Suppression of soilborne pathogens in mixed cropping systems

Gerbert Hiddink

Promotor:
Prof. Dr. Ir. A.H.C. van Bruggen
Hoogleraar in Biologische bedrijfssystemen
Wageningen Universiteit

Co-promotoren:
Dr.Ir. A.J. Termorshuizen
Senior product manager bodemkwaliteit
Blgg Wageningen
Dr.Ir. J.M. Raaijmakers, Universitair docent, Laboratorium voor Fytopathologie
Wageningen Universiteit

Promotiecommissie:
Prof. Dr. W. van der Putten, NIOO-KNAW te Heteren en Wageningen Universiteit
Prof. Dr. Ir. P.C. Struik, Wageningen Universiteit
Prof. Dr. L.C. van Loon, Universiteit Utrecht
Dr. P.A.H.M. Bakker, Universiteit Utrecht
Dr. J. Kohl, Plant Research International en Rheinische Friedrich-Wilhelms Universität

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Abstract

Since the green revolution, agricultural production has increased tremendously due to synthetic fertilizers, chemical crop protectants and high yielding plant varieties. However, soilborne pathogens remain yield-limiting factors in agricultural production. Hardly any sustainable solutions are available to control soilborne diseases and effective chemicals are limited and often have unwanted environmental side effects. Therefore, more sustainable ways of cultivating crops and agricultural production are needed.

The general goal of this thesis research was to investigate the effect of mixed cropping on soilborne diseases in organically managed soils and to unravel the mechanism(s) involved. First, the disease suppressiveness of organically managed soils against the soilborne fungal pathogen *Gaeumannomyces graminis* var. *tritici* and the role of antagonistic *Pseudomonas* bacteria were investigated. Take-all disease severity was lower in organically compared to conventionally managed soils. Naturally occurring populations of 2,4-diacetylphloroglucinol producing pseudomonads were lower in organically managed soils. The initial establishment of an introduced fluorescent pseudomonad was hampered in these soils and its biocontrol efficacy was lower in the organically than in the conventionally managed soils, indicating that these pseudomonads do not play an important role in disease suppression in organically managed soils. Rather, microbial activity was likely important for disease suppression in the organically managed sandy soils.

Disease levels are often lower in mixed crops compared to single crops. In this thesis we present an overview of soilborne pathogens in mixed crops and discuss the disease suppressive mechanisms that are involved.

Furthermore, we studied the effects of mixed cropping (Brussels sprouts-barley and triticale-white clover) in organically managed soils on suppressiveness against three soilborne pathogens. Mixed cropping did not result in enhanced disease suppressiveness compared to the single-cropped soils. Also soil microbial community structure and activity in mixed cropped soils were indistinguishable from single-cropped soils.

Finally we investigated the effects of mixed cropping triticale-clover on the development of Take-all disease under field and greenhouse conditions and the mechanisms involved. Under field conditions, Take-all disease severity increased for both single- and mix-cropped triticale over three consecutive years. Disease increase tended to be lower in the mixed- than in the single-cropped triticale. In the fourth year this trend was however reversed, probably due to bad clover establishment. In the greenhouse, Take-all disease was lower in the mix- than in the single-cropped triticale during five consecutive cycles. In both the field and greenhouse experiments clover biomass was negatively correlated with Take-all disease severity. Potential disease suppressive mechanisms stimulated by the clover biomass could be a change in triticale root architecture, host dilution, enhanced microbial activity, stimulation of antagonists in the rhizosphere and possibly increased ammonium concentrations in the rhizosphere.

We conclude that effects of mixed cropping systems on soilborne pathogens depend more on the crops or cultivars growing at that moment than on the mixed crops grown previously. Disease suppression in mixed crops is therefore a plant-driven process and effects are mainly observed in the rhizosphere and not in the bulk soil. Root architecture might have a large influence on the interactions that can take place in mixed

cropping and the selection of the crops or cultivars to be included in the mixture should be made with great care to have significant effects on soilborne plant pathogens.

Voorwoord (foreword)

De oorsprong van dit proefschrift ligt in de conventie van Rio de Janeiro (1992), waar door een groot aantal landen het biodiversiteitsverdrag is ondertekend. In dit verdrag is overeengekomen dat deze landen investeren in kennis naar het behoud en de toepasbaarheid van biodiversiteit. Naar aanleiding van deze afspraken is door de Nederlandse overheid het 'stimuleringsprogramma Biodiversiteit' in werking gesteld, waarbinnen het onderzoek 'Suppression of soilborne pathogens in mixed cropping systems', een klein onderdeeltje was.

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Gerbert

Contents

Chapter 1

General Introduction

Introduction

Since the green revolution, agricultural productivity has increased due to the availability of artificial fertilizers, chemical crop protectants and high yielding varieties (Matson et al., 1997). This, however, has resulted in an array of negative environmental side effects including pollution and eutrophication of ground and surface waters (Gliessman et al., 2001). Several of these negative effects are the result of chemical control measures against pathogens and pests. Therefore, more and more hazardous chemical crop protectants like methyl bromide have been banned or limited (Liu et al., 2007). As a consequence, this reduced availability of pesticides constitutes a challenge for the management of plant pathogens, especially soilborne pathogens. For soilborne pathogens, resistance in plants is generally not available and fungicides are not sufficiently effective, leaving physical-cultural and biological measures as the main management options.

One of the oldest and most effective cultural measures to control soilborne pathogens is crop rotation (Cook and Veseth, 1991), *i.e.* the cultivation of different crops in sequence as opposed to continuous cultivation of the same crop on the same field. By growing different crops in rotation, the inoculum of pathogens is gradually reduced due to the absence of hosts or to specific effects of the hosts or their organic residues on the pathogens or their antagonists (Hoitink and Boehm, 1999). The more extended the non-host period is, the more reduced the amount of inoculum (Garret and Cox, 2006). The design of the rotation in relation to the specific pathogens present is crucial (Krupinsky et al., 2002). Crop rotation can be regarded as application of crop diversity in time on the same area of land. Application of (agro)diversity in space, e.g. growing multiple crops on the same area of land might have similar effects. Crop diversity and therefore more diverse crop residues likely affect microbial diversity in soil and rhizosphere and may in turn affect soilborne pathogens (Hoitink and Boehm, 1999, Garbeva et al., 2004). Thus, different forms of agrobiodiversity can be regarded as management tools against soilborne pathogens.

