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

Journal Article

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Publication date:
2018-08

Permanent link:
https://doi.org/https://doi.org/10.3929/ethz-b-000283970

Originally published in:
FEMS Microbiology Ecology 94(8), https://doi.org/10.1093/femsec/fiy075

Funding acknowledgement:
- Sustaining and improving soil health with plant-beneficial bacteria ()

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

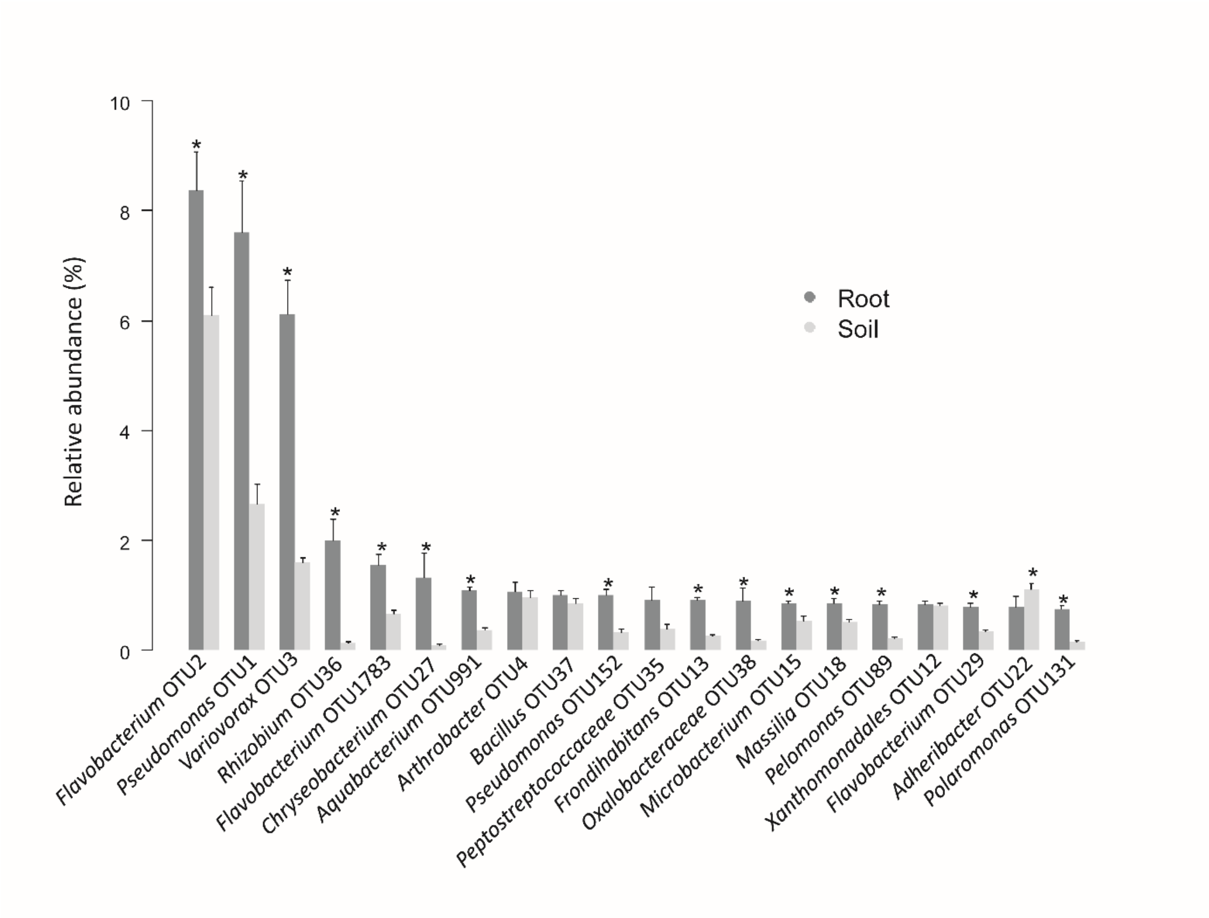

Figure S1 | The twenty most abundant bacterial operational taxonomic units (OTUs) detected on wheat roots and in bulk soil based on 16S rRNA V5-V7 region amplicon sequencing. Taxonomic assignments were determined with the SILVA database. The highest assigned taxonomic rank is shown. Sequencing was performed 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, $p<0.05$).

