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Effect of organic management of soils on suppressiveness to *Gaeumannomyces graminis* var. *tritici* and its antagonist, *Pseudomonas fluorescens*

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Abstract

Organic management of soils is generally considered to reduce the incidence and severity of plant diseases caused by soil-borne pathogens. In this study, take-all severity on roots of barley and wheat, caused by *Gaeumannomyces graminis* var. *tritici*, was significantly lower in organically-managed than in conventionally-managed soils. This effect was more pronounced on roots of barley and wheat plants grown in a sandy soil compared to a loamy organically-managed soil. Fluorescent *Pseudomonas* spp. and in particular *phlD*$^+$ pseudomonads, key factors in the take-all decline phenomenon, were represented at lower population densities in organically-managed soils compared to conventionally-managed soils. Furthermore, organic management adversely affected the initial establishment of introduced *phlD*$^+$ *P. fluorescens* strain Pf32-*gfp*, but not its survival. In spite of its equal survival rate in organically- and conventionally-managed soils, the efficacy of biocontrol of take-all disease by introduced strain Pf32-*gfp* was significantly stronger in conventionally-managed soils than in organically-managed soils. Collectively, these results suggest that *phlD*$^+$ *Pseudomonas* spp. do not play a critical role in the take-all suppressiveness of the soils included in this study. Consequently, the role of more general mechanisms involved in take-all suppressiveness in the organically-managed soils was investigated. The higher microbial activity found in the organically-managed sandy soil combined with the significantly lower take-all severity suggest that microbial activity plays, at least in part, a role in the take-all suppressiveness in the organically-managed sandy soil. The significantly different bacterial composition, determined by DGGE analysis, in organically-managed sandy soils compared to the conventionally-managed sandy soils, point to a possible additional role of specific bacterial genera that limit the growth or activity of the take-all pathogen.

Abbreviations: 2,4-DAPG – 2,4-diacetylphloroglucinol; CFU – colony forming units; Ggt – Gaeumannomyces graminis var. tritici; Pf32-gfp – Pseudomonas fluorescens strain (Pf32) gfp tagged

Introduction

Organic farming is regarded as a more sustainable way of food production, causing less environmental side effects than the conventional ways of farming (Mäder et al., 2002; Fravel, 1999). Soils that are organically-managed are considered to be less conducive to root diseases (van Bruggen and Termorshuizen, 2003). Several studies have shown a lower incidence or severity of soil-borne diseases in organically than in conventionally-managed soils (reviewed by van Bruggen, 1995; van Bruggen

and Termorshuizen, 2003). For example, corky root (caused by *Pyrenochaeta lycopersici*) was less severe on roots of organically grown tomatoes (Workneh and van Bruggen, 1994), and also root rot of organically grown grapes, caused by various fungi, was suppressed (Lotter et al., 1999).

Take-all is an important root pathogen of several cereals worldwide and is caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*). Take-all severity can be reduced by microbial antagonism (Gerlagh, 1968; reviewed by Weller et al., 2002) which can be maintained or increased by organic matter management, including the incorporation of animal and green manures or shallow soil tillage (reviewed by Whipps, 1997). High levels of organic matter not only increase the activity but also the diversity of the resident microbial community in soil (Gunapala and Scow, 1998; Mäder et al., 2002). Organic farmers rely heavily on organic matter management to control root diseases. In addition to increasing the general suppressiveness of soils, disease suppression by specific microbial genera can also be an important constituent of the natural suppressiveness of soils (Weller et al., 2002). For example, recent studies have shown that fluorescent *Pseudomonas* spp., and in particular those that produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG), play a key role in soils that are naturally suppressive to take-all disease of wheat (Raaijmakers and Weller, 1998; Weller et al., 2002). Antibiotic-producing strains of *P. fluorescens* isolated from take-all suppressive soils have been introduced onto wheat seeds for biological control of *G. graminis*, but disease suppression has not been consistent so far and depends on many biotic and abiotic factors (Weller, 1988; Larkin and Fravel, 2002). Soil management practices may indeed affect the performance of introduced antagonists. Organic management strategies are intended to create a more diverse and stable microbial community, which may be highly competitive and therefore be relatively resistant to inundative applications of specific antagonistic microorganisms. Consequently, establishment and biocontrol efficacy of an introduced antagonist might be more difficult in organically-managed soils than in conventionally-managed soils.

Although it was previously demonstrated that root diseases, including take-all of cereal crops, are generally less severe in organically- than in conventionally-managed soils (reviews by van Bruggen and Termorshuizen, 2003; Whipps, 1997), the mechanisms underlying disease suppression in organically-managed soils have not been investigated in detail so far. The objectives of this study were: (1) to investigate the effect of organic management of soils on suppression of take-all severity on barley, wheat and triticale; (2) to determine the role of fluorescent *Pseudomonas* spp. and in particular 2,4-DAPG-producing *Pseudomonas* spp. in take-all suppressiveness in these soils; and (3) to assess differences in bacterial diversity and microbial respiration between organically- and conventionally-managed soils and to relate possible differences to take-all disease severity.

Materials and Methods

Overview of experiments

Four main experiments were conducted to investigate the effect of organic and conventional management on the suppressiveness against *Ggt* and on the population dynamics of its antagonist *Pseudomonas fluorescens*. In the first experiment (Exp. 1), *Ggt* disease severity was determined on four different barley cultivars grown in two pairs of organically- and conventionally-managed neighbouring soils (OS, CS, OL and CL, Table 1). In the second experiment (Exp. 2), *Ggt* disease severity was measured on roots of triticale grown in a conventionally managed soil (CsL), an organically managed soil (OS) and a soil in transition from conventional to organic management (OtS, Table 1). In this second experiment, the rhizosphere population densities of naturally occurring 2,4-DAPG-producing Pseudomonads were assessed. In the third experiment (Exp. 3), the establishment and survival of introduced *phlD*$^+$ *P. fluorescens* strain Pf32-*gfp* was determined for three paired organically- and conventionally-managed neighbouring soils (OS, CS, OL, CL, OLL and CLL, Table 1). In the fourth experiment (Exp. 4), the efficacy of introduced *phlD*$^+$ *P. fluorescens* strain Pf32-*gfp* to control take-all disease of wheat was determined in two pairs of organically- and conventionally-managed neighbouring soils (OS, CS, OL and CL, Table 1).

All the organically managed soils were sampled on SKALL-accredited organic farms and a

Table 1. Description of soil samples collected from organic and conventional farms at various locations in the Netherlands

Experiment[a]	Code	Location	Management	Soil	Previous crop	Sampling Year	pH	N-NO_3 mg kg^{-1}	N-NH_4 mg kg^{-1}	N_{tot} mg kg^{-1}	C-org mg kg^{-1}	OM %	Clay %	Silt %	Sand %
Exp. 1	OS	Marknesse	Organic	Sand	Potatoes	2000	7.1	47.5	5.3	64.0	n.d.[b]	n.d.	3.2	33.3	63.5
	CS	Marknesse	Conventional	Sand	Potatoes	2000	7.2	21.1	3.3	27.2	n.d.	n.d.	3.2	32.4	64.5
	OL	Ens	Organic	Loam	Potatoes	2000	7.6	19.4	2.4	24.0	n.d.	n.d.	8.3	54.5	37.2
	CL	Ens	Conventional	Loam	Potatoes	2000	7.6	6.8	2.3	10.7	n.d.	n.d.	7.7	51.7	40.6
Exp. 2	CsL[c]	Woensdrecht	Conventional	Silty Loam	Triticale	1998	7.6	3.2	5.3	n.d.	23002	3.6	6.1	59.5	34.4
	OS	Marknesse	Organic	Sand	Triticale	2001	7.3	2.4	5.9	n.d.	21674	3.0	2.1	21.9	76.0
	O_tS[d]	Wageningen	Organic in transition	Sand	Triticale	2001	5.4	1.2	6.6	n.d.	26346	4.9	2.1	11.9	86.0
Exp. 3 and 4	OS	Marknesse	Organic	Sand	Potatoes	2002	6.7	10.7	5.2	23.6	24236	n.d.	3.2	33.3	63.5
	CS	Marknesse	Conventional	Sand	Potatoes	2002	6.9	22.1	3.5	29.7	14314	n.d.	3.2	32.4	64.5
	OL	Ens	Organic	Loam	Onions	2002	7.3	22.4	2.3	28.3	16707	n.d.	8.3	54.5	37.2
	CL	Ens	Conventional	Loam	Onions	2002	7.4	22.5	2.6	33.8	16640	n.d.	7.7	51.9	40.4
	OLL[e]	Langeweg	Organic	Loam	Onions	2002	7.4	20.0	2.6	28.0	17646	n.d.	11.4	43.8	44.8
	CLL[e]	Langeweg	Conventional	Loam	Onions	2002	7.4	39.6	2.6	45.0	17463	n.d.	9.3	39.5	51.2

[a]Exp. 1 = barley, exp. 2 = wheat, exp. 3 = triticale, exp. 4 = survival experiment.
[b]N.d. = not determined.
[c]Soil continuously cropped with wheat for 14 years (de Souza and Raaijmakers, 2003).
[d]Soil cropped to triticale for the second year.
[e]Only used in the Pf32-*gfp* survival experiment.

description of the management practices at both the organically- and conventionally-managed farms is given in Table 2. Soil samples (10 kg per soil) were collected with a spade (about 15 cm deep). Soil samples were stored on ice immediately after sampling and stored at 4 °C. The soils were used within 1 month after sampling. Soils sampled for the triticale experiment were sampled and air-dried and stored until further use in plastic bags.