This thesis focuses on studying the effects of agrobiodiversity on the development of soilborne pathogens. The main goal of this thesis is to explore the prospects of mixed cropping as a tool to manage soilborne pathogens and to unravel the mechanisms involved. In this introductory chapter, organic management as a tool to enhance agrobiodiversity and disease suppression of soilborne pathogens are introduced. Then, mixed cropping is defined and its potential to contribute to suppression of soilborne pathogens is presented.

Organic agriculture

The National Organic Standards Board of the USA has defined organic agriculture in 1996 as: *"an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain and enhance ecological harmony"*. It is often regarded as one of the most sustainable forms of agriculture. Organic agriculture leads to ecological benefits (less pollution of harmful substances, less eutrophication,

higher biodiversity), and possibly human health benefits (direct via nutrition and indirect via decreased pollution of the environment) (Heaton, 2001). These benefits prompt consumers to buy organic products. Besides the ecological benefits and their own aversion against conventional production principles, higher prices stimulate farmers to convert to organic agriculture. Mäder et al. (2002) showed that organic agriculture can be both sustainable and profitable. Still, organic production comprises only a minor part (0.7% or 30.4 Mha) of the area under agricultural production worldwide (Willer et al., 2008). In the EU, the percentage of the agricultural area managed according to organic principles is one of the highest in the world: 4% of the agricultural area or 6.8 Mha (Willer et al., 2008). The US and Europe are the most important markets for organic products (97%) and turnover in 2006 has doubled since 2000 to 38.6 million dollars (Willer et al., 2008).

The management tools applied to produce organic products are fundamentally different from those used for conventional products. The most important difference is the rejection of the use of synthetic fertilizers and chemical crop protectants in organic agriculture (EEG Nr. 2092/91 – Article 6; Carpenter-Boggs et al., 2000). Instead, an integrated approach of cultural and biological methods is used to protect crops (Letourneau and van Bruggen, 2006). Natural products, like plant extracts and biological control agents, are rarely used in practice. A limited number of fungicides are permitted in organic agriculture (EEG No. 2092/91 – addendum II), primarily copper and sulfur fungicides, but their effectiveness and sustainability relative to synthetic organic fungicides have been debated, especially in the case of copper based products (Van Zwieten et al., 2004). No pesticides are available in organic agriculture to control soilborne pests and diseases.

To maintain and increase soil fertility, wide crop rotations including leguminous crops are used. In addition, soils are amended with organic residues such as cover crops and animal manure. Organic matter management is an important production practice to maintain and increase soil fertility and it is the basis of sustainable organic production (Mäder et al., 2002). Amendment with organic matter serves, besides maintaining soil fertility, several other goals, such as promotion of soil quality and soil health (Doran and Zeiss, 2000; Janvier et al., 2007). Soil quality is "*the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health*" (Doran et al., 1996). Soil health can be regarded as a subset of ecosystem health, which is characterized by (i) integrity of nutrient cycles, (ii) stability in terms of amplitude of fluctuations and resilience to disturbance or stress, (iii) high biodiversity and/or connectedness between functional groups (van Bruggen and Semenov, 1999). Outbreaks of plant and animal diseases can be considered as indicators of instability and poor ecosystem health. Soil quality is determined by soil physical, chemical and biological characteristics, while soil health is primarily determined by biological characteristics.

In organic agriculture, soil physical properties such as the water holding capacity and soil structure are improved by organic amendments. Organically managed soils are generally regarded to be of a higher quality and healthier than conventionally managed soils (Workneh and van Bruggen, 1994), although a link between soil quality indicators and soil function (such as microbial activity and mineralisation or plant disease severity) is not always straightforward (Nannipieri et al., 2003; Janvier et al., 2007). In general, the mineral N-content is limited in organically managed soils (reviewed by Walters and Bingham,

2007; Van Diepeningen et al., 2006). Likely this is an important reason for reduced disease severity and reduced N-leaching in those soils (Walters and Bingham, 2007). In organically managed soils, N is gradually released from particulate organic matter during mineralisation (Tu et al., 2006), and substantial amounts of N can become available due to a high organic matter content and associated microbial biomass and activity. Thus, the yield of organic crops can approach that of conventional crops provided that there are no other limiting factors (Clark et al., 1999). The asymptotic relationship between the N-content and yield, where higher N-availability above a threshold only marginally increases the yield, is another reason for a potentially small difference in yield between conventional and organic production (Walters and Bingham, 2007).

An important constraint that limits organic production are plant diseases caused by an array of pathogens. These include soilborne pathogens, although under certain conditions they are often suppressed to some extent in organic crop production (van Bruggen and Termorshuizen, 2003; Hiddink et al., 2005a). Nevertheless, damping-off diseases can be quite problematic, especially if crops are planted within a few weeks after incorporation of fresh organic matter (van Bruggen, 1995). As only few fungicidal (natural) products are allowed in organic production, and none of these control soilborne pathogens, other ways of reducing or suppressing root pathogens are needed. Some organic amendments like pig slurry (Tenuta et al., 2002) or composted cotton-gin trash (Liu et al., 2007) have been shown to have direct suppressive activity against soilborne pathogens (Bailey and Lazarovits, 2003). However, the required levels of application are unsustainable, and are not allowed and/or uneconomic in most EU countries. Also other amendments, especially designed for use in biodynamic agriculture, a holistic and regenerative form of organic agriculture, can increase various aspects of soil quality (Zaller and Köpke, 2004). Biodynamic field sprays with a combination of cow manure, ground silica and horse tail (*Equisetum arvense* L.) increased mineral C and showed minor changes in microbial community profiles based on FAME (Carpenter-Boggs et al., 2000). However, the efficacy of biological dynamic amendments is not unequivocal (Raupp et al., 1999, Carpenter-Boggs et al., 2000), and scientific proof of many products is meagre at best. In this thesis (Chapter 2) we investigated how organic management practices influence soilborne pathogens and antagonist establishment and survival, as well as their relation to soil health indicators. Furthermore we investigated another cultural method that is considered to be sustainable and a potential tool to reduce the detrimental effects of soilborne pathogens: “mixed cropping”.

Mixed cropping

Mixed cropping or intercropping is generally defined as the cultivation of two (or more) crops at the same time in the same field (Vandermeer, 1990; Geno and Geno, 2000). It is probably the most ancient agricultural practice and nowadays many farmers still practice mixed cropping, especially in tropical regions (Vandermeer, 1990). Typical examples are the cultivation of cotton-sorghum, sorghum-pigeon pea, beans-maize but also coconut-pineapple as mixed crops. Also in ‘modern’ agriculture, renewed attention is given to mixed cropping such as wheat-clover and grass-clover but also cultivar mixtures and multilines of cereals.