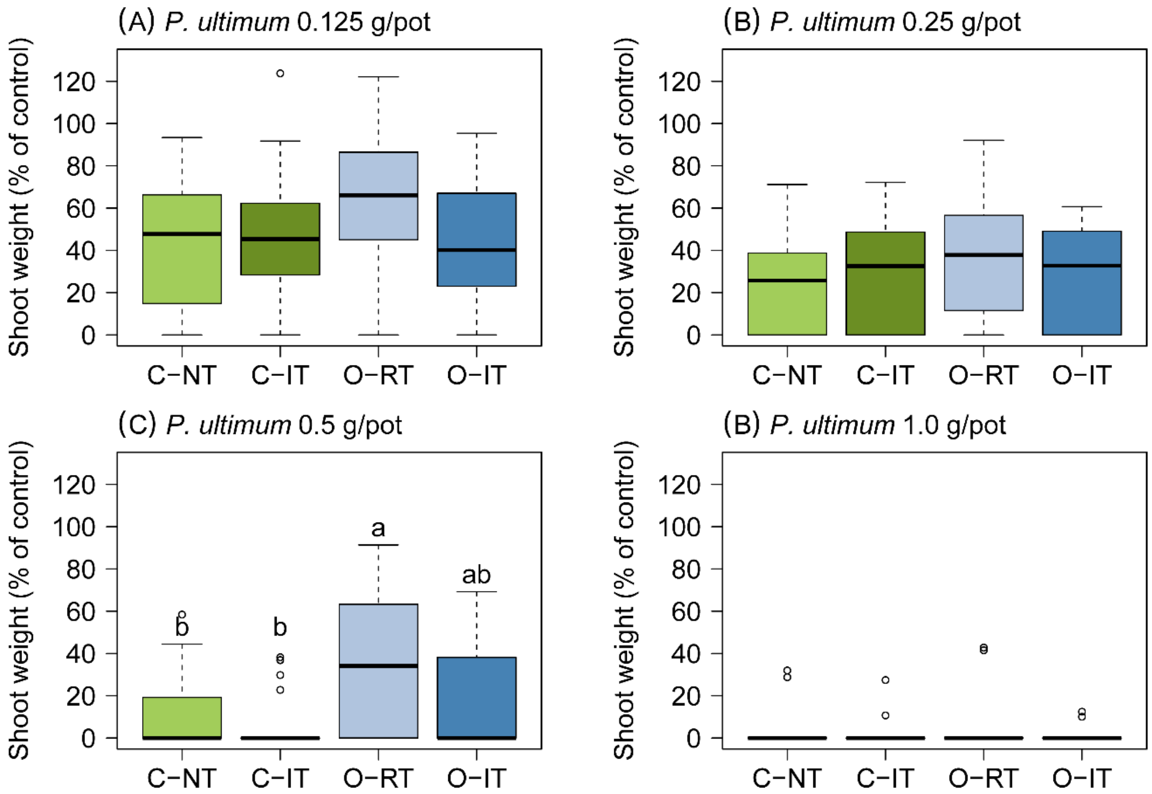

Figure S2 | Relative resistance of soils from different cropping systems to the soil-borne pathogen *Pythium ultimum* at different inoculum quantities. Increasing concentrations of pathogen inoculum were added to the soil before planting with cucumber seedlings. **(A)** 0.125 g/pot, **(B)** 0.25 g/pot, **(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.

