IT and C-IT treatments, although here the differences were significant only in 2014 (Figs 2B and 3B). The abundance of pseudomonads carrying *phzF* (biosynthesis of PHZ) was not significantly different between treatments in both years of sampling (Figs 2C and D and 3C and D). For *prnD* (biosynthesis of pyrrolnitrin) results differed between the two years. In 2014, there were no significant differences found for the roots (Fig. 2E), while in bulk soil, the abundance of bacteria carrying *prnD* was significantly lower in C-NT compared to C-IT and O-RT (Fig. 2F). However, in 2013, *prnD*+ bacteria abundances were significantly higher on the roots of C-IT compared to both organic treatments and on the roots of C-NT compared to O-IT (Fig. 3E) and in bulk soil of C-IT compared to O-RT (Fig. 3F). Overall, the highest gene abundances on roots were associated with *phlD*+ in 2014 (median abundance: 8.8×10^4 cells/g root, Fig. 2) and in 2013 (median abundance: 1.5×10^5 cells/g root, Fig. 3). *phzF*+ *Pseudomonas* on roots were 8-fold and 70-fold less frequent than *phlD*+ *Pseudomonas* in 2014 and 2013, respectively. *prnD*+ bacteria on roots were 41-fold less frequent than *phlD*+ *Pseudomonas* in 2014 and 6-fold less frequent in 2013.

Expression of *Pseudomonas* antimicrobial metabolite biosynthesis genes

In addition to the in planta abundances of *phlD*, *phzF* and *prnD* genes in 2013 and 2014, the expression of the antimicrobial biosynthesis genes *phlA*, *phzA* and *prnA* on the roots of wheat plants was measured with a reporter strain based assay using soils collected in 2014 (Fig. 4). The investigated cropping systems had no impact on the expression of the genes *phlA* (biosynthesis of 2,4-diacetylphloroglucinol), *phzA* (biosynthesis of PHZ) and *prnA* (biosynthesis of pyrrolnitrin). The levels of root colonisation and gene expression at single cell level were measured as previously described by Imperiali *et al.* (2017), but no differences could be observed in the different treatments (data not shown). Moreover, results are consistent with those obtained by Imperiali *et al.* (2017), since the gene expression values are in the same range of those obtained in the previous study. These results indicate that the investigated cropping systems have no impact on antimicrobial activity of the employed reporter strains.

Soil disease resistance and pathogen abundance

Complementary to the assessment of beneficial pseudomonads, we also investigated if cropping systems impacted the abundance of *P. ultimum* and *G. tritici* or the disease resistance of the soils to these pathogens. The abundance of naturally present *P. ultimum* and *G. tritici* was assessed with qPCR on roots and bulk soil. While in 2014 *P. ultimum* could be detected in all biological replicates from all treatments, in bulk soil as well as on roots, *G. tritici* was only occasionally detected, in bulk soil more frequently than on roots (Fig. 5). In 2013, both pathogens were only sporadically detected at lower abundances than in 2014 (Fig. 6). In both years, no significant differences in pathogen abundance were detected between cropping systems.

We tested the resistance of the soils to *P. ultimum* and *G. tritici* in a greenhouse experiment, where the pathogen load in the soils collected in 2014 was manipulated. At lower *P. ultimum* concentrations, plants grown in soil from O-RT plots tended to have higher shoot weights compared to the other treatments (Fig. S2, Supporting Information). This difference was more pronounced under higher pathogen pressure. When 0.5 g *P. ultimum* had been added per pot, relative shoot weights of plants grown soil from O-RT plots were significantly higher than those of both conventional treatments (Fig 7A; Fig. S2, Supporting Information). The soils sampled from all cropping systems were completely resistant to *G. tritici* and no reduction of shoot weight in comparison to untreated control plants was observed even at the highest pathogen concentration (Fig 7B; Fig. S3, Supporting Information). We excluded the possibility that this lack of plant infection was due to a lack of virulence of the inoculum by conducting an experiment with autoclaved soil (Fig. S4, Supporting Information). Adding *G. tritici* to autoclaved soil strongly reduced the shoot weight of wheat plants.

Summary of results

To summarise the diverse information obtained in this study, we report the normalised medians for each measured trait in

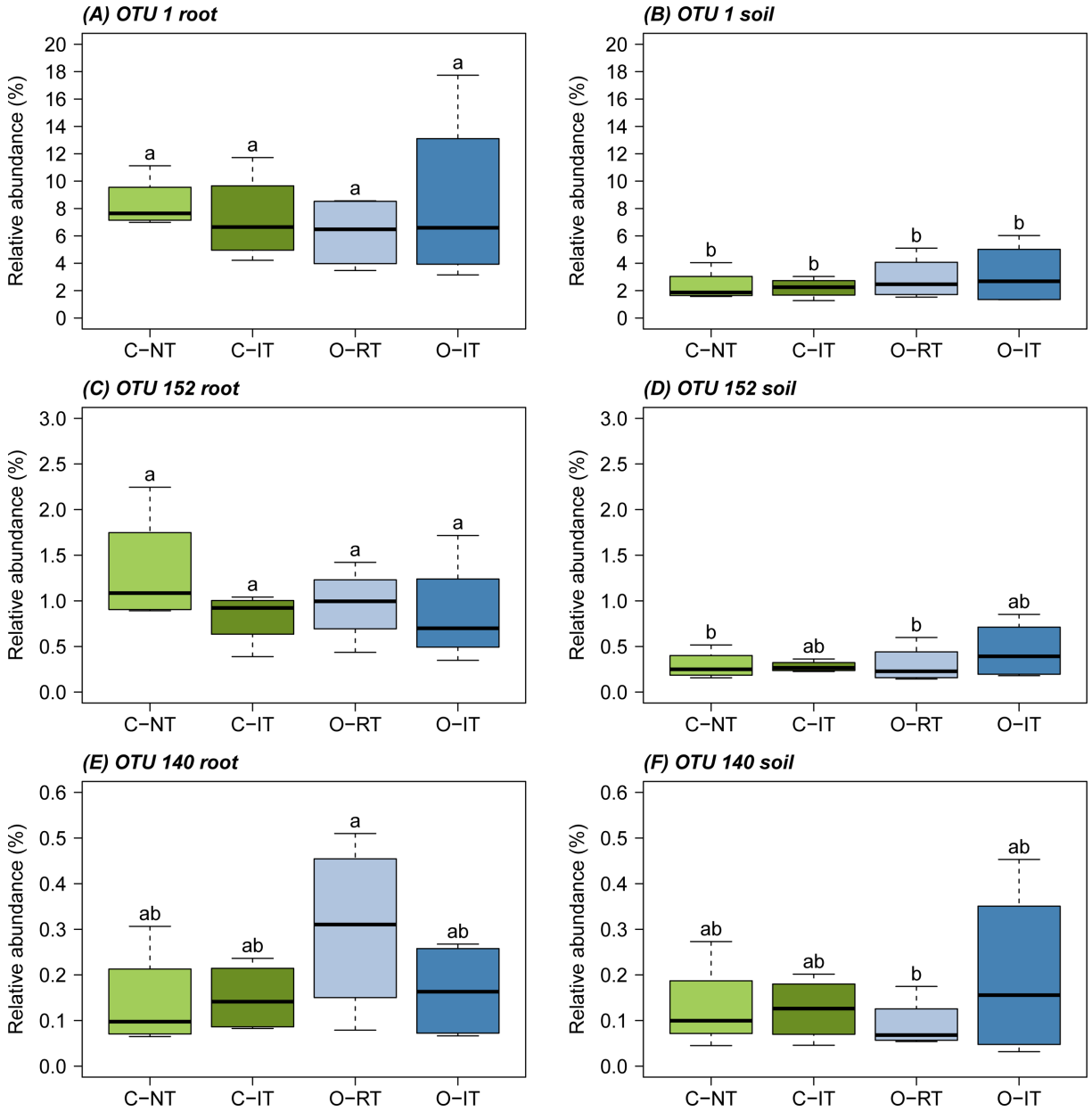

Figure 1. Relative abundance of OTUs assigned to the genus *Pseudomonas* on the roots of wheat and in soil in different agricultural management systems. Amplicon sequencing of the 16S rRNA gene V5–V7 regions was performed on four replicates per cropping system. OTUs with a relative abundance greater than 0.1% are shown. Letters show significant differences (Kruskal–Wallis test followed by Dunn post-hoc test, $P < 0.05$). For each OTU data presented in root and soil panels were analysed together. Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Sequencing was performed with samples collected from the field experiment FAST II, 2014. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length.