Soil analyses

Air-dried soil samples (2 g, sieved over a 2 mm sieve) were added to 100 ml 0.01 M $CaCl_2$ and shaken for 2 h. Nitrate and ammonium concentrations (Table 1) were determined with an Auto-analyzer II manifold (Technicon TM) according to Houba and Novozamsky (1998). The pH was determined with an Ino-lab pH-level-1 (WTW, Weilheim, Austria/Germany) in the same solution. Total nitrogen and carbon content was measured as a percentage of dry matter (Nieuwenhuizen et al., 1994) using an EA 1110 Element analyzer (CE instruments, Milan, Italy). The organic matter content was determined by the glow loss method according to Ball (1964). Soil texture (Table 1) was determined by the Department of Soil Quality, Wageningen University, (Wageningen, The Netherlands). The soils used in the experiments differed in texture, but most had relatively low clay contents (Table 1). The N content was higher in the conventionally-managed than in the organically managed soils used for the wheat experiment, but the reverse was true for the barley experiment (mostly due to recent incorporation of grass-clover in one organically-managed soil).

Take-all experiments

In the barley assay, infested oat kernels inoculum (0.5% v/v) of *Gaeumannomyces graminis* var. *tritici* (*Ggt*; Isolate R3-111-a-1) (Raaijmakers and Weller, 1998) were placed 2 cm below the soil surface and covered with a layer of non-infested soil. Autoclaved inoculum served as control. Two surface-sterilized barley seeds were sown per pot (*Hordeum aestivum*). The modern cultivars were Barke and Reggae, and the old cultivars were Goudgerst and Kenya. Old cultivars were developed (around 1920) under conditions of organic management, before the use of pesticides and artificial fertilizers became common practice. Modern cultivars were developed under conditions of conventional management (1990 or later). Old cultivars were hypothesized to perform better in the organically-managed soils while the modern cultivars perform better in the conventionally-managed soils. Seeds were provided by the Centre for Genetic Resources, the Netherlands (CGN) (Wageningen, The Netherlands).

In the wheat and triticale experiments, soils were amended with ground 0.5% w/w oats inoculum (particle size 200–500 μm). Pots were filled with 150 g (dw) infested (viable or autoclaved inoculum) soil and this was covered with 25 g original field soil. Ten surface-sterilized (treated with 1% (v/v) NaOCl for 1 min and rinsed for 5 min in running tap water) and pre-germinated seeds of wheat (*Triticum aestivum* cv. Vivant, Plant Breeding Int, Cambridge Ltd., Trumpington, Great Britain) or triticale (*X Triticosecale* cv. Galtjo, Svalöf–Weibull B.V., Emmeloord, The Netherlands) were sown, and covered with another 25 g of original, non-infested soil (in total 50 g non-infested soil). Pots were placed in the greenhouse in a completely randomized block design with ten (barley) or five (wheat and triticale) replications. Plants were watered when necessary. Take-all disease severity was scored after four weeks on a 0–8 scale (0 = healthy, 8 = dead; Thomashow and Weller, 1988). Plant growth was measured at harvest by measuring plant weight (wheat) or shoot height (barley). Root parts with lesions were checked for *Ggt* colonization on a semi-selective and diagnostic medium for *Ggt* (Duffy and Weller, 1994).

Fluorescent pseudomonads

Population densities of native fluorescent pseudomonads were determined in triticale rhizospheres (Cs-, OtS- and OS-soil) after four weeks of plant growth in non-infested soil and, in the same soil, four weeks after infestation and resowing. For each soil, five replicate samples of 1.0 g of roots with adhering soil were suspended in 5 ml sterile distilled water (SDW), vortexed vigorously (1 min), sonicated (1 min) in an ultrasonic cleaner (Bransonic 12, Branson Ultrasonics Corp., Geneva, Switzerland), serially diluted and plated three-fold on King's medium B amended with cycloheximide (100 mg l^{-1}), ampicillin (40 mg l^{-1},

Table 2. Description of the management practices on the organic and conventional farms

Experiment[a]	Code	Management[b]	Organic since	Soil type	Sampling year	Crop	Sampling year −1	Sampling year −2	Fertilizer[c]	Amount[d] applied	Use of cover crops[e]	Weed management[f]	Crop protection[g]
Exp. 1	OS	Organic	1987	Sand	2000	Potatoes	Grass-clover	Grass-clover	M and S	40[h]	yes	M	n.a.
	CS	Conventional	–	Sand	2000	Potatoes	Tulip	Wheat	S and C	25	yes	M and C	C
	OL	Organic	1989	Loam	2000	Potatoes	–	–	M	20	yes	M	n.a.
	CL	Conventional	–	Loam	2000	Potatoes	Sugarbeet	Barley	S and C	25	yes	M and C	C
Exp. 2	CsL	Conventional	–	Silty Loam	1998	Triticale[i]	Wheat	Wheat	n.k.[j]	n.k.	n.k.	n.k.	n.k.
	OS	Organic	1987	Sand	2001	Triticale[i]	Carrots	n.k.	M and S	50[k]	yes	M	n.a.
	O_tS	Organic in transition	2000	Sand	2001	Triticale[i]	Barley	Fallow	M	30	no	M	n.a.
Exp. 3 and 4	OS	Organic	1987	Sand	2002	Potatoes	Cale/lettuce	Carrots	M and S	20 + 50[l]	yes	M	n.a
	CS	Conventional	–	Sand	2002	Potatoes	Tulip	Wheat	S and C	25	yes	M and C	C
	OL	Organic	1989	Loam	2002	Onions	Oats-white clover	Carrots	M	20	yes	M	n.a.
	CL	Conventional	–	Loam	2002	Onions	Potatoes	Sugarbeet	S and C	25	yes	M and C	C
	OLL	Organic	1995	Loam	2002	Onions	Spinach/oats	Herbs	n.k.	n.k.	no	n.k.	n.a.
	CLL	Conventional	–	Loam	2002	Onions	Potatoes	Chicory	M and C	40	no	M and C	C

[a]Exp. 1 = barley, Exp. 2 = triticale, Exp. 3 = survival experiment, Exp. 4 = wheat.
[b]Farms practicing organic management are all SKALL-accredited and are not allowed to use chemical fertilizer, pesticides or genetically modified organisms.
[c]M – manure, S – slurry and C – chemical fertilizer.
[d]Applied organic fertilizer in ton ha^{-1}, not all amounts were applied in the year of sampling, but are depending on the crop and crop-rotation.
[e]Cover crops were not necessarily grown in the year of sampling.
[f]M – mechanical weed eradication, C – chemical weed eradication.
[g]C – chemical crop protection, n.a. – not applied.
[h]Total of 20 ton deep litter house manure and 20 ton humus earth were applied.
[i]Triticale was grown for 4 weeks to revitalize the microbial community in the dried soils.
[j]Not known.
[k]Humus earth.
[l]Ton ha^{-1} organic manure and 50 ton ha^{-1} humus earth.

Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and chloramphenicol (13 mg l^{-1}, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) (Simon and Ridge, 1974). After 48 h of incubation at 25 °C, the number of colony forming units (CFU) was counted. Plates were stored at 4°C until they were assayed for the presence of *phlD*, a key gene involved in the biosynthesis of 2,4-DAPG (Raaijmakers and Weller, 1998). The frequency of indigenous 2,4-DAPG-producing *Pseudomonas* spp. was determined by colony hybridization followed by PCR using *phlD*-specific probe and primers (Raaijmakers et al., 1997).