The most evident reason for mixed cropping is that it is considered as a more

sustainable and secure way of producing agricultural products as compared to monoculture (Vandermeer, 1990). If one crop performs less, the accompanying crop can compensate for the yield loss. In less developed regions, the need for more secure cropping systems is provoked by the low availability and high prices of artificial fertilizers, chemicals and high yielding varieties. Mixed cropping is therefore often linked with organic agriculture (Vandermeer, 1995), although it is used only occasionally and not certified as such. Besides a better yield guarantee, an important and interesting benefit of mixed cropping is an increase in the combined yield that is often observed per unit of land area compared to growing single crops. Additional aspects of mixed cropping that are advantageous include more efficient nutrient use (especially through reduced leaching of nutrients), improved weed and disease suppression. While the effects of mixed cropping on the epidemic spread of airborne pests and pathogens has been documented relatively well, the effects on the fate of soilborne pathogens has received only limited attention.

Whether a mixed cropping system has a yield advantage over single crops depends on many factors. Vandermeer (1990) proposed two production principles that can apply to mixed cropping, the Competitive Production Principle (CPP, (Vandermeer, 1981; cited in Vandermeer, 1990)) and the Facilitative Production Principle (FPP, (Vandermeer, 1984; cited in Vandermeer, 1990)). The Competitive Production Principle (CPP) operates when within-crop species competition exceeds between-crop species competition. Diluting a single crop or replacing it in part by another non-competing crop will then lower competition and thus increase the productivity in the mixed crops. Under CPP, two crops occupy different niches *e.g.* with respect to light requirement or rooting depth, thus facing reduced competition. The opposite effect, when crops share similar niches, has been exploited to suppress weeds (Banik et al., 2006). For example, in a mixed crop pea-false flax (*Camelina sativa*), smothering reduced establishment of annual weeds ((Sauke and Ackermann, 2006). Furthermore, both crops showed increased yields because of resource allocation that stimulated competiveness.

The Facilitative Production Principle (FPP) is the opposite of CCP (Vandermeer, 1990): it is used to explain the effect of one crop on the environment, resulting in benefits for the accompanying crop. A well-known example is N supplying legume – non-legume mixes (Haugaard-Nielsen, 2005): nitrogen fixation causes an increase in the amount of available N available for the accompanying crop (Känkänen and Eriksson, 2007; Song et al., 2007). Legumes sown under a standing main crop function as storage for the surplus of available N in soil. This results in less weed development in a pea-barley intercrop and leads to higher yields as compared to the sole crops (Haugaard-Nielsen et al., 2001).

In mixed crops, soil erosion is reduced because of prolonged and more intense soil coverage (De Bie, 2005). This, of course, is most evident in soils that are sensitive to erosion. For example, in Jordan the run-off coefficient was reduced by 43% in mixed crops and as a result yield increased by more than 50% (Sharaiha and Zaidat, 2008). Also mixed cropping led to more efficient use of water. Microclimatic changes within the canopy might be regarded as a FPP in cases where it reduces pest insects (reviewed by Hooks and Johnson, 2003; Bukovinsky, 2004; Pitan and Otalunde, 2006) or when the environment changes such that it can support higher parasitoid densities (Ayalew and Ogol, 2006).

Airborne diseases are well-known to be reduced in multilines and cultivar mixtures in grains (reviewed by Mundt, 2002). Host dilution in the mixed crops is generally regarded

as the mechanism responsible for lower disease in the mixed crops (Burdon and Chilvers, 1982). Dilution can be accomplished in different ways. The use of multilines and cultivar mixtures varying in resistance against pathovars of poly-cyclic foliar pathogens reduce establishment and dispersal of these pathogens (reviewed by Mundt, 2002). Airborne spores are intercepted on unsusceptible tissues when accompanying crops function as a physical barrier. If crop mixtures affect the microclimate, lower wind speeds reduce dispersal distances and a changed microclimate can affect pathogen dynamics. In contrast, reduced evaporation can result in a more humid and warm microclimate, which can stimulate *e.g.* grey mould caused by *Botrytis cinerea*.

Although often referred to in general terms (Mundt, 2002; Hiddink et al., 2005b), the effects of mixed cropping on soilborne pathogens are less studied. This is due, in part, to the fact that belowground interactions are not easily observed. Quantifying the spatio-temporal development of soilborne pathogens and their host roots is time-consuming and cumbersome. Only rarely can the occurrence of soilborne pathogens be diagnosed directly based on field observations. In this thesis, we investigate the effects of mixed cropping on soilborne pathogens and study the underlying mechanisms. Finally we aim to establish how disease suppressive effects are linked to soil health and how these can be used and managed in both conventionally and organically managed agricultural soils.

Research objectives

The **general goal** of this thesis research was to investigate the effect of mixed cropping on soilborne diseases and to unravel the mechanism(s) involved. Insight into the mechanisms related to disease suppression are likely to provide management tools to control soilborne pathogens. More specifically, we aimed to:

- Investigate the disease suppressiveness of organically managed soils against the soilborne fungal pathogen *Gaeumannomyces graminis* var. *tritici* and the role of antagonistic *Pseudomonas* bacteria. The hypothesis was that organic management of soils results in higher soil biodiversity which in turn affects the dynamics of both the take-all pathogen *G. graminis* and that of its antagonist *Pseudomonas fluorescens*.
- Provide an up-to-date review of the effects of mixed cropping on soilborne pathogens and the underlying mechanisms.
- Study the effects of mixed cropping (Brussels sprouts/barley and triticale/white clover) in organically managed soils on disease suppressiveness against three soilborne pathogens and to unravel the mechanisms involved.
- Investigate the effects of mixed cropping triticale-clover on the development of take-all disease under field conditions and and the mechanisms involved in disease suppressiveness against the take-all pathogen *Gaeumannmyces graminis* var. *tritici*.

Thesis outline

In **Chapter 2,** disease suppressiveness of organically and conventionally managed soils against the soilborne pathogen *G. graminis* var. *tritici* is investigated. The effectiveness of antagonistic *Pseudomonas* bacteria introduced into organically and conventionally managed soils to control take-all disease was assessed. The hypothesis was that a higher microbial diversity and activity in the organically managed soils contributes to a higher level of disease suppressiveness.