the four tested cropping systems (Fig. 8). In 2014, the heat map shows a trend that conventional cropping systems, especially with no tillage, support higher levels of DAPG and PHZ producers, whereas PRN producers were especially abundant in the organic treatment with intensive tillage. Interestingly, the organic cropping system with reduced tillage displayed the highest resistance to *P. ultimum*, but also the highest natural *P. ultimum* abundance and at the same time, harboured the lowest numbers of the investigated groups of antimicrobial pseudomonads. In 2013, similar trends were observed for the abundance of pseudomonads harbouring DAPG and PHZ biosynthesis genes, but in contrast to 2014, *P. ultimum* was below the detection limit in most samples of all treatments (Fig. 8). This may indicate that DAPG, PRN and PHZ might not be involved in the suppression of this pathogen in the soil of the FAST experiment. No differences between organic and conventional treatments were detected for antimicrobial gene expression. There was no trend observed for conservation tillage systems (reduced and no tillage), where neither the abundance of antimicrobial pseudomonads on roots, nor expression of antimicrobial genes, nor the disease resistance to *P. ultimum* and *G. tritici* were significantly different from the respective intensive tillage treatment (Figs 2fig3.fig –4, 7; Figs S2 and S3, Supporting Information).

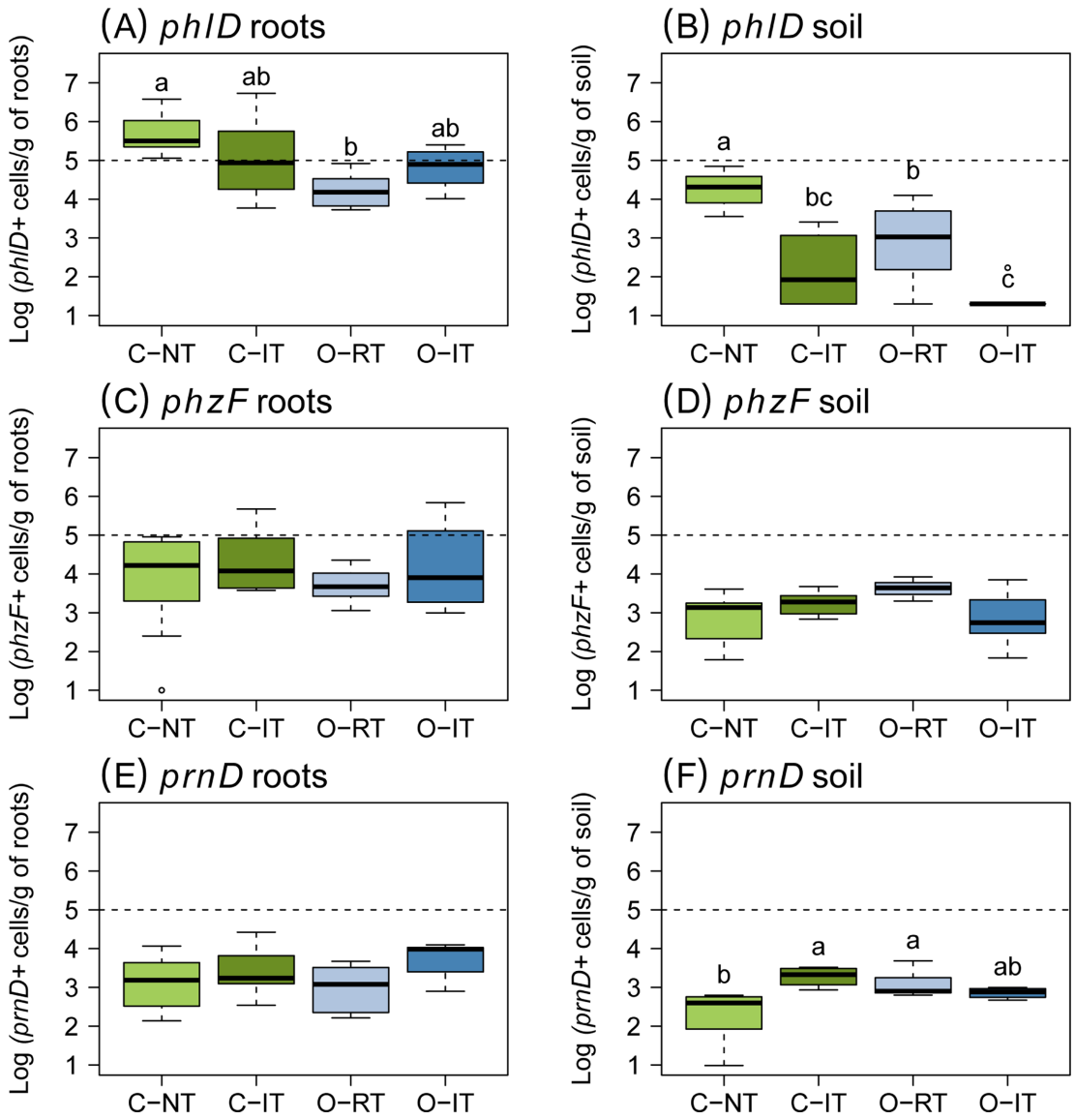

Figure 2. Abundance of bacterial cells harbouring biosynthesis genes for antimicrobial compounds in soils with different agricultural management systems in FAST II, 2014: (**A**, **B**) *Pseudomonas* harbouring *phlD* (2,4-diacetylphloroglucinol biosynthesis), (**C**, **D**) *Pseudomonas* harbouring *phzF* (biosynthesis of PHZ) and (**E**, **F**) bacteria harbouring *prnD* (pyrrolnitrin biosynthesis), (A, C, E) wheat root, (B, D, F) bulk soil. The dotted line indicates 10^5 cells per g of dry roots (A, C, E) or per g of soil (B, D, F). Letters in the graphs indicate significant differences between cropping systems ($P < 0.05$). For graphs C, D and E no significant differences were detected. Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers.

DISCUSSION

Pseudomonas spp. in the root and soil microbiome

In this study, we investigated relationships between cropping systems, bacterial diversity, abundance and activity of plant-beneficial pseudomonads and soil disease resistance.

The 16S rRNA gene amplicon sequencing revealed that *Flavobacterium*, *Variovorax* and *Pseudomonas* were among the most abundant taxa on wheat roots (Fig. S1, Supporting Information). Earlier studies reported *Pseudomonas* among the abundant bacteria on roots of various plant species, including *Arabidopsis* (Bulgarelli *et al.* 2012), barley (Bulgarelli *et al.* 2015), maize (Hacquard *et al.* 2015), clover (Hartman *et al.* 2017), as well as cucumber and wheat (Ofek-Lalzar *et al.* 2014). To our knowledge, the present study is the first to compare the relative abundances of *Pseudomonas* in soil and in wheat root microbiomes between different cropping systems in a common experimental setup under field conditions. We did not detect an impact of tillage or organic farming on the relative abundance of *Pseudomonas* on the roots or in bulk soil. However, in another study *Pseudomonas* were found to be more abundant in soil from a conventionally managed field, compared to soil from an adjacent organically managed field (Pershina *et al.* 2015).