Pseudomonas fluorescens *(Pf32*-gfp*)*

Pseudomonas fluorescens strain 32 was isolated from roots of wheat growing in a Dutch take-all suppressive soil where wheat had been grown in monoculture for 27 years (Souza et al., 2003). This strain effectively controls *Ggt* and produces 2,4-DAPG. A spontaneous rifampicin-resistant derivative of strain 32 was transformed (R. Sayler, 601 SCEN Department of Biological Sciences, University of Arkansas, Fayetteville, USA) with plasmid pVSP61TIR by triparental mating (Koch et al., 2001). The plasmid was kindly provided by Dr. S. Lindow (UC Berkeley, USA). This plasmid harbours a constitutively expressed *gfp*-construct and was stably maintained in *P. fluorescens* strain 32, from now on referred to as Pf32-*gfp*. The expression of the green fluorescent protein was confirmed by epi-fluorescence microscopy, and strain integrity was verified by comparing the rep-PCR DNA fingerprint of the wild type strain of *P. fluorescens* 32 to that of the transformed strain Pf32-*gfp*. Pf32-*gfp* was cultured at 25 °C on Pseudomonas agar No 3. (Difco laboratories, Detroit, USA) supplemented with 50 mg l^{-1} kanamycin (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and 50 mg l^{-1} rifampicin (Duchefa, Haarlem, The Netherlands), from now on referred to as $PA3^+$. The transformed strain was stored at −80 °C.

Survival of P. fluorescens *Pf32*-gfp *in organically- and conventionally-managed soils*

Cell suspensions of two-day-old Pf32-*gfp* cultures were prepared in SDW (pH 7) and adjusted to a final concentration of 5×10^8 cells ml^{-1}; 0.5 ml of the suspension was sprayed onto 20 g of soil from each of three pairs of organically- and conventionally-managed farms and mixed in 50 ml screw cap tubes (Greiner Bio-one, Kremsmünster, Austria) to a final concentration of approximately 10^7 cells g^{-1} dry soil. Each soil and management type was replicated 3 times for each sampling date resulting in a total of 126 tubes. Tubes were covered loosely with the screw caps and incubated in the dark at 20 °C. Tubes were opened daily for several seconds to maintain atmospheric gas levels. Initial soil moisture content was 17.6% as percentage of fresh weight. Soil samples were taken from three pairs of tubes, before amending, immediately after mixing, and 1, 3, 5, 10 and 17 days after the start of the experiment. To determine Pf32-*gfp* densities, a dilution series of soil samples (1 g) was prepared as described above. The suspension was serially diluted and 50 μl aliquots were plated onto $PA3^+$ for enumeration. Fluorescent bacterial colonies were counted after incubation at 25 °C for 48 h under a UV lamp (365 nm UV-A, PL-S, Philips, Eindhoven, The Netherlands). Colony-forming units (CFU) were calculated per gram of dry soil; non-fluorescing colonies (about 5%) were subtracted from the total colony counts.

Dilutions 10^{-1} and 10^{-2} were used for direct microscopic counts of Pf32-*gfp* in both conventionally- and organically-managed soils. A 10 μl suspension was prepared for each microscopic observation and 100 fields per slide were checked under an epi-fluorescent microscope ('Axioscop', Zeiss, Jena, Germany). Blue UV light (450–490 nm) was used to visualize *gfp* (Miller and Lindow, 1997). Background fluorescence was checked in suspensions from control tubes without Pf32-*gfp*.

Take-all suppression by Pf32-gfp *in organically- and conventionally-managed soils*

Ggt-infested soil and pots were prepared as described above. Part of the *Ggt*-infested soil was mixed with a cell suspension of *P. fluorescens* Pf32-*gfp* as described previously, with a final density of approximately 10^8 cells g^{-1} dry soil. There were seven pots infested with *Ggt* and amended with Pf32-*gfp* for each soil and management type, seven pots were amended with Pf32-*gfp* alone, five pots infested with *Ggt* alone and five control pots (not infested with *Ggt* nor amended with Pf32-*gfp*).

Wheat seeds were planted and handled as described before, and take-all disease severity was scored after 28 days as described for the previous experiments. Pf32-*gfp* densities were determined immediately and 10 days after introduction (two pots per treatment only) both in bulk soil and rhizosphere soil.

PCR and DGGE

PCR and DGGE were performed on sub-samples of soil taken at day zero and day ten of the Pf32-*gfp* survival experiment (CL, OL, CS and OS soils only). Soil samples to be used for PCR were first stored at −20 °C. DNA was extracted using the FastDNA SPIN Kit for soils (Bio101 systems, USA) according to manufacturer's protocol, except for the elution step, which was extended for 20 min at 65 °C. DNA quality and quantity was tested on a 1.2% agarose gel and samples were diluted to a final concentration of approximately 1 ng μl^{-1} prior to PCR. PCR amplification was performed as described by Rosado et al. (1998) with some small adjustments, using the U968 (40-mer GC-clamp) and L1401 universal eu-bacterial primers (Heuer and Smalla, 1997). Each reaction (49 μl) consisted of: 32.46 μl MilliQ water; 5 μl 10 × Stoffelbuffer (Applied Biosystems, Foster City, CA); 7.5 μl 25 mM $MgCl_2$; 1 μl dNTP mix (10 mM of each dNTP, Roche Diagnostics GmbH, Mannheim, Germany); 1 μl (10 μM) U968; 1 μl (10 μM) L1401; 0.5 μl 100% formamide (Sigma-Aldrich); 0.04 μl T4 gene 32 protein (5 $\mu g\ \mu l^{-1}$, USB Corporation, Cleveland, Ohio, USA); and 0.5 μl AmpliTaq Stoffelfragment (10 U μl^{-1}, Applied Biosystems, Foster City, CA). The master-mix was irradiated with UV light during 3 min. Then, 49 μl mastermix and 1 μl of DNA-extract (1 ng μl^{-1}) were mixed together and the DNA was amplified in a PTC-200 PCR machine (MJ research DNA Engine Gradient cycler, BIOzym, Landgraaf, The Netherlands). The initial denaturation step was performed at 94 °C for 3 min. Strand separation was carried out at 94 °C for 1 min every cycle. The annealing temperature was initially set at 60 °C and then decreased by 1 °C every second cycle until it reached 55 °C (11 cycles). Then 19 additional cycles were carried out with the annealing temperature of 55 °C (1 min) and extension at 72 °C (2 min). The final extension step was 10 min at 72 °C, after which the reaction mixtures were cooled to 4 °C. DNA quality and quantity were checked on 1.2% (w/v) MP agarose gel in 0.5 × TBE and stained with ethidium bromide. Amplification products were stored at −20 °C until further handling.

DGGE analysis was carried out using the DCode universal mutation detection system (Bio-rad Laboratories, Hercules, CA) according to the manufacturer's protocol. Polyacrylamide gels (6%, 37.5:1 Acrylamide: bisacrylamide) with a vertical denaturing gradient from 45% to 60% (100% denaturant defined as 7 M urea plus 40% formamide) and an 8% stack were used to run PCR-products (0.5 μg DNA per sample) for 16 h at 100 V in 60 °C 0.5 × TAE-buffer. Gels were silver stained according to the manufacturer's protocol (Bio-rad Laboratories, Hercules, CA). Gels were analyzed with Phoretix 1D Advanced version 4.00 (Non Linear Dynamics Ltd, Newcastle upon Tyne, UK). Background was subtracted using the rolling disc method (radius 40 pixels). Minimum peak height (after background subtraction) was set at two pixels and peak width was fixed at five pixels. Diversity indices were calculated using the relative band intensity (peak height × fixed width) according to the following equation $H' = -P_i \log P_i$, where $P_i = n_i/N$ and n_i = the peak intensity and N the sum of all peak intensities in a lane profile (Eichner et al., 1999). For the samples obtained at day 0 and day 10 of the survival experiment, similarity between lanes was calculated using the method of Nei and Li (1979) in the Treecon programme (Van de Peer and de Wachter, 1994). Dendrograms were constructed using UPGMA. Bootstrap values were based on 1000 replicates.