In **Chapter 3,** an up-to-date literature review is given with special emphasis on the effects of mixed cropping systems on the management of soilborne fungal pathogens. Various mixed cropping systems are described and the potential mechanisms of disease reduction in mixed cropping systems are discussed.

In **Chapter 4,** the effects of mixed cropping on disease suppressiveness against three different soilborne pathogens is presented. The soilborne pathogens studied were *Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *lini* and *Gaeumannomyces graminis* var. *tritici*. To unveil potential mechanisms involved in disease suppressiveness, soil microbial diversity was studied by Denaturing Gradient gel Eelctrophoresis (DGGE) and also microbial activity and nitrogen dynamics were monitored.

In **Chapter 5**, the effects of mixed cropping on take-all disease of triticale was studied in a long-term field experiment and in greenhouse bioassays. Besides disease severity and incidence, also yield, biomass, microbial diversity and activity, and nitrogen dynamics were measured and related to take-all disease severity. Also the phenomenon of take-all decline that occurs naturally during continuous cultivation of barley and wheat, was investigated.

Finally, in **Chapter 6** the results presented in this thesis are summarized and discussed. Recommendations for implementation of the obtained results in agricultural management are given.

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Chapter 2

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

Hiddink, G.A., van Bruggen, A.H.C.,
Termorshuizen, A.J., Raaijmakers, J.M., and Semonov, A.V.
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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.

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 (*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 neighboring 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).

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

Experiment[1]	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.[2]	n.d.	3.17	33.3	63.5
	CS	Marknesse	Conventional	Sand	Potatoes	2000	7.2	21.1	3.3	27.2	n.d.	n.d.	3.15	32.4	64.5
	OL	Ens	Organic	Loam	Potatoes	2000	7.6	19.4	2.4	24.0	n.d.	n.d.	8.27	54.5	37.2
	CL	Ens	Conventional	Loam	Potatoes	2000	7.6	6.8	2.3	10.7	n.d.	n.d.	7.74	51.7	40.6
Exp. 2	CsL[3]	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[4]	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.	3.17	33.3	63.5
	CS	Marknesse	Conventional	Sand	Potatoes	2002	6.9	22.1	3.5	29.7	14314	n.d.	3.15	32.4	64.5
	OL	Ens	Organic	Loam	Onions	2002	7.3	22.4	2.3	28.3	16707	n.d.	8.27	54.5	37.2
	CL	Ens	Conventional	Loam	Onions	2002	7.4	22.5	2.6	33.8	16640	n.d.	7.74	51.9	40.4
	OLL[5]	Langeweg	Organic	Loam	Onions	2002	7.4	20.0	2.6	28.0	17646	n.d.	11.4	43.8	44.8
	CLL[5]	Langeweg	Conventional	Loam	Onions	2002	7.4	39.6	2.6	45.0	17463	n.d.	9.26	39.5	51.2

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

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 neighboring 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 neighboring soils (OS, CS, OL and CL, Table 1).

All the organically managed soils were sampled on SKAL-accredited organic farms and a 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* (Sacc.) v. Arx & Olivier var. *tritici* Walker (*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* L.). The modern cultivars were Barke and Reggae, and the old cultivars were Goudgerst and Kenya. Old cultivars stand model for cultivars that have been developed (around 1920) under conditions of organic management, i.e. before the use of pesticides and artificial fertilizers became common practice. Modern cultivars stand model for cultivars that have been 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* L, cv. Vivant, Plant Breeding Int, Cambridge Ltd., Trumpington, Great Britain) or triticale (*X Triticosecale* Wittm. 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 five ml sterile distilled water, vortexed vigorously (1 min), sonicated (1 min) in an ultrasonic cleaner (Bransonic 12, Branson Ultrasonics Corp., Geneva, Switzerland), serially diluted and plated in three-fold on King's medium B amended with cycloheximide (100 mg l^{-1}), ampicillin (40 mg l^{-1}, 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). P. 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

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

Experiment[1]	Code	Management[2]	Organic since	Soil type	Sampling year	Crop	Sampling year -1	Sampling year -2	Fertilizer[3]	Amount[4] applied	Use of cover crops[5]	Weed management[6]	Crop protection[7]
Exp. 1	OS	Organic	1987	Sand	2000	Potatoes	Grass-clover	Grass-clover	M and S	40[8]	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[9]	Wheat	Wheat	n.k.[10]	n.k.	n.k.	n.k.	n.k.
	OS	Organic	1987	Sand	2001	Triticale[9]	Carrots	n.k.	M and S	50[11]	yes	M	n.a.
	O_tS	Organic in transition	2000	Sand	2001	Triticale[9]	Barley	Fallow	M	30	no	M	n.a.
Exp. 3	OS	Organic	1987	Sand	2002	Potatoes	Cale/lettuce	Carrots	M and S	20+50[12]	yes	M	n.a
and	CS	Conventional	-	Sand	2002	Potatoes	Tulip	Wheat	S and C	25	yes	M and C	C
Exp. 4	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

[1] Exp. 1 = barley, Exp. 2 = triticale, Exp. 3 =su rvival experiment, Exp. 4 = wheat
[2] Farms practicing organic management are all SKAL -accredited and are not allowed to use chemical fertilizer, pesticides or genetically modified organisms.
[3] M = manure, S = slurry and C = chemical fertilizer.
[4] 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
[5] cover crops were not necessarily grown in the year of sampling
[6] M = mechanical weed eradication, C = chemical weed eradi cation
[7] C = chemical crop protection, n.a. = not applied
[8] Total of 20 ton deep litter house manure and 20 ton humus earth were applied
[9] Triticale was grown for 4 weeks to revitalize the microbial community in the dried soils
[10] Not known
[11] Humus earth
[12] Ton ha^{-1} organic manure and 50 ton ha^{-1} humus earth

strain was stored at -80^{o}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 sterile distilled water (pH 7) and adjusted to a final concentration of 5 x 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 10x 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 µg μl^{-1}, USB Corporation, Cleveland, Ohio, USA); and 0.5 µl AmpliTaq Stoffelfragment (10U 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 0.5xTBE 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 100V in 60˚C 0.5X 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 x 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 program (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.

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*.