We identified three OTUs that could be assigned to the genus *Pseudomonas* (OTU1, OTU152 and OTU140). We found that OTU1 and OTU152 were significantly more abundant on roots than in bulk soil (Fig. 1). It is assumed that fluorescent pseudomonads are enriched in the rhizosphere compared to bulk soil (Dennert and Schlaeppi, unpublished). Moreover, many type strains in the *P. fluorescens* group have been isolated from plant roots (Flury

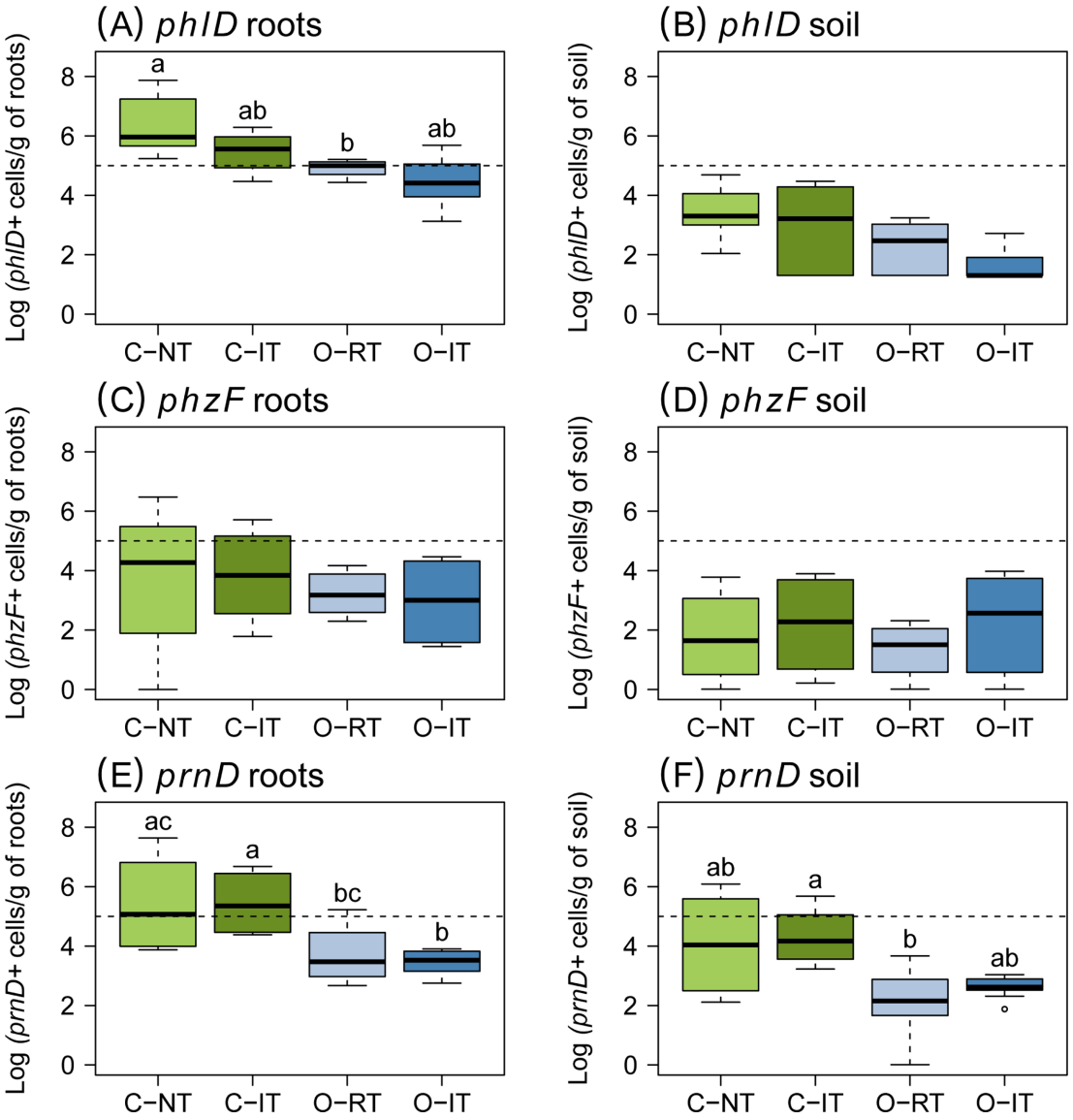

Figure 3. Abundance of bacterial cells harbouring biosynthesis genes of antimicrobial compounds in soils with different agricultural management systems in FAST I, 2013: (**A**, **B**) *Pseudomonas* harbouring *phlD* (2,4-diacetylphloroglucinol biosynthesis), (**C**, **D**) *Pseudomonas* harbouring *phzF* (biosynthesis of PHZ) and (**E**, **F**) bacteria harbouring *prnD* (pyrrolnitrin biosynthesis), (A, C, E) wheat root, (B, D, F) bulk soil. The dotted line indicates 10^5 cells per g of roots. Letters in the graphs indicate significant differences between cropping systems ($P < 0.05$). For graphs C, D and E no significant differences were detected. Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers.

et al. 2016; Garrido-Sanz *et al.* 2016). In contrast, OTU140, was equally abundant on roots and in soil.

Pseudomonas spp. harbouring biosynthesis genes of antimicrobial metabolites

While the abundance of *Pseudomonas* OTUs, as determined by amplicon sequencing, was not influenced by tillage or organic management, the abundance of pseudomonads carrying antimicrobial genes differed between cropping systems. Our findings that *phlD*+ pseudomonads are more abundant in C-NT compared to O-RT, in both investigated years are in agreement with a previous study (Hiddink *et al.* 2005), where DAPG producers were also more abundant in conventionally managed fields compared to organically managed fields. In contrast, we could not detect differences in the abundance of *phlD*+ pseudomonads on roots between conventional and organic management in an earlier investigation (Dennert *et al.* 2016).

In the present study, we did not observe an effect of tillage on the abundance of *phlD* carrying pseudomonads on plant roots. Rotenberg *et al.* (2007), on the contrary, found that *phlD*+ pseudomonads were more abundant in the rhizosphere of maize grown in no tillage plots compared to moderately tilled plots. We obtained similar results but only for bulk soil and only in one year. In 2014, bulk soil from the C-NT and O-RT treatments harboured significantly higher numbers of *phlD*+ pseudomonads than the respective intensive tillage treatments (Fig. 2). This suggests that cropping systems with reduced tillage intensity can favour the abundance of these bacteria in soil.

For pseudomonads carrying the PHZ biosynthetic gene *phzF,* no significant differences between cropping systems were

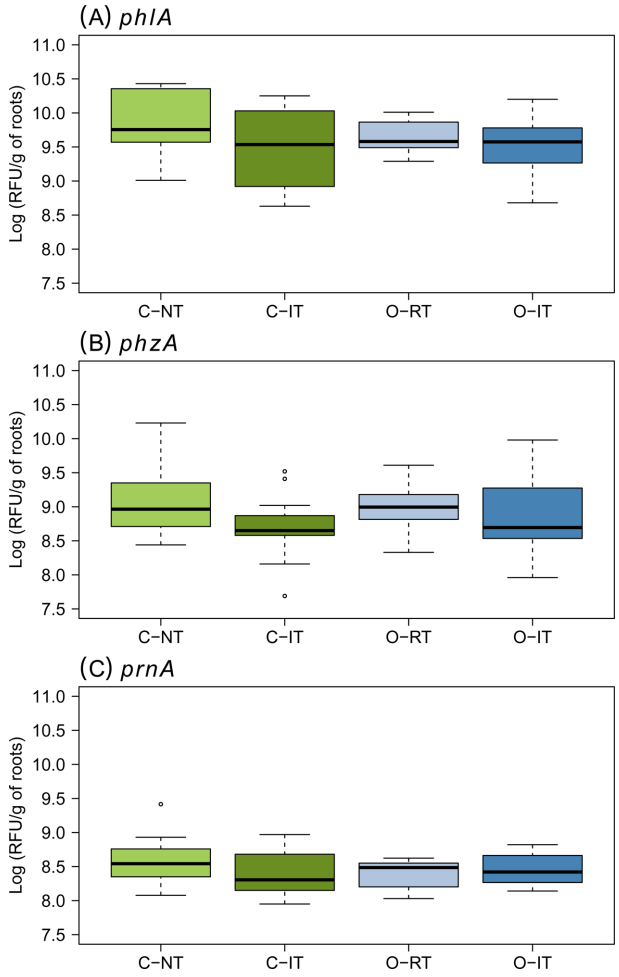

Figure 4. Relative expression of genes required for the biosynthesis of antimicrobial compounds (**A**) 2,4-diacetylphloroglucinol (*phlA*), (**B**) PHZ (*phzA*), (**C**) pyrrolnitrin (*prnA*) in soils from different cropping systems planted with spring wheat. Expression was monitored by fluorescence-activated cell-sorting based flow cytometry using GFP-tagged strains of *Pseudomonas protegens* (CHA0-*gfp*) carrying reporter plasmids pME9012 (*phlA-mcherry*), or pME11011 (*prnA-mcherry*) and *Pseudomonas chlororaphis* (PCL1391-*gfp*) carrying reporter plasmid pME11017 (*phzA-mcherry*). Data are shown as relative fluorescence units per gram of root dry weight and were calculated as the median mCherry expression per GFP tagged *Pseudomonas* cell multiplied with the total number of GFP-tagged *Pseudomonas* cells per gram of root. No significant differences between cropping systems were found ($P < 0.05$). Soils were sampled from **FAST II, 2014**. Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers.

found, neither in soil nor on the root surface (Figs 2 and 3). To our knowledge, this is the first study measuring the abundance of pseudomonads carrying PHZ biosynthesis genes in soils from different cropping systems.