Microbial respiration

Microbial respiration was determined in soils used for the wheat-*Ggt* experiment (CL, OL, CS and OS soils only). For each soil type microbial respiration was measured in duplicate. Microbial respiration was measured according to the protocol described by Heinemeyer et al. (1989) for an infrared gas analyzer. For each soil type, 50 g of moist soil was placed in closed horizontal tubes with a volume of 250 ml. The tubes were connected to a continuous flow system where moisture saturated air was blown over the soil. Released CO_2 was measured using an infrared gas analyzer (ADC 7000 gas

analyzer, Hoddesdon England). The amount of CO_2 released was calculated by subtracting CO_2 concentration of the tubes filled with soil from an unfilled control tube.

Statistical analyses

Statistical analysis was performed using the SAS system for Windows (SAS institute Inc, Cary, NC, USA). Disease severity scores were ranked and analyzed using the ANOVA F procedure (Proc Mixed procedure) as described by Shah and Madden (2004). The necessary macros were obtained from the website of the University of Gottingen, Germany (http://www.ams.med.uni-goettingen.de/de/sof/ld/makros.html). Contrast analyses were carried out to compare organically- versus conventionally managed soils and loamy soils versus sandy soils; when appropriate, individual treatments were also compared with contrast analyses. To analyze the effect of introduction of Pf32-*gfp* on *Ggt* severity, two methods were used. First a model including the introduction effect (Proc Mixed), secondly a model where the dependent variable was the percentage disease reduction and the weight increase (Proc GLM). The disease reduction was calculated as: [log (1 – (the disease on roots from soils with *Ggt* + Pf32-*gfp* divided by the disease rating on roots from soils infested with *Ggt* alone))]. Plant weight increase was calculated in a similar way. Plant growth parameters and *P. fluorescens* numbers in the biocontrol experiment with *Ggt* were analyzed with the Proc GLM procedure (ANOVA) and where necessary contrast analyses were performed.

To describe survival of Pf32-*gfp* in soil, an exponential decay model was used, $C_t = a + (m - a) \times e^{(-b \times t)}$, where C_t = CFU g^{-1} dry soil for plate or direct counting, a = asymptote (CFU g^{-1} dry soil or cells g^{-1} dry soil), m = number of introduced Pf32-*gfp* cells g^{-1} dry soil, b = decrease rate ($days^{-1}$) and t = time (days). Parameters for the survival curves (six curves in total) of Pf32-*gfp* in the different soils were estimated with the NLIN procedure and the parameters were analyzed with MANOVA.

Shannon-Weaver indices calculated from DGGE-community profiles obtained from the survival experiment were analyzed with Proc Mixed (Schabenberger and Pierce, 2002) for repeated measurements to determine the effects of introduction on the microbial communities in time.

Results

Take-all suppression in organically- versus conventionally-managed soils

In soils not infested with *Ggt*, disease severity caused by resident populations of the take-all pathogen was low to insignificant, ranging from a disease rating of 0 (no disease) in the experiments with wheat to a rating of 2 (a few lesions on one or two seminal roots) in the experiments with barley and triticale (data not shown). In soils artificially infested with the take-all pathogen (0.5% (w/w) *Ggt*-inoculum), substantial levels of take-all severity were observed for barley, wheat and triticale (Figure 1). Disease severity was significantly ($P < 0.0001$) lower on roots of barley (exp. 1, Table 3) grown in organically- than in conventionally-managed soils (Figure 1). This effect was more pronounced on roots of barley plants grown in the sandy than in loamy soil (Figure 1). In the experiments with barley, four cultivars were tested representing two old and two modern barley cultivars. For each of the individual soils tested, take-all severity was similar for the four cultivars (data not shown). For each of the four cultivars, take-all severity was significantly lower when grown in organically-managed soils than in conventionally-managed soils. With respect to disease severity, there was no interaction between cultivar characteristics (old versus modern) and organic or conventional management (data not shown). In the barley experiment, there was a significant correlation between disease severity and plant growth parameters. The average shoot height of barley plants grown in organically-managed soils was 32.6 cm and was significantly higher than shoot height of plants grown in conventionally-managed soils (24.9 cm, $P < 0.0001$). Also barley plants grown in the organically-managed loamy soil were taller (27.6 cm) than those grown in the conventionally-managed loamy soil (22.8 cm).

In the experiment with wheat, there was a soil type × management interaction ($P = 0.0133$, Table 4a) and a treatment × management interaction ($P = 0.0755$) with respect to disease severity

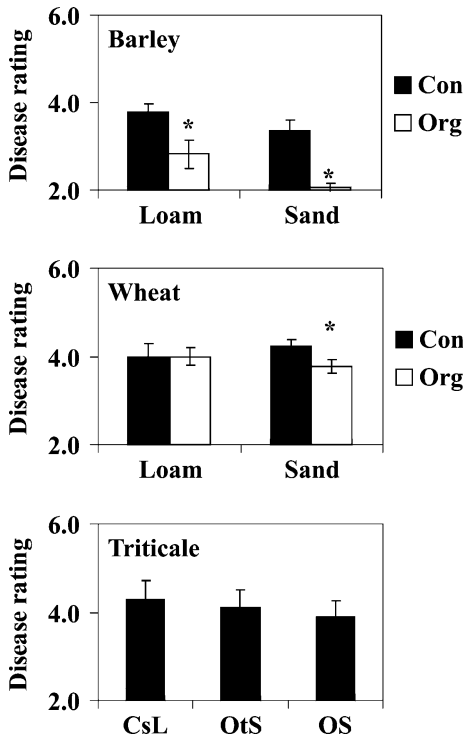

Figure 1. Mean severity of take-all disease on barley (Exp. 1) and wheat (Exp. 3) in two pairs of soil infested with *G. graminis* var. *tritici* (0.5% oat inoculum w/w). Triticale (Exp. 2) was grown in three soils. (CsL = conventional loam, OtS = conventional sandy soil in transition to organic, OS = organic sandy soil). An asterisk indicates a statistically significant difference at $P = 0.05$. Error bars indicate the standard deviation of the mean.

(Figure 1). In loamy soils, take-all severity was not different between organic and conventional management (average disease rating was 4.0 for both). However, disease severity was significantly lower ($P = 0.0208$, Table 4a) on roots of wheat grown in the organically-managed sandy soil (average disease rating 3.8) than in the conventionally-managed sandy soil (average disease rating 4.2). Although the reduction of take-all severity of wheat grown in organically-managed sandy soil was relatively low, plant fresh weight showed a significant treatment × management × soil type interaction ($P = 0.0077$). Plant fresh weight in soils infested with *Ggt*-alone was higher in the organically-managed sandy soil (average 1.1 g) than in the other three soils (plant weight in conventionally-managed sandy soil was 0.89 g ($P = 0.0031$), in conventionally-managed loamy soil 0.88 ($P = 0.0022$), and in organically-managed loamy soil 0.94 g ($P = 0.0147$)).

In the experiment with triticale, take-all severity did not differ significantly among the three soil types tested (Figure 1). In this latter experiment, no significant differences in shoot height of triticale were observed between the soils tested (CsL: 23.2 cm, OtS: 22.6 cm, OS: 21.7 cm).

Native fluorescent Pseudomonas *populations in organically- versus conventionally-managed soils*

Rhizosphere populations of fluorescent *Pseudomonas* spp. were monitored on roots of triticale grown in three soils with different management types (CsL-, OtS- and OS-soil). In soils not amended with the take-all pathogen, average population densities of total fluorescent *Pseudomonas* spp. ranged from 7.4 (OtS) to 6.0 (OS) log

Table 3. Experiment 1 (barley), ANOVA-table of the disease severity ratings and shoot lengths of barley plants grown in organically and conventionally managed soils infested with 0.5% (v/v) *Ggt*

	Effect	Disease severity[a]				Shoot length[b]		
		Nom DF[c]	Den DF	*F*-value	Pr > *F*	DF	*F*-value	Pr > *F*
Disease severity 0.5 % *Ggt*	Block	9.00	67.4	7.02	< 0.0001	9	1.91	0.0562
	Cultivar	2.94	115.0	1.40	0.2463	3	7.65	< .0001
	Soil type	1.00	115.0	10.47	0.0004	1	51.27	< .0001
	Cultivar × Soil type	2.94	115.0	0.26	0.7961	3	1.89	0.1340
	Management	1.00	115.0	40.89	< 0.0001	1	108.49	< .0001
	Cultivar × Management	2.94	115.0	0.42	0.6549	3	1.85	0.1417
	Soil type × Management	1.00	115.0	1.53	0.1641	1	0.80	0.3714
	Cultivar × Soil type × Management	2.94	115.0	0.29	0.7675	3	1.46	0.2278

[a]Anova's obtained with the ANOVAF-procedure and Mivque0-methode as described by Shah and Madden (2004).
[b]Anova's obtained using GLM.
[c]Corrected degrees of freedom (Shah and Madden, 2004).