	Disease severity[1]				**Shoot length[2]**		
Effect	**Nom DF[3]**	**Den DF**	**F-value**	**Pr>F**	**DF**	**F-value**	**Pr>F**
Block	9.00	67.4	7.02	<.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 x Soil type	2.94	115.0	0.26	0.7961	3	1.89	0.1340
Management	1.00	115.0	40.89	<.0001	1	108.49	<.0001
Cultivar x Management	2.94	115.0	0.42	0.6549	3	1.85	0.1417
Soil type x Management	1.00	115.0	1.53	0.1641	1	0.80	0.3714
Cultivar x Soil type x Management	2.94	115.0	0.29	0.7675	3	1.46	0.2278

[1] Anova's obtained with the ANOVAF-procedure and Mivque0-method as described by Shah and Madden (2004)
[2] Anova's obtained using GLM
[3] Corrected degrees of freedom (Shah and Madden, 2004).

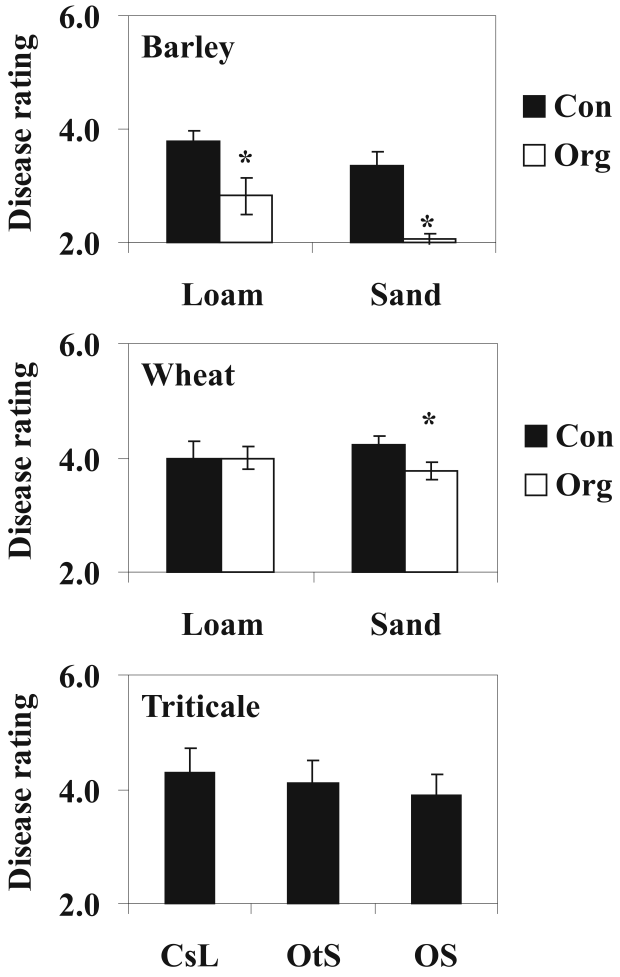

Figure 1. Mean severity of take-all disease on barley (Exp. 1) and wheat (Exp. 3) in two pairs of soil infested with *G. graminis* var. *tritici* (0.5% oat inoculum w/w). Triticale (Exp. 2) was grown in three soils. (CsL = conventional loam, OtS = conventional sandy soil in transition to organic, OS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

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 x management interaction ($P = 0.0755$) with respect to disease severity (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 x management x 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 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.

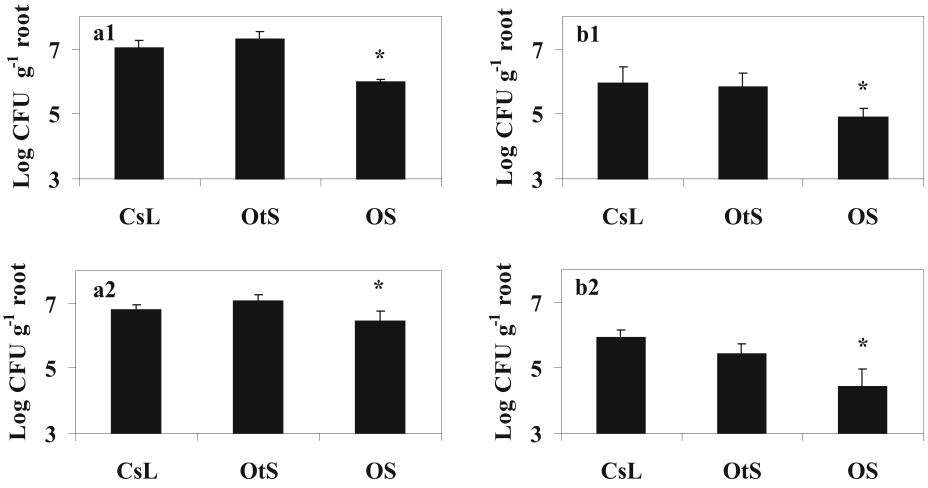

Figure 2. Mean population density (log CFU g^{-1} root) of fluorescent pseudomonads (a) and fluorescent pseudomonads harboring the *phlD* gene (b) in triticale (Exp. 2) rhizosphere without the take-all pathogen *Gaeumannomyces graminis* var. *tritici* (2a1 and 2b1) and in soils, 4 wks after addition 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.

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 neighboring 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

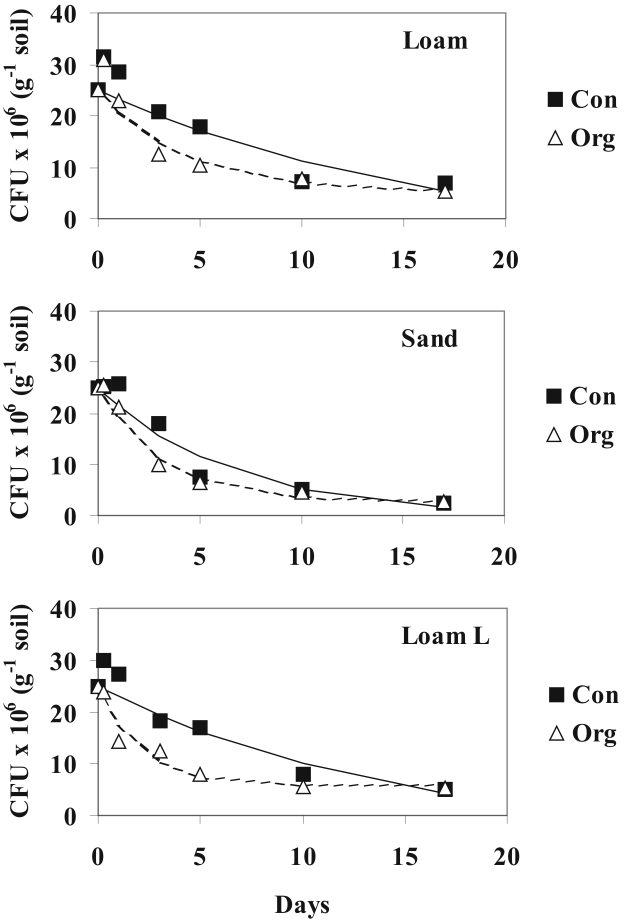

Figure 3. Survival of introduced *Pseudomonas fluorescens* strain Pf32-*gfp* (CFU g^{-1} dry soil, direct counts, Exp. 3) in paired organically- and conventionally-managed soils, CS and OS (Sand), CL and OL (Loam) and CLL and OLL (Loam L) from three locations (Marknesse, Ens and Langeweg) in The Netherlands.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

significantly different between organically- and conventionally-managed soils (P = 0.57, data not shown). After 10 days, the densities (based on 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.