While the abundance of PRN producers was previously compared in grassland and arable land (Garbeva, Voesenek and Elsas 2004), the effect of organic management or reduced tillage on *prnD+* bacteria is not well known. Previously, we found *prnD+* bacteria to be significantly less abundant in samples from organic compared to conventional soil (Dennert *et al.* 2016), similarly to the results obtained here for FAST I in 2013 (Fig. 3). However, in 2014 (Fig. 2) this trend was not confirmed.

All the three investigated groups of *Pseudomonas* harbouring antimicrobial metabolite biosynthesis genes tended to be more abundant on roots in 2013 compared to 2014 in all treatments indicating that the climatic conditions in the year of sampling could be an important factor shaping antifungal pseudomonads populations. Fluorescent pseudomonads are sensitive to drought. In 2014, there was long period without rainfall and the upper 5–8 cm of the soil were very dry at the time of sampling. These results highlight the need of studies over multiple growing seasons to understand the link between cropping systems and the abundance of specific groups of microorganisms.

Expression of *Pseudomonas* antimicrobial metabolite biosynthesis genes

The quantification of antimicrobial genes from pseudomonads is an indication for the size of the bacterial population potentially able to produce certain antimicrobial metabolites, but they do not indicate if different cropping systems influence expression levels of these genes. Therefore, we monitored the expression of antimicrobial genes using FACS-based flow cytometry and GFP-marked *Pseudomonas* model strains carrying mCherry-based reporter plasmids. To our best knowledge, the present study is the first assessing expression of antimicrobial metabolite genes in response to different cropping systems. However, we did not detect any significant differences in the expression of DAPG, PHZ or PRN biosynthesis genes on roots of wheat planted in soil sampled from plots with different cropping systems (Fig. 4), suggesting that at the FAST field site the investigated agricultural practices have at most minor impacts on antimicrobial gene expression. Nevertheless, our results only give first indications since they are obtained with two reporter strains and not by quantifying the expression of naturally present *phlA*, *phzA* and *prnA* genes.

Only little is known on expression of antimicrobial genes in agricultural soils, mainly because of methodological challenges associated with the recovery of sufficient quantities of the specific mRNAs from natural soil. Still, some of the factors influencing antimicrobial gene expression in *Pseudomonas* have already been identified. For instance, a recent study (Imperiali *et al.* 2017) found correlations between *phlA*, *phzA* and *prnA* expression in reporter strains and organic matter, clay, silt, magnesium, potassium and manganese contents in soil. Another study by Almario *et al.* (2013b) also showed that expression of *phlA* was influenced by the type of clay present in an artificial soil. Antimicrobial gene expression is strongly influenced by the plant species and as determined in different studies (Notz *et al.* 2001; de Werra *et al.* 2008; Rochat *et al.* 2010). Moreover, expression of DAPG biosynthetic genes is also modulated by different metabolites produced by bacteria itself, like gluconic acid (de Werra *et al.* 2011), DAPG, salicylate and pyoluteorin (Schnider-Keel *et al.* 2000; Maurhofer *et al.* 2004; Yan *et al.* 2017), or by the presence of plant pathogens, e.g. *P. ultimum* and *Fusarium* and by fusaric acid, a toxin produced by the pythopathogenic fungus *Fusarium* (Schnider-Keel *et al.* 2000; Notz *et al.* 2002). To date, however, still little is known on the regulation of clusters responsible for PHZ and PRN production in *Pseudomonas* strains.

All these results indicate that soil physical and chemical properties might have a stronger impact on antimicrobial gene expression than the cropping system. However, the expression of antimicrobial genes will have to be addressed in additional field experiments in order to obtain a deeper insight into the

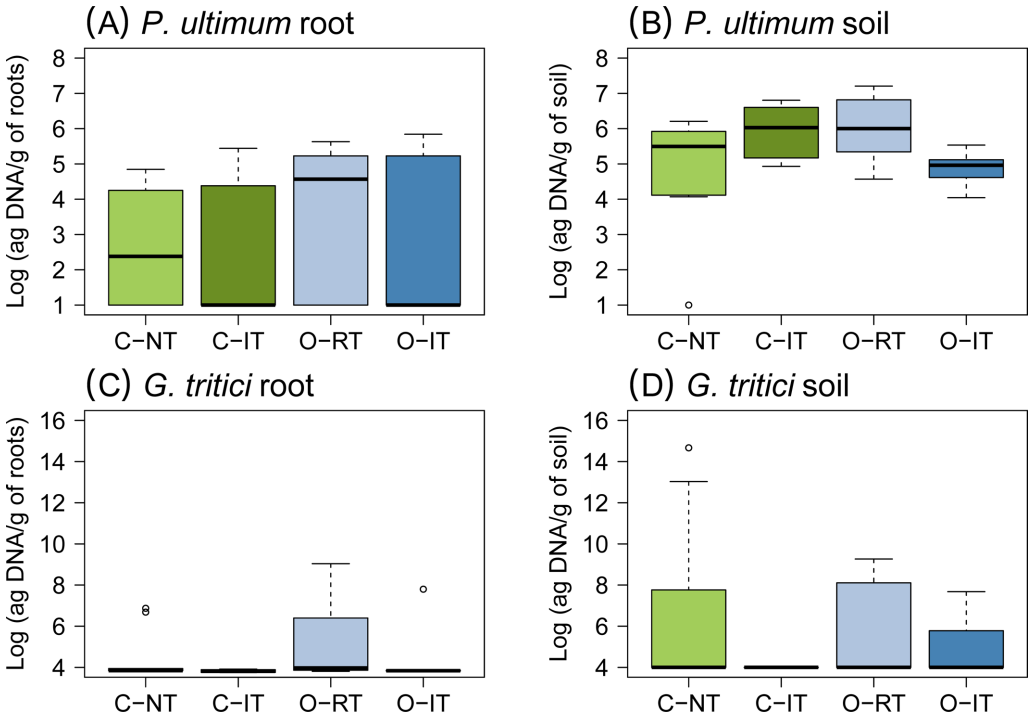

Figure 5. Natural abundance of the pathogens *P. ultimum* and *G. tritici/G. avenae* in soils from different cropping systems planted with winter wheat in FAST II, 2014. (**A**) *P. ultimum* on wheat roots; (**B**) *P. ultimum* in bulk soil; (**C**) *G. tritici/G. avenae* on wheat roots; (**D**) *G. tritici/G. avenae* in bulk soil. Abundance is shown as quantity of pathogen DNA (attograms) per gram of soil or root (dry weight). The detection limits of the qPCR assays were 10 attogram DNA/g of root or soil (*P. ultimum* assay) and 10^4 attogram/ g root or soil (*G. tritici/G. avenae* assay). For each cropping system, four biological replicates (four replicate plots) with three technical replicates each were analysed. No significant differences between cropping systems could be found for both pathogens ($P < 0.05$). Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers.