Table 4. Experiment 2 (wheat), ANOVA-tables of experiment 2: A. The Disease severity on roots and plant weights in soils infested with or without 0.5% *Ggt* and with or without Pf32-*gfp* (complete experiment). B. Disease severity on roots in soils infested with 0.5 % *Ggt* and with or without the *Pseudomonas* treatment introduced. C. Analysis of the difference between the treatments *Ggt* and *Ggt*(Pf32-*gfp*), expressed as (*Ggt* /*Ggt*(Pf32-*gfp*)) * 100%

	Effect	Disease severity[a]				Plant weight[b]		
		Nom DF[c]	Den DF	*F*-value	Pr > *F*	DF	*F*-value	Pr > *F*
a ANOVA	Block	4.00	53.00	0.04	0.9971	4	1.89	0.1247
	Treatment[d]	1.86	18.10	404.01	< .0001	3	110.05	< .0001
	Soil type	1.00	18.10	14.37	0.0013	1	213.65	< .0001
	Treatment × Soil type	1.86	18.10	14.73	0.0002	3	29.47	< .0001
	Management	1.00	18.10	2.19	0.1562	1	0.09	0.7662
	Treatment × Management	1.86	18.10	3.05	0.0755	3	4.79	0.0050
	Soil type × Management	1.00	18.10	7.53	0.0133	1	0.28	0.6009
	Treatment × Soil type × Management	1.86	18.10	2.67	0.0993	3	4.40	0.0077
Contrasts	Sand *Ggt* vs Sand *Ggt* + Pf32-*gfp*	1	9.71	0.53	0.4850	1	68.47	< .0001
	Loam *Ggt* vs Loam *Ggt* + Pf32-*gfp*	1	7.87	50.59	0.0001	1	6.80	0.0118
	Organically man. sand *Ggt* vs conventionally man. sand *Ggt*	1	3.32	17.28	0.0208	1	9.62	0.0031
	Organically man. loam *Ggt* vs conventionally man. loam *Ggt*	1	7.27	0.00	0.9713	1	0.33	0.5683
	Organically man. sand *Ggt* + Pf32-*gfp* vs conventionally man. sand *Ggt* + Pf32-*gfp*	1	6.60	0.49	0.5086	1	11.90	0.0011
	Organically man. loam *Ggt* + Pf32-*gfp* vs conventionally man. loam Ggt + Pf32-*gfp*	1	13.00	27.66	0.0002	1	0.21	0.6525
	Organically man. sand *Ggt* vs organically man. sand *Ggt* + Pf32-*gfp*	1	4.01	0.32	0.6034	1	6.27	0.0153
	Organically man. loam *Ggt* vs organically man. loam Ggt + Pf32-*gfp*	1	2.87	20.12	0.0227	1	2.94	0.0923
	Conventionally man. sand *Ggt* vs conventionally man. sand *Ggt* + Pf32-*gfp*	1	6.78	2.77	0.1415	1	88.79	< .0001
	Conventionally man. loam *Ggt* vs conventionally man. loam Ggt + Pf32-*gfp*	1	5.51	30.62	0.0019	1	3.94	0.0522
b ANOVA	Block	4.00	0.14	21.80	0.6785	4	9.59	< .0001
	Pf32-*gfp*-introduction	1.00	10.70	25.75	0.0004	1	63.75	< .0001
	Soil type	1.00	10.70	14.39	0.0029	1	51.73	< .0001
	Pf32-*gfp*-introduction × Soil type	1.00	10.70	15.06	0.0025	1	17.66	0.0003
	Management	1.00	10.70	2.35	0.1555	1	0.25	0.6224
	Pf32-*gfp*-introduction × Management	1.00	10.70	3.36	0.0814	1	12.39	0.0016
	Soil type × Management	1.00	10.70	8.05	0.0185	1	0.38	0.5451
	Pf32-*gfp*-introductio × Soil type × Management	1.00	10.70	0.25	0.6152	1	11.20	0.0025
Contrasts	Organically man. *Ggt* vs Organically man. *Ggt* + Pf32-*gfp*	1.00	4.83	4.41	0.0916		9.39	0.0050
	Conventionally man. *Ggt* vs Conventionally man. *Ggt* + Pf32-*gfp*	1.00	5.83	26.05	0.0024		70.49	< .0001
	Sand *Ggt* vs sand *Ggt* + Pf32-*gfp*	1.00	5.64	0.47	0.5204		47.59	< .0001
	Loam *Ggt* vs loam *Ggt* + Pf32-*gfp*	1.00	6.18	55.08	0.0003		16.72	0.0004
	Conventionally man. clay vs organically man. Clay	1.00	5.64	1.07	0.3388		–	–
	Conventionally man. sand vs organically man. Sand	1.00	6.18	7.24	0.0382		–	–

c ANOVA[e]	Block	4.00	–	0.63	0.6549	4	0.52	0.7257
	Soil type	1.00	–	27.17	0.0004	1	67.43	< .0001
	Management	1.00	–	5.96	0.0347	1	23.49	0.0007
	Soil type × Management	1.00	–	0.06	0.8172	1	14.84	0.0032

[a]Anova's obtained with the ANOVAF-procedure and Mivque0-methode as described by Shah and Madden (2004).
[b]Anova's obtained using GLM.
[c]Corrected degrees of freedom to obtain the correct F-value (Shah and Madden, 2004).
[d]Treatments were: Control (soil infested with sterilized oats inoculum and no Pf32-*gfp* was added), *Ggt* (soils infested with 0.5% *Ggt* but no Pf32-*gfp* was added), Pf32-*gfp* (soil infested with sterilized oats inoculum and Pf32-*gfp* was added), Ggt(Pf32-*gfp*) (soils infested with 0.5% *Ggt* and Pf32-*gfp* was added).
[e]Analysis performed with Proc GLM, percentages were log transformed to obtain normality.

CFU g^{-1} root (Figure 2a1). In the OS-soil, densities of total fluorescent *Pseudomonas* spp. were significantly ($P < 0.0001$) lower than in the CsL- and OtS-soil. Also the number of 2,4-DAPG-producing *Pseudomonas* spp., assessed by colony hybridization followed by PCR with *phlD*-specific probe and primers, was significantly lower in the OS-soil compared to the CsL-soil ($P = 0.01$) and the OtS-soil ($P = 0.02$). In *Ggt*-infested soils, densities of fluorescent *Pseudomonas* spp. (Figure 2a2) were comparable to densities found in the non-infested soils. Densities of 2,4-DAPG-producers (Figure 2b2) were highest in the conventionally-managed soil (CsL) soil and lowest in the organically-managed soil (OS). Densities of 2,4-DAPG-producers in the CsL- and OtS-soils were significantly higher than the densities in the OS-soil ($P = 0.006$ and $P = 0.012$, respectively). No significant differences were observed in population densities of 2,4-DAPG producers between the CsL- and OtS-soils ($P = 0.64$). Collectively, these results suggest that fluorescent *Pseudomonas* spp. and in particular 2,4-DAPG producers were not represented at higher population densities in the organically-managed soil compared to the conventionally-managed soil. In fact, the results suggest that organic management adversely affects the population densities of 2,4-DAPG producing *Pseudomonas* spp.