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 conventionally-managed 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 amended with Pf32-*gfp*, while in sandy soil the difference was not significant (P = 0.3388). The soil type x 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 x 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 stronger (P = 0.0347) in conventionally-managed soils than in organically-managed soils (Figure 4b, Table 4c).

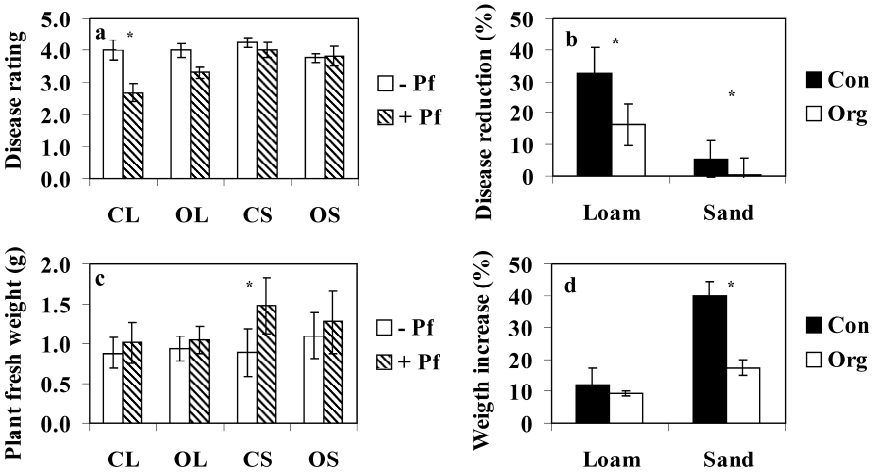

Figure 4. Effect of introduced *Pseudomonas fluorescens* (Pf32-*gfp*) on take-all severity on roots of wheat (Exp. 4) grown in soils from neighboring organic and conventional farms artificially infested with *Gaeumannomyces graminis* var. *tritici* (0.5% w/w) (4a). Mean disease reduction (*Ggt*/*Ggt*(Pf)*100%) in soils from neighboring 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; an asterisk indicates a statistically significant difference at P = 0.05.

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* x soil type x 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 to 20% in the other soils. Introduction of Pf32-*gfp* in soils not infested with *Ggt* also resulted in 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.

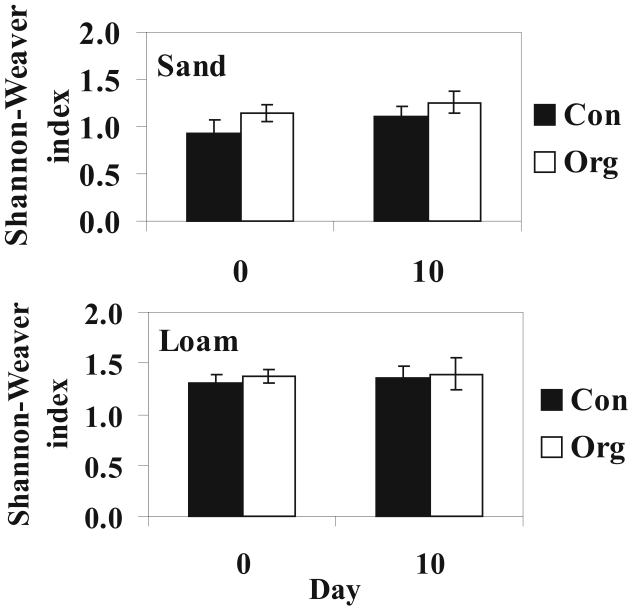

Figure 5. Mean Shannon-weaver indices obtained from 16SrDNA PCR-DGGE profiles of DNA extracted from loamy and sandy soils from neighboring organic and conventional farms before (day 0) and 10 days after introduction of *Pseudomonas fluorescens* (Pf32-*gfp*) in soils (Exp. 3). Error bars represent standard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

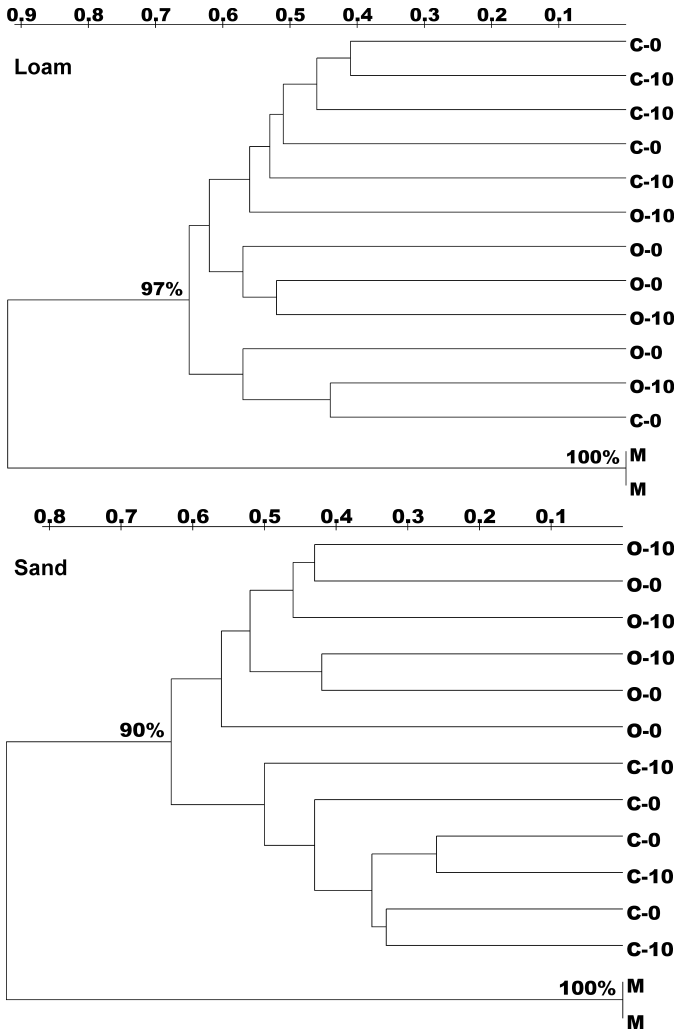

Figure 6. Dendrograms based on 16S-rDNA PCR-DGGE analyses performed on DNA isolated from soil (Exp. 3). DNA was obtained at the start (0) of the experiment and 10 days after introduction of *P. fluorescens* strain Pf32-*gfp* in the loamy and sandy soils. O-0 refers to organically-managed soil at t = 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.