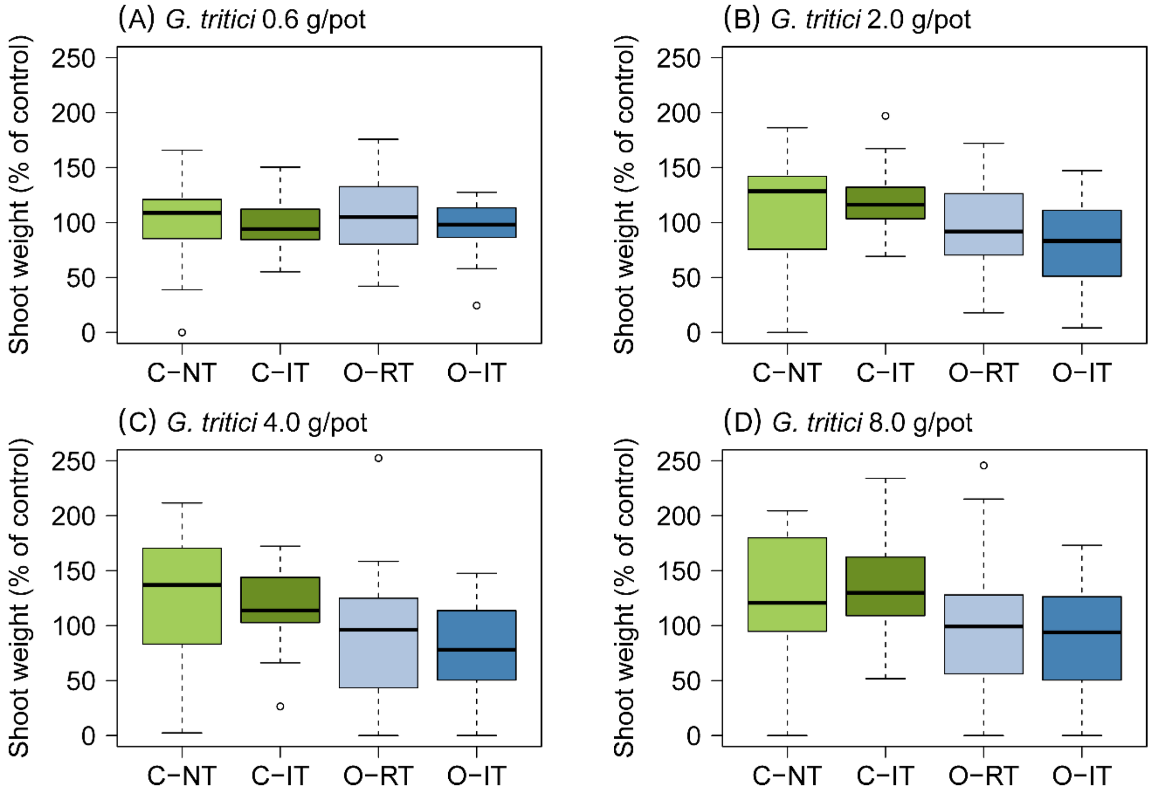

Figure S3 | Relative resistance of soils from different cropping systems to the soil-borne pathogen *Gaeumannomyces tritici* at different inoculum quantities. Increasing concentrations of pathogen inoculum were added to the soil before planting with spring wheat seedlings. **(A)** 0.6 g/pot, **(B)** 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.

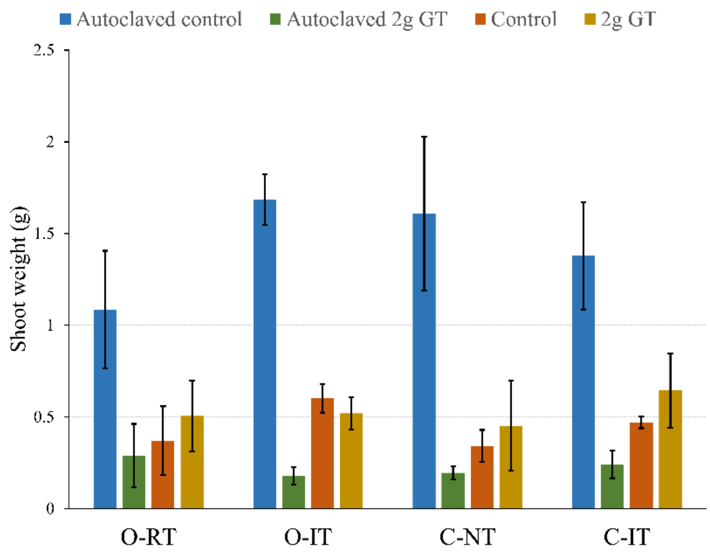

Figure S4 | Virulence of *Gaeumannomyces tritici* inoculum used for greenhouse assays. Autoclaved (green bar) or natural (yellow bar) soil was infested with 2 g/pot of *G. tritici* strain I-17 inoculum, and planted with spring wheat var. "Rubli". The fresh shoot weight was measured after 21 days and 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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