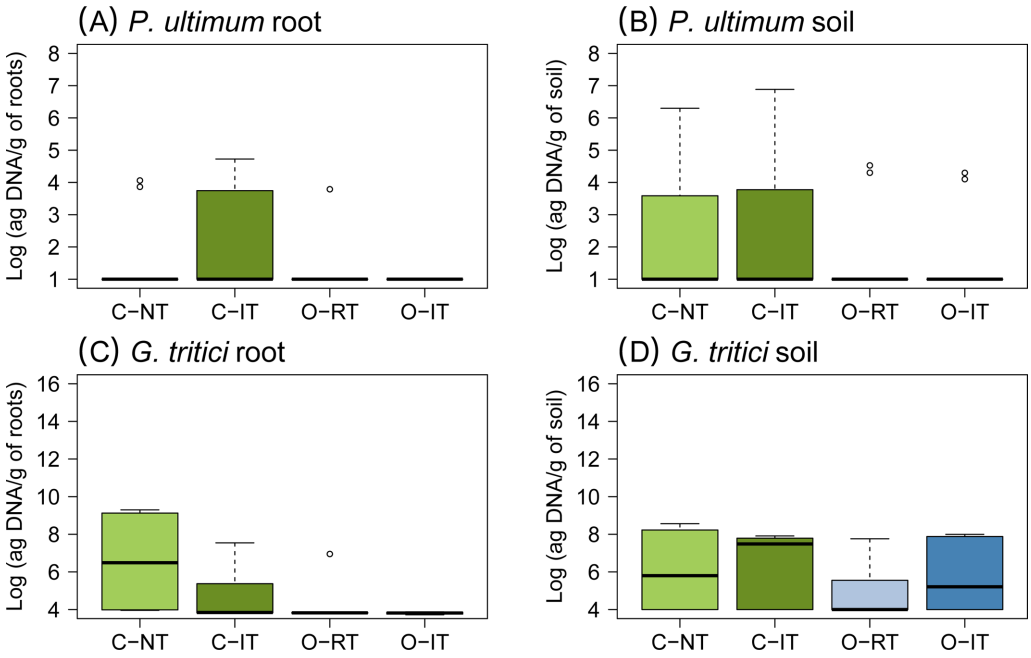

Figure 6. Natural abundance of the pathogens *P. ultimum* and *G. tritici/G. avenae* in soils from different cropping systems planted with winter wheat in FAST I, 2013. (**A**) *P. ultimum* on wheat roots; (**B**) *P. ultimum* in bulk soil; (**C**) *G. tritici/G. avenae* on wheat roots; (**D**) *G. tritici/G. avenae* in bulk soil. Abundance is expressed as quantity of pathogen DNA (attograms) per gram of soil or root (dry weight). The detection limits of the qPCR assays were 10 attogram DNA/ g of root or soil (*P. ultimum* assay) and 10^4 attogram/ g root or soil (*G. tritici/G. avenae* assay). No significant differences between cropping systems could be found for both pathogens ($P < 0.05$). Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers.

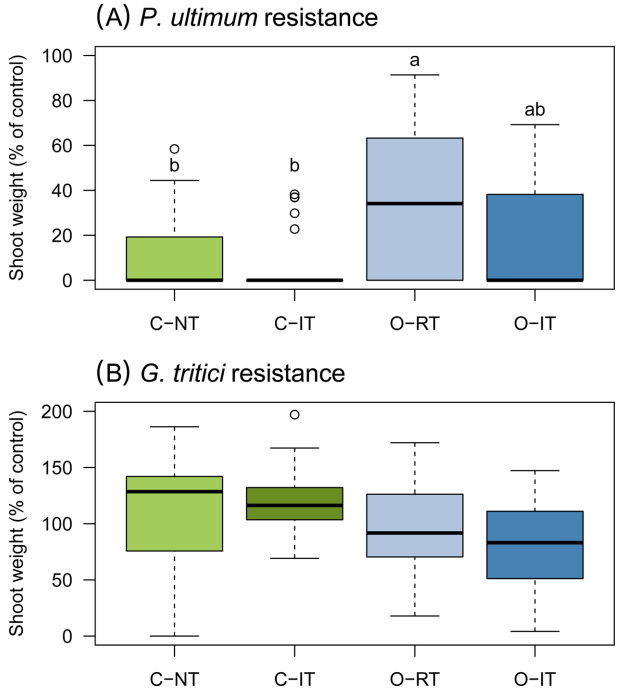

Figure 7. Relative resistance of soils from different cropping systems to the soil-borne pathogens (**A**) *P. ultimum* (*Pythium*) and (**B**) *G.itici* (*Gaeumannomyces*) in FAST II, 2014. Increasing concentrations of pathogen inoculum were added to the soil before planting with cucumber (*Pythium* experiment) or spring wheat (*Gaeumannomyces* experiment) seedlings. Data shown here are for 0.5 g *Pythium* and 2.0 g *Gaeumannomyces* per pot. Results for the other inoculum concentrations are shown in Figs S2 (*Pythium* experiment) and S3 (*Gaeumannomyces* experiment) (Supporting Informating). Soil resistance is shown as fresh shoot weight of plants in artificially pathogen-infested soil compared to fresh shoot weight of control plants grown in non-infested soil. Letters indicate significant differences between management systems ($P < 0.05$). For resistance to *G. tritici* no significant differences were detected. Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5∗ box length; open circles, outliers.

interplay of agricultural practices and activity of plant-beneficial soil bacteria.

Soil disease resistance and pathogen abundance

Dissecting bacterial communities and analysis of known plant-beneficial bacteria in soil delivers information on how certain environmental factors, in this study cropping systems, influence soil ecology. But most important from an agronomical point of view and a prerequisite for the implementation of conservation biocontrol strategies is the knowledge on how cropping systems impact on plant performance and plant health. To this end, we tested the resistance of soils sampled in the FAST experiment to two soilborne pathogens, *P. ultimum,* which is a major seedling and root pathogen, for many different crops, and *G. tritici,* a pathogen attacking wheat roots and causing the take-all disease. While the soil from organically managed plots with reduced tillage was significantly more resistant to *P. ultimum* than soil from conventionally managed plots (Fig. 7), the *P. ultimum* qPCR data showed that there were no differences in abundance of resident *P. ultimum* between FAST treatments (Figs 5 and 6). This indicates that the naturally present *P. ultimum* population did not affect the outcome of our disease resistance tests and that the investigated cropping systems do not impact on the numbers of this pathogen in soil. The increased *P. ultimum* resistance of the O-RT plots cannot be accredited to pseudomonads since these plots neither harboured higher numbers of total pseudomonads, nor of antimicrobial *Pseudomonas* groups, nor did soils of these plots support elevated levels of antimicrobial gene expression. We assume that other microorganisms than pseudomonads were responsible for the higher resistance to *P. ultimum* in the O-RT treatment. This hypothesis is supported by a recent study investigating the abundance of *Pseudomonas* harbouring DAPG, and PHZ biosynthetic genes in 10 representative Swiss agricultural soils, where no significant correlation between the level of soil disease resistance to *P. ultimum* and the abundance of DAPG+ and PHZ+ *Pseudomonas* was found (Imperiali *et al.* 2017).

Organic fertilisation is often described as a means to lower disease incidence. A review by van Bruggen and Finckh (2016) summarises descriptions of organically managed soils displaying higher resistanceto soilborne pathogens than conventionally managed soils. They describe a reduced disease severity in organically managed plots for *Fusarium* infections, damping off caused by *Rhizoctonia solani* and stalk rot caused by *Sclerotinia sclerotiorum*. In organically managed soils, the competition for organic resources is higher, which is suggested to impair certain soilborne pathogenic fungi. In addition, Hiddink *et al.* (2005) found that take-all disease severity was lower in organically managed compared to conventionally managed fields. In the FAST experiment, we did not observe such an effect, since all the soils sampled from all treatments were completely resistant to *G. tritici* (Fig. 7; Fig. S3, Supporting Information). Similarly as for *P. ultimum*, the *G. tritici/G. avenae* qPCR results showed that cropping systems had no impact on abundance of naturally present *G. tritici*. Abundance of naturally present *G. tritici* was lower in most samples from our study (Figs 5 and 6), compared to other studies on soils from New Zealand (Bithell *et al.* 2012a; Keenan *et al.* 2015). Accordingly, the roots of the sampled plants did not show any symptoms caused by *G. tritici*. For the *P. ultimum* abundance, no other studies quantifying this pathogen in wheat systems with qPCR were found in the literature, but we hypothesise that the abundance in the FAST trial is low, since the sampled plants did not show any *P. ultimum* symptoms. Our experiment comparing autoclaved with natural soils from all FAST treatments with and without addition of *G. tritici* showed that first, the pathogen inoculum we used was virulent, and second, that autoclaved soils had lost their *G. tritici* resistance (Fig. S4, Supporting Information). This indicates that the soil of the FAST experiment is indeed resistant to *G. tritici* and that the soil resistance is probably due to biological factors. Whether DAPG producing pseudomonads, which are known to play a key-role in take-all decline soils (Weller *et al.* 2002) and which we found to be abundant in the FAST experiment, are involved in the *G. tritici* resistance, remains subject to further studies.

We did not detect any differences in disease resistance between no- or reduced tillage systems and the respective intensive tillage treatments. The influence of reduced tillage on the severity of root diseases is not well studied, although there are indications that no tillage might favour soilborne pathogens by helping them persist on crop residues and roots of volunteer plants (Paulitz 2006). Moreover, in a study by Steinkellner and Langer (2004), it was found that *Fusarium* spp. were more abundant and diverse in soils managed with conservation tillage than in soils managed with conventional tillage.