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.

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; Carpenter-Boggs et al., 2000, Drinkwater et al., 1995). These management practices can indeed result in less disease caused by soil-borne pathogens (van Bruggen and Termorshuizen, 2003). For example, corky root severity (*Pyrenochaeta lycopersici*) was 37% lower in organically than in conventionally grown tomato plants (Workneh and van Bruggen, 1994). Likewise, Koch (1991) found less eyespot (*Pseudocercosporella herpotrichoides*) and root rot in winter wheat grown on organic farms. Disease incidence of foot rot (*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 without crop-specific differences. Also the two ‘old’ and two ‘new’ barley cultivars did not significantly differ with respect to take-all

Table 4. Experiment 4 (wheat), ANOVA-tables of experiment 4: 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%.

			Disease[1] severity				Plant weight[2]		
		Effect	Nom DF[3]	Den DF	F-value	Pr>F	DF	F-value	Pr>F
a	ANOVA	Block	4.00	53.0	0.04	0.9971	4	1.89	0.1247
		Treatment[4]	1.86	18.1	404.01	<.0001	3	110.05	<.0001
		Soil type	1.00	18.1	14.37	0.0013	1	213.65	<.0001
		Treatment x Soil type	1.86	18.1	14.73	0.0002	3	29.47	<.0001
		Management	1.00	18.1	2.19	0.1562	1	0.09	0.7662
		Treatment x Management	1.86	18.1	3.05	0.0755	3	4.79	0.0050
		Soil type x Management	1.00	18.1	7.53	0.0133	1	0.28	0.6009
		Treatment x Soil type x Management	1.86	18.1	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	6.6	0.49	0.5086	1	11.90	0.0011
		Organically man. loam *Ggt*+Pf32-*gfp* vs conventionally man. loam Ggt+Pf32-*gfp*	1	13	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	10.7	25.75	0.0004	1	63.75	<.0001
		Soil type	1.00	10.7	14.39	0.0029	1	51.73	<.0001
		Pf32-*gfp*-introduction x Soil type	1.00	10.7	15.06	0.0025	1	17.66	0.0003
		Management	1.00	10.7	2.35	0.1555	1	0.25	0.6224
		Pf32-*gfp*-introduction x Management	1.00	10.7	3.36	0.0814	1	12.39	0.0016
		Soil type x Management	1.00	10.7	8.05	0.0185	1	0.38	0.5451
		Pf32-*gfp*-introduction x Soil type x Management	1.00	10.7	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. loam vs organically man. loam	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	Block[5]	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 x Management	1.00	-	0.06	0.8172	1	14.84	0.0032

[1] Anova's obtained with the ANOVAF-procedure and Mivque0-method as described by Shah and Madden (2004)
[2] Anova's obtained using GLM
[3] Corrected degrees of freedom to obtain the correct F-value (Shah and Madden, 2004)
[4] Treatments were: Control (soil infested with steril ized 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)
[5] Analysis performed with Proc GLM, percentages were log transformed to obtain normality

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* (Ownley et al., 2003; Duffy and Defago, 1999).

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, 1992ab), 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 significantly (P=0.06) 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, 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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Chapter 3

Mixed cropping and suppression of soilborne diseases

G.A. Hiddink, A.J. Termorshuizen, and A.H.C. van Bruggen
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Abstract

Soilborne plant pathogens are major yield-limiting factors in the production of food, fiber and ornamental crops. In this review, we assess the potential of mixed cropping in managing soilborne pathogens. Three types of cropping systems are recognized that may adversely affect soilborne pathogens: i) continuous cultivation of single crops (monoculture), ii) crop rotation (*i.e.* sequential cultivation of different crops), and iii) mixed cropping (*i.e.* cultivation of multiple crops in the same field at the same time). Continuous cultivation of the same single crop in the same field is practiced worldwide, especially in areas where the number of crops that can be grown is agronomically and economically limited. Although monoculture generally leads to a build-up of soilborne pathogens, there are several intriguing and well-studied examples where continuous cultivation of single crops induces the natural decline of specific soilborne diseases. Crop rotation is widely practised to avoid the build-up of soilborne pathogens, and especially alternations of dicotyledonous with monocotyledonous crops are effective at limiting and reducing the inoculum levels of many soilborne plant pathogens. Mixed cropping is practiced to optimize nutrient acquisition/uptake, control soil erosion, suppress the epidemic spread of airborne pathogens and improve crop yields per unit of area. While mixed cropping has received attention for its effects on airborne pests and pathogens, the effects on soilborne pathogens barely have attracted attention. Mixed cropping is defined as the cultivation of a mixture of two (or more) crops together in the same field. Mixed cropping varies from sowing mixtures of seeds *at random* (mixed cropping *sensu stricto*) to row (mixed cropping in single rows) and strip mixed cropping (mixed cropping in multiple rows), relay mixed cropping (cultivation of multiple crops simultaneously during only part of their field period) and multi-storey mixed cropping (combining tall perennials with shorter biannual or annual crops). In 30 out of 37 publications, mixed cropping showed a significant reduction of soilborne disease and in seven, no or a negative effect was found. Diseases caused by splash-dispersed pathogens were reduced in mixed cropping systems in 10 out of 15 studies. Host dilution appeared to be among the most important mechanisms of disease suppression for both soilborne and splash-dispersed pathogens (12 and 5 observations, respectively). Other mechanisms involved in the disease suppression in mixed crops are allelopathy and microbial antagonism. Although the use of mixed cropping for disease suppression is still in its infancy, the wide range of biological effects and interactions observed holds promise for further optimization and management of soilborne diseases. Among the strategies proposed to further optimize disease suppression by mixed cropping is the selection of plant species and/or cultivars that provide an optimal combination of root architectures.