Establishment and survival of P. fluorescens *in organically- versus conventionally-managed soils*

Establishment and survival of a *gfp*-tagged 2,4-DAPG-producing *P. fluorescens* strain 32, referred to as Pf32-*gfp*, was studied by introducing this strain in three pairs (organically-managed versus conventionally-managed) of neighbouring soils. The results showed small but consistent differences between management types (Figure 3). Based on direct microscopic counts, densities of Pf32-*gfp* declined significantly faster in the organically-managed than in the conventionally-managed soils (intercept: $P = 0.0338$, asymptote $P = 0.0170$, MANOVA a and b: $P = 0.0814$). Based on plate counts, the rate of decline of Pf32-*gfp* showed a similar trend as the direct microscopic counts but was not significantly different between organically- and conventionally-managed soils ($P = 0.57$, data not shown). After 10 days, the densities (based on

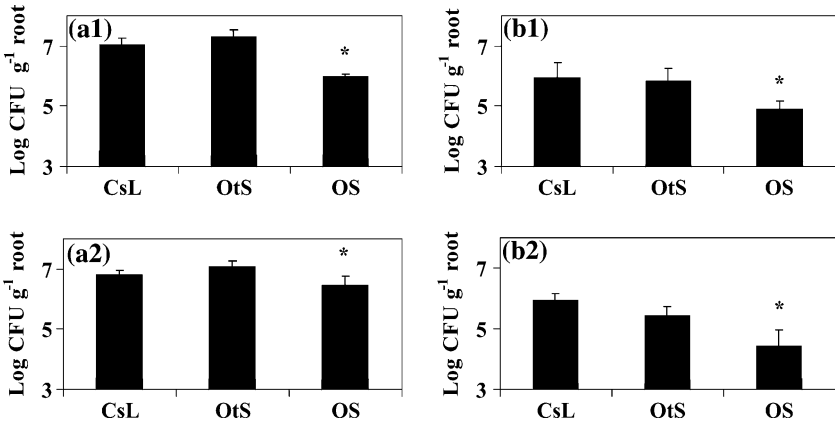

Figure 2. Mean population density (log CFU g^{-1} root) of fluorescent pseudomonads (a) and fluorescent pseudomonads harbouring the *phlD* gene (b) in triticale (Exp. 2) rhizosphere without the take-all pathogen *Gaeumannomyces graminis* var. *tritici* (2a1 and 2b1) and in soils, 4 weeks after addition of *Gaeumannomyces graminis* var. *tritici* (0.5% w/w oats inoculum) (2a2 and 2b2). CsL = conventionally-managed loamy soil, OtS = sandy soil in transition to organic management, OS = organically-managed sandy soil; error bars indicate standard deviations of the mean; an asterisk indicates a statistically significant difference at $P = 0.05$.

both plate and microscopic counts) of Pf32-*gfp* remained stable at approximately 5×10^6 CFU g^{-1} soil for both organically- and conventionally-managed soils. The final densities (measured by both plate and direct counts) were not significantly different between organically- and conventionally-managed soils.

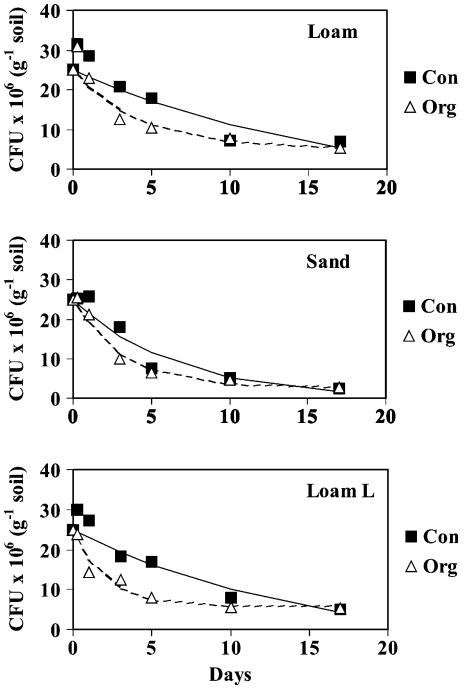

Figure 3. Survival of introduced *Pseudomonas fluorescens* strain Pf32-*gfp* (CFU g^{-1} dry soil, direct counts, Exp. 3) in paired organically- and conventionally-managed soils, CS and OS (Sand), CL and OL (Loam) and CLL and OLL (Loam L) from three locations (Marknesse, Ens and Langeweg) in The Netherlands. Lines are drawn based on the exponential decay equation used in the analysis ($C_t = a + (m - a) \times e^{(-b \times t)}$).

Activity of P. fluorescens *in organically- versus conventionally-managed soils*

In the biocontrol assays with wheat, Pf32-*gfp* was introduced into the two pairs of organically- and conventional soils (amended with 0.5% *Ggt*-inoculum) at an initial density of 10^8 CFU g^{-1} soil. After 10 days of incubation, densities of Pf32-*gfp* in both bulk soil and in the wheat rhizosphere had dropped to approximately 10^6 CFU g^{-1} for all soils tested (data not shown). Take-all severity on wheat roots showed a significant ($P = 0.0185$) soil × management interaction (Figure 4a, Table 4b). Disease severity was significantly lower ($P = 0.0032$) in conventionally- than in organically-managed loamy soil, while in sandy soil the difference was not significant ($P = 0.3388$). The soil type × Pf32-*gfp* interaction ($P = 0.0025$) showed that introduction of Pf32-*gfp* had significantly more effect against take-all of wheat in the loamy soil ($P = 0.0003$) than in the sandy soil ($P = 0.5204$). The management × Pf32-*gfp* interaction ($P = 0.0814$) indicates that introduction of the antagonist has less effect in the organically managed soil ($P = 0.0916$) than in the conventionally managed soil ($P = 0.0024$). This was supported by analysis of the relative disease reduction by Pf32-*gfp*, which was significantly

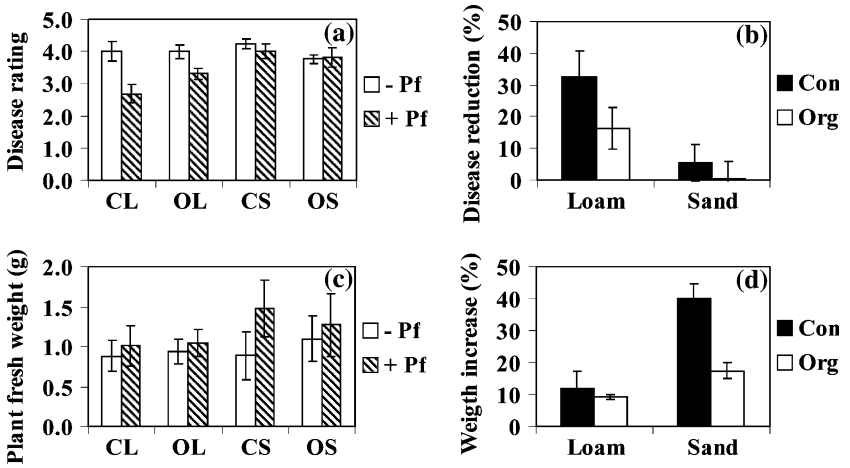

Figure 4. Effect of introduced *Pseudomonas fluorescens* (Pf32-*gfp*) on take-all severity on roots of wheat (Exp. 4) grown in soils from neighbouring organic and conventional farms artificially infested with *Gaeumannomyces graminis* var. *tritici* (0.5% w/w) (4a). Mean disease reduction (*Ggt*/*Ggt*(Pf)*100%) in soils from neighbouring organic and conventional farms (4b). Mean plant fresh weight of wheat plants grown in soils infested with *Gaeumannomyces graminis* var. *tritici* (0.5% w/w) and amended with or without Pf32-*gfp* (4c). Mean increase in plant fresh weight (4d, (100% − (*Ggt*/*Ggt*(Pf)*100%))). Error bars indicate standard deviations of the mean.

stronger ($P = 0.0347$) in conventionally-managed soils than in organically-managed soils (Figure 4b, Table 4c).

In the treatments with *Ggt*, but without the introduced strain Pf32-*gfp*, a higher disease severity resulted in significantly lower plant fresh weights (Pearson's $R = -0.39$, $P < 0.0001$). In presence of Pf32-*gfp*, however, the correlation was less clear (Pearson's $R = 0.14$, $P = 0.054$). This indicates that not only disease was influenced, but that plant growth was additionally stimulated by the introduction of Pf32-*gfp*.

Introduction of Pf32-*gfp* increased plant weight (CL, 26.2%; OL, 25.8%; CS, 45.0% and OS, 29.6%). A Pf32-*gfp* × soil type × management-interaction was significant ($P = 0.0025$, Table 4b). Plant weight was higher in all soils amended with Pf32-*gfp* and infested with *Ggt* than in soils infested with *Ggt* alone (Figure 4c, Table 4c), and the effect was more pronounced in the sandy soils (Figure 4d). Analysis of the percent increase in plant weight (Figure 4d) showed this as a significant soil type × management-interaction ($P = 0.0032$, Table 4c) in soils infested with 0.5 % (w/w) *Ggt*. Addition of Pf32-*gfp* (in the presence of *Ggt*) resulted in a 40% increase in plant weight of wheat grown in conventional sandy soil and 10–20% in the other soils. Introduction of Pf32-*gfp* in soils not infested with *Ggt* resulted in even significantly ($P < 0.0001$) higher plant weights compared to the non-amended controls, ranging from 2-fold in the conventionally-managed sandy soil to 1.6-fold in the loamy organically-managed soil (data not shown).