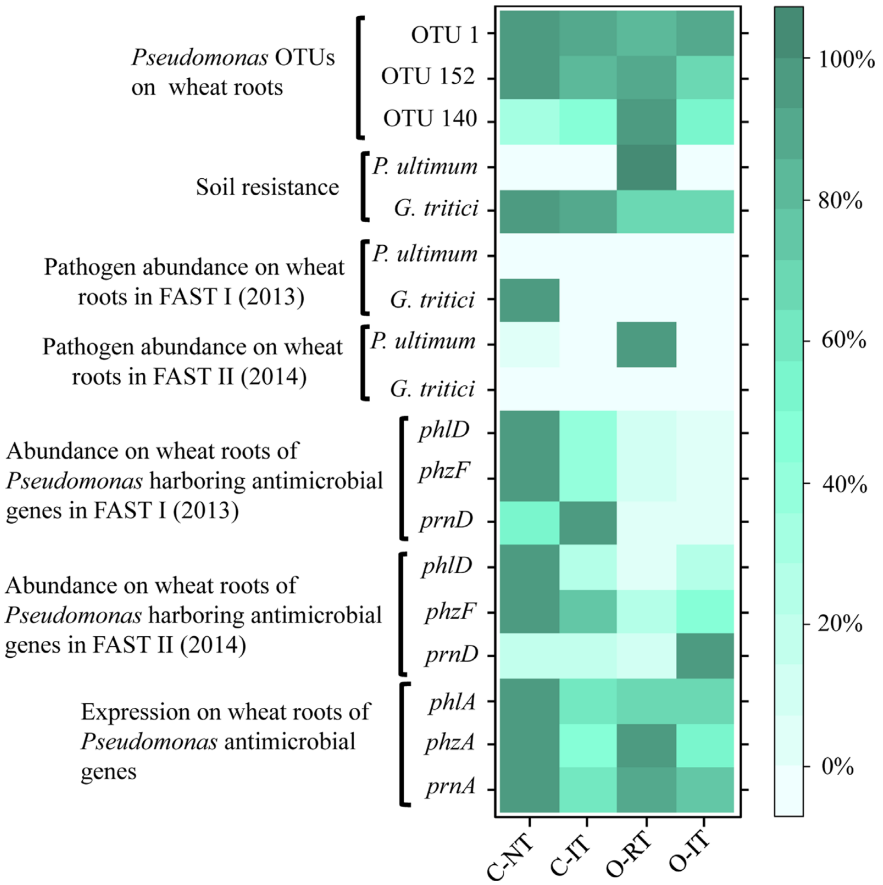

Figure 8. Heat map showing normalised medians of relative abundance of *Pseudomonas* OTUs, resistance to pathogens, pathogen abundance in soil, abundance of cells harbouring antimicrobial genes on roots and expression of antimicrobial genes on roots. Medians were normalised by setting the highest median for each measured trait to 100% and showing the other medians of a given trait in % of the highest median. Values can only be compared within rows. Data from FAST II, 2014 and from FAST I, 2013 were used. Presented data: soil resistance to *P. ultimum* and *G. tritici* (see Fig. 7), natural abundance of *P. ultimum* and *G. tritici/avenae* on wheat roots (see Figs 5 and 6), abundance of antimicrobial gene harbouring *Pseudomonas* on roots of wheat (see Figs 2 and 3), expression of antimicrobial genes on roots (see Fig. 4) and relative abundance of *Pseudomonas* OTUs on wheat roots (see Fig. 1). *Gaeumannomyces tritici* abundance in 2014 and *P. ultimum* abundance in 2013 were below the detection limits in most samples; therefore, these data were included as 0% in all cropping systems. Cropping systems: ≪C-NT≫ is conventional without tillage, ≪C-IT≫ is conventional with tillage, ≪O-RT≫ is organic with reduced tillage, ≪O-IT≫ is organic with tillage.

CONCLUSION

Taken together, our results suggest that *Pseudomonas* are among the dominant taxa in the soil as well as on wheat roots in all the studied cropping systems (Fig 1; Fig. S1, Supporting Information). While bacteria community sequencing did not reveal any differences in the relative abundance of *Pseudomonas* on wheat roots between cropping systems (Fig. 1), *Pseudomonas* spp. producing specific antimicrobial metabolites, i.e. DAPG were more abundant on the roots of wheat grown in conventional systems (Figs 2, 3 and 8). These results highlight that it may indeed be possible to selectively favour specific groups of plant-beneficial *Pseudomonas* by adapting the cropping system. However, resistance to *P. ultimum* was highest in O-RT soils, which supported the lowest abundance of DAPG-producing *Pseudomonas* on roots (Fig. 8A) and were not supportive of PHZ and PRN producers either. This indicates that single taxa of known biocontrol microorganisms cannot be used as bio-indicators for the evaluation of conservation biocontrol strategies. Disease resistance, natural biocontrol of soilborne pathogens, is most probably based on the interplay of several beneficial microorganisms and their complex interactions with plant pathogens are influenced by a multitude of biotic and abiotic factors, such as soil physical and chemical characteristics (Imperiali *et al.* 2017), plant species (Latz *et al.* 2015) and cropping history (Landa *et al.* 2006). In particular, our data show that there are variations between cropping seasons, and that clear trends can probably only be detected in long-term studies. Despite the complex interactions that determine disease resistance in soils, our results indicate that certain cropping systems might increase the resistance of soils to specific pathogens. Studies over multiple cropping seasons and field sites, which focus on various plant-beneficial functions within the root-associated microbiome, are needed to identify strategies for conservation biocontrol of soilborne plant pathogens.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Supplementary Information

Conservation tillage and organic farming induce minor variations in *Pseudomonas* abundance, their antimicrobial function and soil disease resistance

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Supplementary Tables

Table S1: Primers and probes used for quantitative PCR (qPCR) in this study

Target gene	Primers and probes	Sequence (5'-3')[1]	Annealing (°C)	Reference
phlD	PhlD_65F_DEG	GGT RTG GAA GAT GAA RAA RTC	50°C	Imperiali, et al. (2017)
	PhlD_153P_DEG	FAM-ATG GAG TTC ATS ACV GCY TTG TC-BHQ1		
	PhlD_236R_DEG	GCC YRA BAG YGA GCA YTA C		
phzF	PhzF_2Fm	ACC GGC TGT ATC TGG AAA CC	62°C	Imperiali, et al. (2017)
	PhzF_2Pm	FAM-GCC GCC AGC ATG GAC CAG CCG AT-BHQ1		
	PhzF_2Rm	TGA TAG ATC TCG ATG GGA AAG GTC		
prnD	PrnD_F	TGC ACT TCG CGT TCG AGA C	60°C	Garbeva, et al. (2004)
	PrnD_P	FAM-CGA CGG CCG TCT TGC GGA TC-BHQ1		
	PrnD_R	GTT GCG CGT CGT AGA AGT TCT		
P. ultimum (ITS)	92F	TGT TTT CAT TTT TGG ACA CTG GA	60°C	Cullen, et al. (2007)
	116T	FAM-CGG GAG TCA GCA GGA CGA AGG TTG-BHQ1		
	166R	TCC ATC ATA ACT TGC ATT ACA ACA GA		
G. tritici/G. avenae (ITS)	tritici_avenae_F	AAC TCC AAC CCC TGT GAC CA	60°C	Bithell, et al. (2012)
	tritici_avenae_P	FAM-TCG TCC GCC GAA GCA-BHQ1		
	tritici_avenae_R	CGC TGC GTT CTT CAT CGA TGC C		
Cassava mosaic virus (internal standard)	CMV_1F	TCA TCA TTT CCA CTC CAG GCT C	62°C	Von Felten, et al. (2010)
	CMV_1R	TCA TCC CTC TGC TCA TAC GAC TG		

[1]TaqMan probes were labelled with fluorescin (FAM) at the 5' end and with the black hole quencher 1 (BHQ-1) at the 3' end

Table S2: Reaction setup and cycling conditions of qPCR assays[1]

Reagent	**Quantity in reaction mix (final reaction volume=20 µL)**	**Concentration of stock**	**Manufacturer**
Forward primer	2 µL	10 µM	Microsynth, Balgach, Switzerland
Reverse Primer	2 µL	10 µM	Microsynt
TaqMan Probe	2 µL	2.5 µM	Microsynth
Bovine Serum Albumin	0.5 µL	20 mg mL^{-1}	New England Biolabs, Ipswich, USA
GeneExpression Mastermix	10 µL	According to manufacturer's indications	Applied Biosystems, Foster City, USA
Template DNA	2 µL	10-50 ng μL^{-1}	
H_2O	1.5 µL		
Cycling conditions	**Step**	**Temperature**	**Duration**
	Uracyl Glycosylase Activation	50°C	2 min.
	Initial Denaturation	95°C	10 min.
40 Cycles	Denaturation	95°C	15 sec.
	Annealing	See Table S1	30 sec
	Elongation	72°C	30 sec

[1]Reaction mix and cycling conditions were the same for all qPCR assays used in this study targeting the following genes: *phlD* (2,4-diacetylphloroglucinol biosynthesis), *phzF* (biosynthesis of phenazines), *prnD* (pyrrolnitrin biosynthesis), ITS (*P. ultimum*), ITS (*G. tritici/avenae*).

Table S3: Function of genes studied with quantitative PCR and in in-situ reporter strain assay

Antimicrobial metabolite(s)	Experiment[1]	Gene	Function[2]	Reference
DAPG[3]	Abundance-quantitative real-time PCR	*phlD*	Synthesis of phloroglucinols from malonyl-CoA	Bangera and Thomashow (1996); Achkar, et al. (2005)
DAPG	Expression-*in situ* reporter strain assay	*phlA*	Condensation of monoacetylphloroglucinol to DAPG[5]	Bangera and Thomashow (1996)
Phenazines	Abundance-quantitative real-time PCR	*phzF*	Synthesis of phenazine-1-carboxylic acid	Mavrodi, et al. (1998); Blankenfeldt, et al. (2004)
Phenazines	Expression-*in situ* reporter strain assay	*phzA*	Synthesis of the intermediate product 6-amino-5-oxocyclohex-2-ene-1-carboxylic	Mentel, et al. (2009)
Pyrrolnitrin	Abundance-quantitative real-time PCR	*prnD*	Catalyzation of the oxidation in the final step of pyrrolnitrin biosynthesis	Kirner, et al. (1998)
Pyrrolnitrin	Expression-*in* situ reporter strain assay	*prnA*	Chlorination of L-tryptophan in the first step of the pyrrolnitrin biosynthesis	Kirner, et al. (1998)

[1]Experiment in which the gene was studied (see chapter Material and Methods).

[2]Function of the gene in the biosynthesis pathway of the antimicrobial metabolite.

[3]DAPG: 2,4-diacetylphloroglucinol.

Supplementary Figures

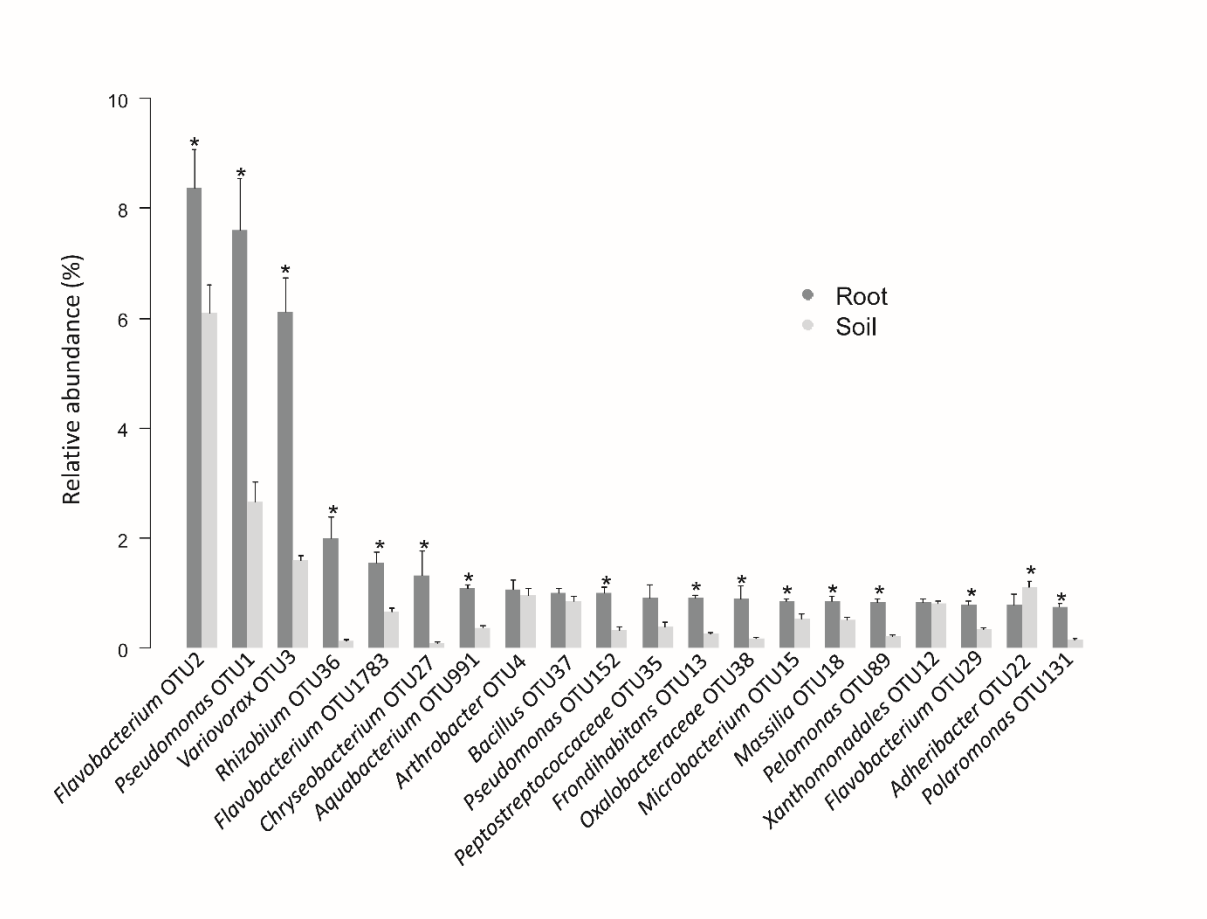

Figure S1 | The twenty most abundant bacterial operational taxonomic units (OTUs) detected on wheat roots and in bulk soil based on 16S rRNA V5-V7 region amplicon sequencing. Taxonomic assignments were determined with the SILVA database. The highest assigned taxonomic rank is shown. Sequencing was performed with samples from the field experiment **FAST II (sampling in 2014**). Data from different cropping systems (conventional without tillage (C-NT), conventional with tillage (C-IT), organic with reduced tillage (O-RT), organic with tillage (O-IT) were pooled. Four replicates per treatment were sequenced. Bars show the average relative abundance and standard errors. Asterisks denote taxa that are significantly more abundant on roots than in bulk soil (Kruskal-Wallis test, $p<0.05$).