Collectively these results indicate that introduction of a 2,4-DAPG-producing *P. fluorescens* strain resulted in small to no reduction of take-all disease in organically-managed soils supporting the hypothesis that fluorescent *Pseudomonas* spp. and in particular 2,4-DAPG-producers do not contribute to take-all suppressiveness observed in organically-managed soil. The results therefore suggest that other disease suppressive mechanisms operate in organically-managed soils.

Microbial diversity and respiration in organically- versus conventionally-managed soils

Several other mechanisms have been proposed to contribute to disease suppressiveness of soils, including total microbial diversity and activity (Weller et al., 2002; Whipps, 1997). In the present study, bacterial diversity was determined on the basis of 16S-rDNA community profiles (Exp. 3) and expressed as the Shannon-Weaver indices (Figure 5). These indices ranged from 0.92

(conventional sand) to 1.37 (organic loam) just before introduction of Pf32-*gfp*. Ten days after introduction, the index ranged from 1.11 (conventional sand) to 1.40 (organic loam). Shannon-Weaver indices were higher for organically-managed soils ($P = 0.06$) than for conventionally-managed soils. Sandy soils had a lower bacterial diversity than the loamy soils ($P = 0.0003$). Introduction of Pf32-*gfp* did not result in a significant change in the Shannon-Weaver index as determined by DGGE. Dendrograms showed that similarity between soil samples was low (distances ranging from 0.42 to 0.65). For the loamy soils, there was no clear discrimination between organic and conventional management (Figure 6). In the sandy soils, however, a significant (bootstrap value 90%) difference in microbial communities was observed between organic and conventional management. In spite of the variation between the samples taken from the same soil, the DGGE profiles from the organically-managed sandy soil clustered together as did the profiles from the conventionally-managed sandy soil (Figure 6).

Both sandy and loamy conventional soils had similar microbial respiration rates (Exp. 4) of approximately 0.74 μg CO_2 g^{-1} soil h^{-1}. Microbial respiration in organically-managed loamy soil (0.85 μg CO_2 g^{-1} soil h^{-1}) was not significantly different from respiration measured in the sandy and loamy conventionally-managed soils. However, microbial respiration was more than twice as high (1.82 μg CO_2 g^{-1} soil h^{-1}) in the organically-managed sandy soil than in the three other soils. The higher microbial activity in the organically-managed sandy soil combined with the significantly lower take-all disease severity on roots of wheat grown in the organically-managed sandy soil (Figure 1), may suggest that microbial activity plays, at least in part, a role in the take-all suppressiveness observed in the organically-managed sandy soil.

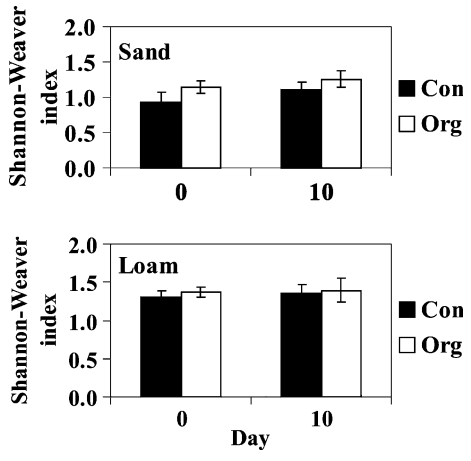

Figure 5. Mean Shannon-weaver indices obtained from 16SrDNA PCR-DGGE profiles of DNA extracted from loamy and sandy soils from neighbouring organic and conventional farms before (day 0) and 10 days after introduction of *Pseudomonas fluorescens* (Pf32-*gfp*) in soils (Exp. 3). Error bars represent standard deviations of the mean.

Discussion

Soil-borne pathogens are considered to be generally less problematic in organically- than in conventionally-managed soils (van Bruggen and Termorshuizen, 2003). Organic management is directed toward prevention of soil-borne diseases and improvement of soil health. High levels of organic matter, one of the main goals of organic soil management, increases faunal and microbial diversity (Mäder et al., 2002), microbial competition and antagonism (Reganold et al., 1993; Drinkwater et al., 1995; Carpenter-Boggs et al., 2000). These management practices can indeed result in less disease caused by soil-borne pathogens (van Bruggen and Termorshuizen, 2003). For example, corky root severity (caused by *Pyrenochaeta lycopersici*) was 37% lower in organically than in conventionally grown tomato plants (Workneh and van Bruggen, 1994). Likewise, Koch (1991) found less eyespot (caused by *Pseudocercosporella herpotrichoides*) and root rot in winter wheat grown on organic farms. Disease incidence of foot rot (caused by *Gaeumannomyces graminis* and *Rhizoctonia* spp.) of barley was lower in soils that had been organically-managed for more than 5 years (Hannukkala and Tapio, 1990) even though disease levels were low. The results of our study confirm and extend these observations. The present study showed that suppression of take-all was stronger on barley, and to a lesser extent on wheat, grown in organically-managed soils compared to conventionally-managed soils (Figure 1). Suppression of take-all on roots of triticale was not significantly lower in the organically-managed soil. In contrast to previous research (Hollins et al., 1986), disease severity was of the same magnitude for barley, wheat and triticale

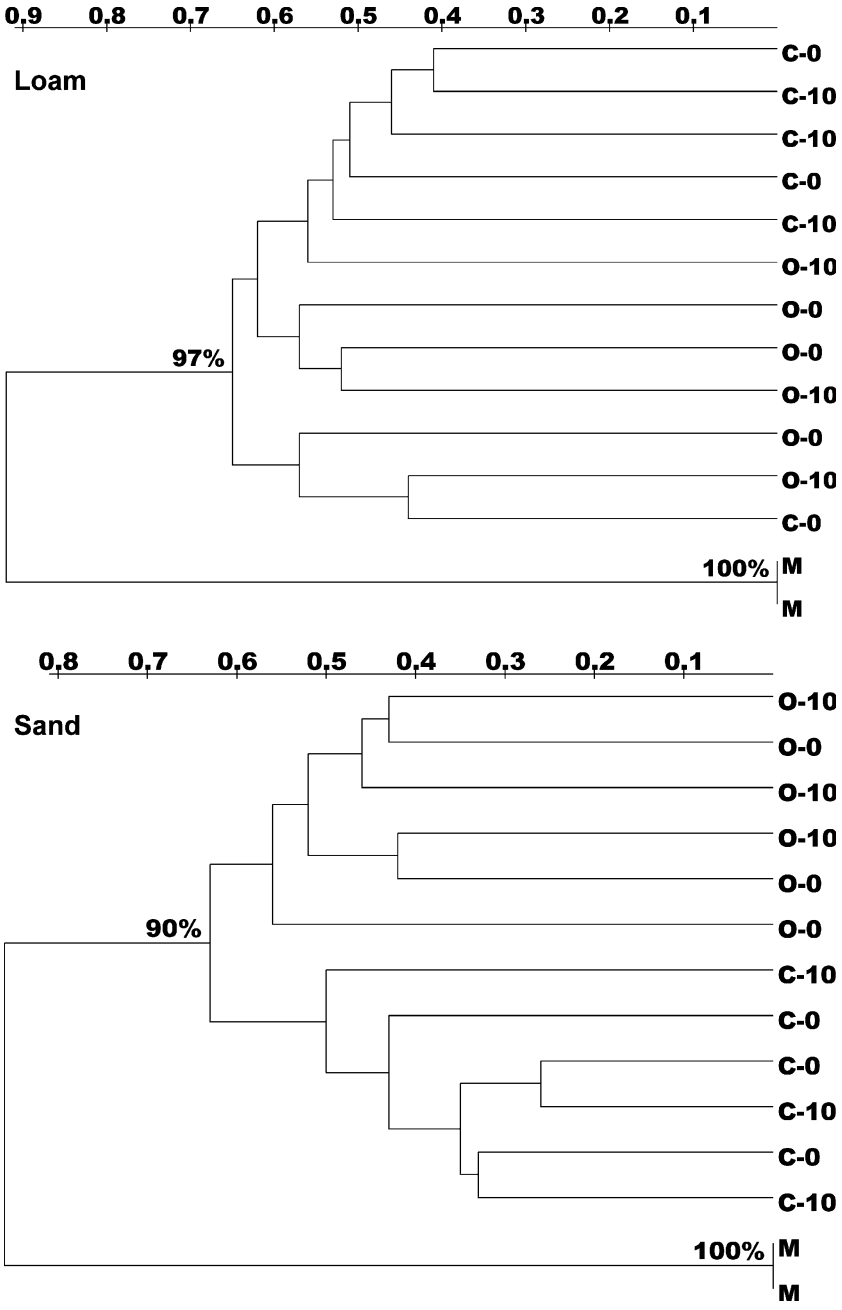

Figure 6. Dendrograms based on 16S-rDNA PCR-DGGE analyses performed on DNA isolated from soil (Exp. 3). DNA was obtained at the start (0) of the experiment and 10 days after introduction of *P. fluorescens* strain Pf32-*gfp* in the loamy and sandy soils. O−0 refers to organically-managed soil at t = 0, O−10 refers to organically-managed soil at t = 10 days, C−0 refers to conventionally-managed soil at t = 0, C−10 refers to conventionally-managed soil at t = 10, M refers to the markers. Bootstrapping was performed 1000 times and values higher than 70% are presented in the dendrogram.

without crop-specific differences. Also the two 'old' and two 'new' barley cultivars did not significantly differ with respect to take-all disease severity. A disease suppressive interaction of the 'old' cultivars with organic management was also not observed. Preference of pathogens (Hollins

et al., 1986) or antagonists for specific host plant genotypes has been reported previously (Smith et al., 1999; Germida and Siciliana, 2001, Mazzola et al., 2004). The results obtained in this study with barley, however, indicate that organic management can have a stronger disease suppressive effect than the genotype of this particular host plant.