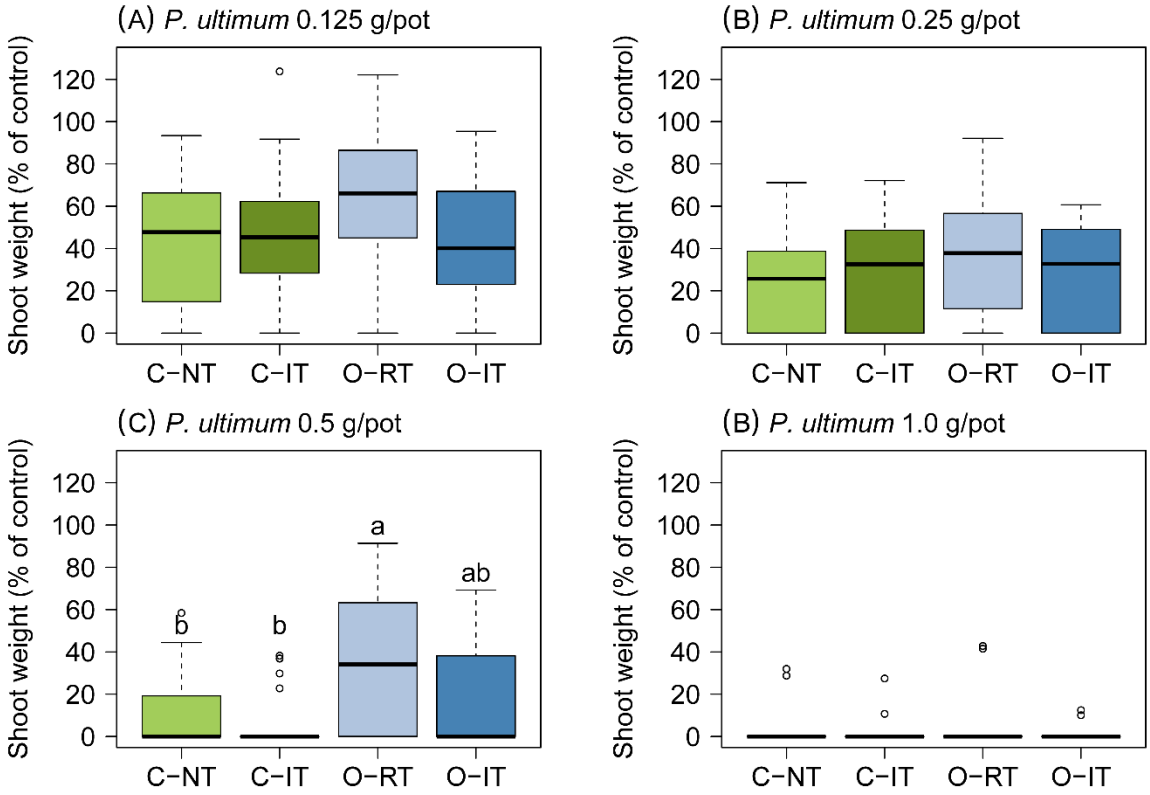

Figure S2 | Relative resistance of soils from different cropping systems to the soil-borne pathogen *Pythium ultimum* at different inoculum quantities. Increasing concentrations of pathogen inoculum were added to the soil before planting with cucumber seedlings. **(A)** 0.125 g/pot, **(B)** 0.25 g/pot, **(C)** 0.5 g/pot, **(D)** 1.0 g/pot. Soil resistance is shown as fresh shoot weight of plants in artificially pathogen-infested soil compared to fresh shoot weight of control plants grown in non-infested soil. Soils from four replicate plots per cropping system were tested. For each plot, each pathogen concentration was tested in six replicate pots. Letters indicate significant differences between cropping systems ($p<0.05$). Soils were sampled from **FAST II in 2014**. Cropping systems: «C-NT» is conventional without tillage, «C-IT» is conventional with tillage, «O-RT» is organic with reduced tillage, «O-IT» is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5* box length; open circles, outliers.

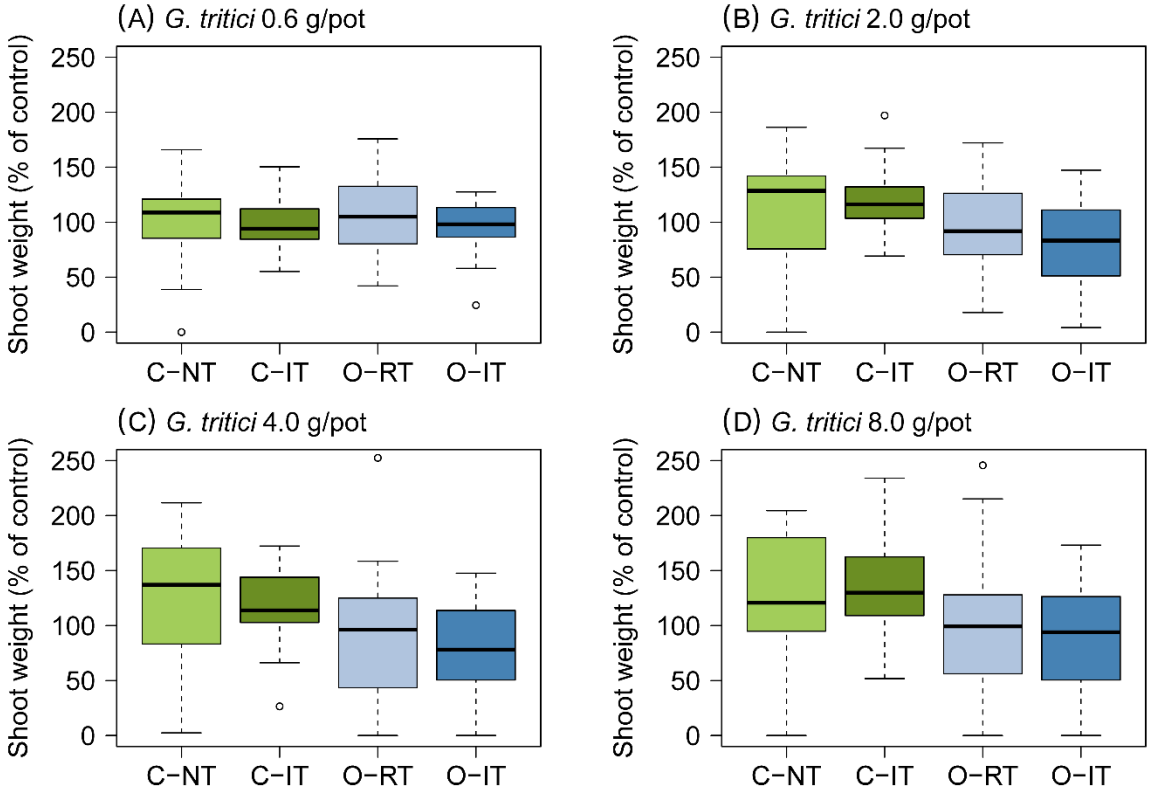

Figure S3 | Relative resistance of soils from different cropping systems to the soil-borne pathogen *Gaeumannomyces tritici* at different inoculum quantities. Increasing concentrations of pathogen inoculum were added to the soil before planting with spring wheat seedlings. **(A)** 0.6 g/pot, **(B)** 2.0 g/pot, **(C)** 4.0 g/pot, **(D)** 8.0 g/pot. Soil resistance is shown as fresh shoot weight of plants in artificially pathogen-infested soil compared to fresh shoot weight of control plants grown in non-infested soil. Soils from four replicate plots per cropping system were tested. For each plot, each pathogen concentration was tested in six replicate pots. Letters indicate significant differences between management systems ($p<0.05$). Soils were sampled from **FAST II in 2014.** Cropping systems: «C-NT» is conventional without tillage, «C-IT» is conventional with tillage, «O-RT» is organic with reduced tillage, «O-IT» is organic with tillage. Boxplots: bold lines, medians; boxes, 25th to 75th percentiles; whiskers, 1.5* box length; open circles, outliers.

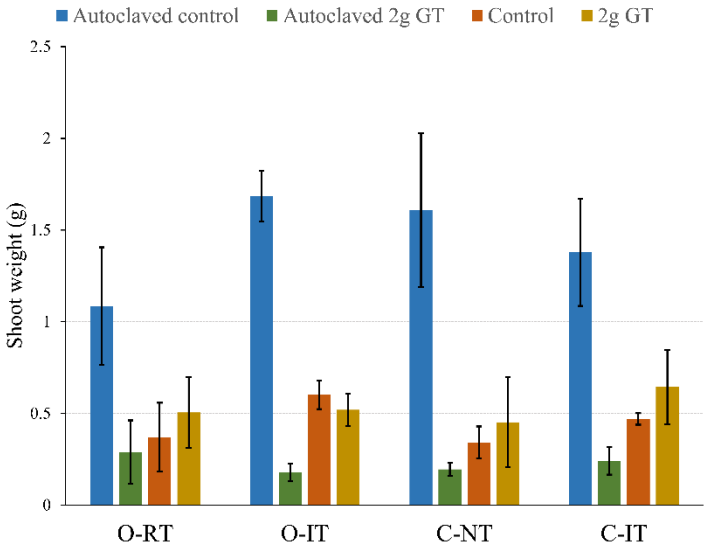

Figure S4 | Virulence of *Gaeumannomyces tritici* inoculum used for greenhouse assays. Autoclaved (green bar) or natural (yellow bar) soil was infested with 2 g/pot of *G. tritici* strain I-17 inoculum, and planted with spring wheat var. "Rubli". The fresh shoot weight was measured after 21 days and compared to the fresh shoot weight of spring wheat plants grown in non-infested autoclaved (blue bar) or natural (orange bar) soil. In autoclaved soils, plants grown in pots inoculated with *G. tritici* had a markedly reduced shoot weight compared to plants from autoclaved control pots. In natural soils, the shoot weight was not reduced by *G. tritici* inoculation. Cropping systems: «C-NT» is conventional without tillage, «C-IT» is conventional with tillage, «O-RT» is organic with reduced tillage, «O-IT» is organic with tillage.

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