To further investigate the disease suppressive mechanism(s) in the organically-managed soils, special emphasis was given to the group of fluorescent *Pseudomonas* spp. and in particular those harboring the *phlD* gene; this group of antibiotic-producing pseudomonads plays a key role in the natural suppressiveness of take-all decline soils in the USA and the Netherlands (reviewed by Weller et al., 2002). The results of the present study, however, showed that this group of antagonistic bacteria does not seem to play an important role in take-all suppressiveness of the organically-managed soils tested. On the contrary, population densities of indigenous *phlD*$^+$-*Pseudomonas* spp. were low and around the detection limit (10^4 CFU g^{-1} root) on roots of triticale grown in organically-managed sandy soil (Figure 2b1 and 2b2). Whether these differences in population densities of indigenous Pseudomonads between organic and conventional management are also representative for wheat and barley was not investigated and will be subject of future studies. The results of this study also showed that organic management adversely affected the initial survival of a *gfp*-tagged *phlD*$^+$ *P. fluorescens* strain (Pf32-*gfp*) introduced into soil (Figure 3). Nevertheless, 17 days after introduction, establishment of the introduced strain Pf32-*gfp* was similar in the soils tested and final densities remained above 10^5 CFU g^{-1}, a threshold density required for take-all control (Raaijmakers and Weller, 1998). However, in spite of similar densities, the introduced strain Pf32-*gfp* showed a reduced efficacy in controlling take-all of wheat grown in organically-managed soils compared to conventionally-managed soils (Figure 4). Therefore, differences in disease suppressive activity between soil types might have been the result from reduced activity of the introduced antagonist caused by environmental conditions rather than survival. Factors like soil type, texture, pH, organic matter and carbon sources are known to influence antibiotic production in *P. fluorescens* (Duffy and Defago, 1999; Ownley et al., 2003).

Despite the limited effect of the introduced strain Pf32-*gfp* against take-all in organically-managed soils, it did promote wheat shoot growth in all soils tested with a maximum increase of 40% in the sandy conventionally-managed soil (Figure 4). A plant growth-promoting effect of *P. fluorescens* has been described before. Pierson and Weller (1994) showed that wheat yield was enhanced when mixtures of fluorescent pseudomonads were added to the soil. *Pseudomonas* spp. stimulated wheat growth in both field and greenhouse experiments (de Freitas and Germida, 1992a,b), where during early plant growth Fe uptake was enhanced. Chickpea plant growth was enhanced after seed treatment with *P. fluorescens* (Landa et al., 2004). It was suggested that competition with minor deleterious microorganisms in the rhizosphere was an important mechanism for plant growth promotion. However, plant growth-promotion by rhizobacteria can be explained by several mechanisms other than suppression of deleterious organisms (Kloepper et al., 1989; Glick, 1995). For example, *P. putida* stimulated root growth of canola and mung bean in absence of a pathogen (Patten and Glick, 2002). Recently, Ryu et al. (2003) showed that also volatiles released by *Bacillus* spp. can induce plant growth in *Arabidopsis*.

Since specific suppression by *phlD*$^+$ pseudomonads apparently does not seem to play an important role in the take-all suppressiveness of soils tested in this study, more general mechanisms may be involved in disease suppression in the organically-managed soils. Higher microbial diversity has been observed in organically-managed soils compared to their conventional counterparts (Workneh and van Bruggen, 1994; Mäder et al., 2002). In the present study, however, diversity indices obtained from 16S PCR-DGGE analyses were not higher in organically-managed soils indicating that diversity of the bacterial community, i.e. diversity within the fraction of the bacterial community that was amplified in PCR-DGGE, could not explain take-all suppression. For the loamy soils, the 16S DGGE profiles from the organically-managed soils could not be distinguished from the conventionally-managed soils. For the sandy soils, however, 16S profiles obtained from samples from the organically-managed soils clustered together and could be separated from profiles obtained from conventionally-managed

soils (Figure 6). These results suggest that management practices like organic farming can indeed change microbial communities. Whether specific microbial genera are preferentially enriched by organic management as indicated by Workneh and van Bruggen (1994) needs to be studied more in depth by the use of, for example, genus-specific primers. Interestingly, the separation of 16S profiles between organically- and conventionally-managed sandy soils and the lack of discrimination between 16S profiles from organically- and conventionally-managed loamy soils correlates well with the difference in disease severity between the organically- and conventionally-managed sandy soils and also with the lack of difference in disease severity between the organically- and conventionally-managed loamy soils.

Increased microbial activity and competition for nutrients are more likely to be major factors in disease suppressiveness in organically-managed soils than one can explain merely by the activity of a single group of antagonistic microorganisms. In organically-managed soils, higher microbial activity and competition by the native microflora has been observed previously (Knudsen et al., 1999; van Bruggen and Termorshuizen, 2003). Also in the present study, higher microbial activity was observed in the organically-managed sandy soil, which further supports and extends the observations made in other organically-managed soils (Reganold et al., 1993; Knudsen et al., 1999; Schjønning et al., 2002). Higher microbial activity has been shown to result in lower take-all disease levels (Gerlagh, 1968). In our study, microbial activity was almost twice as high in the organically-managed sandy soil than in the other soils. We therefore postulate that the microbial community, and specifically microbial activity, plays, at least in part, a role in the suppressiveness of *Ggt* in organically-managed sandy soils. Organic management influences not only the microbial community, but also soil chemical factors that can contribute to take-all suppression. Nitrogen is an important factor in disease development and can directly or indirectly influence *Ggt* (Lucas et al., 1997). Higher ammonium levels in the soil water solution result in lower rhizosphere pH (Smiley and Cook, 1973; Cook, 2003), which stimulates *Pseudomonas* spp. antagonistic to *Ggt* (Smiley, 1978a) and consequently can reduce take-all severity (Smiley, 1978b). In our study, ammonium concentrations tended to be relatively higher in some organically-managed sandy soils (Table 1), but a relationship between observed disease severity and ammonium concentration was not apparent in our experiments (data not shown), which may also provide an explanation that 2,4-DAPG producing pseudomonads were not enriched in the organically-managed sandy soil.

In conclusion, the results of this study showed that organic management resulted in an increase in suppressiveness of soils to take-all disease of barley and wheat. This effect was much more pronounced on roots of barley and wheat plants grown in a sandy than in a loamy organically-managed soil. Fluorescent *Pseudomonas* spp. and in particular *phlD*$^+$ pseudomonads, key factors in the take-all decline phenomenon, were represented at lower population densities in organically- versus conventionally-managed soils and therefore do not seem to play a key role in the take-all suppressiveness of the organically-managed soils in this study. Furthermore, subsequent experiments indicated that organic management adversely affected the initial establishment of a *phlD*$^+$ *Pseudomonas* strain Pf32-*gfp*. The efficacy of biocontrol of take-all disease by strain Pf32-*gfp* was significantly stronger in conventionally-managed soils than in organically-managed soils. The higher microbial activity in the organically-managed sandy soil combined with the significantly lower take-all severity suggest that microbial activity plays, at least in part, a role in the take-all suppressiveness observed in the organically-managed sandy soil. However, the involvement of specific antagonists in take-all suppression cannot be excluded especially since the bacterial composition in the suppressive organically-managed sandy soil was significantly different from that in the conventional sandy soil.

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