Introduction

During the past decades, the intensified mechanization and use of synthetic fertilizers and crop protectants have substantially increased agricultural yields. However, these practices also resulted in an array of adverse environmental side effects, including soil erosion, water pollution, eutrophication and reduced innate soil fertility (Gliessman, 2001). Acquisition of capital-intensive and crop-specific machinery further narrowed rotations. Although these negative side-effects of intensive agriculture counterbalance with an initial increase in food production per unit of area (Matson et al., 1997), ultimately they may lead to a decline in total food production due to land becoming unproductive because of soil erosion and pollution. On the other hand, increasing demands for agricultural products can only be met when high yields per unit of area are achieved, especially when productive land is falling short (Hill, 2007). Therefore, it is necessary to find more sustainable ways of cultivating crops without sacrificing on yield.

Narrow rotations of cash crops have resulted in a high incidence of soilborne diseases (Garrett and Cox, 2006). Although genetic resistance and effective pesticides are insufficiently available, many soilborne pathogens can be managed by wide crop rotations and other cultural measures (Cook, 2001). Soil fumigants can be highly effective, especially for the control of nematodes, but they have a strong negative impact on non-target organisms and therefore their use is discouraged (Schneider et al., 2003; Martin, 2003). Recently, the EU Council of Agriculture Ministers voted in favour of a proposal of the Commission to ban a large number of pesticides including all soil fumigants (http://www.nowpublic.com/health/eu-countries-vote-ban-dozens-pesticides). In addition, soil fumigants are costly and generally too expensive for low-value crops like cereals or for use by subsistence farmers in the developing countries. Also the application of methods specifically designed to control soilborne pathogens, such as biological soil disinfestation, soil solarization and flooding, are often too expensive or have limited efficacy due to environmental constraints.

While mixed cropping has received attention for its effects on airborne pests and pathogens (Wolfe, 1985; Mundt, 2002a), the effects on soilborne pathogens barely have attracted attention. In this review, we evaluate how cropping systems and in particular mixed cropping can affect soilborne pathogens. We first define the different types of cropping systems and specifically continuous single crop cultivation (monoculture), crop rotation (*i.e.* change of crop diversity in time), and mixed cropping (*i.e.* any type of growing multiple crops in the same field at the same time). Then we will shortly assess and discuss how these cropping systems can affect the dynamics of soilborne diseases. The effects of mixed cropping on soilborne and splash-dispersed fungal and bacterial pathogens will be discussed as well as the mechanisms underlying disease suppression by mixed cropping. Finally, recommendations and options for the use of mixed cropping in agro-ecosystems are given that may contribute to improving the sustainability of agricultural production.

Design of cropping systems to manage soilborne diseases

In modern agriculture, cultivation of single crops in a rotation is the most common cropping system for a vast range of crop species worldwide. If properly designed, crop rotation is the most efficient (cultural) practice to reduce the incidence and severity of soilborne diseases

(Cook and Veseth, 1991). However, crop rotation is not always practiced. In highly mechanized productions, continuous cultivation of the same single crop is regularly practiced, whereas in areas where mechanization, artificial fertilizers and crop protectants are too costly, diverse forms of mixed cropping are encountered regularly. Disease suppression related to crop rotation and continuous single crop production has been extensively investigated (Mazzola, 2002; Schneider, 1982; Weller et al., 2002). However, the effects of mixed cropping on soilborne pathogens have received considerably less attention. Where in literature effects of mixed cropping on soilborne pathogens are reported, they often appear just as a co-observation in studies on crop productivity. One important reason why the effects on soilborne pathogens have received little attention is the fact that, in many cases, they remain unnoticed (Cook, 2001) due to a-specific disease symptoms and the inherent difficulty of designing experiments in mixed cropping systems.

Successive cultivation of a single crop. Continuous cultivation of the same single crop in the same field is practiced in areas where the number of crops that can be grown is agronomically and economically limited (Cook, 2001). Under these conditions, mechanization makes cultivation more economically feasible but at the same time hinders the return to a more diversified crop rotation. In continuous crop cultivation, inoculum densities of soilborne pathogens increase without exception and a certain degree of damage has to be accepted (Shipton, 1975). Some cultural measures including reduced tillage can limit the survival of certain pathogens (Pankhurst et al., 2002; Meynard, 2003). Also stimulation of microbial activity through organic amendments can reduce pathogen inoculum or activity (Hoitink and Boehm, 1999). For certain pathosystems, natural disease suppression is known to be induced during continuous cultivation (Schneider, 1982; Weller et al., 2002), *e.g. Gaeumannomyces graminis* in wheat and barley (Gerlagh, 1968; Raaijmakers and Weller, 1998; Weller et al., 2002), *Rhizoctonia solani* in sugar beet (Hyakumachi and Ui in: Sturz and Christie, 2003), *Streptomyces scabies* in potato (Menzies, 1959), and *Fusarium oxysporum* f. sp. *melonis* in melon (Alabouvette et al., 1999). Induction of disease suppression can take multiple years and generally it is lost after growing other crops (Shipton, 1975). The mechanisms involved have been studied extensively and are linked to the microbial community in soil or the rhizosphere. The best-known mechanisms include antibiotic production (*e.g.* by strains of *Pseudomonas fluorescens*), competition by closely related non-pathogenic strains (*e.g.* competition for carbon by nonpathogenic *Fusarium oxysporum*), and parasitism (*e.g.* by *Trichoderma* spp.) (Weller et al., 2002). For these types of disease suppression to develop and to sustain, both the pathogen and a susceptible host plant need to be present and a certain level of damage has to be accepted.

Crop rotation. Crop rotation is the practice of growing crops on the same field sequentially in time. Crop rotation is very generally practiced to avoid the build-up of soilborne pathogens (Cook and Veseth, 1991), to achieve a balanced nutritional soil fertility, and, for root crops, to avoid intensive soil tillage (Termorshuizen, 2001). The beneficial effect of crop rotation against soilborne pathogens is due to their limited host range (Krupinscky et al., 2002). The host-dependent reproduction of most pathogens (Garrett and Cox, 2006) limits inoculum build-up and viability of the inoculum present diminishes in time when

Table 1. Mixed cropping systems (Geno and Geno, 2000; Vandermeer, 1990) and theoretical disease reducing mechanisms.

			Possible disease-reducing mechanisms			
Name	**Sowing Layout**	**Diversity**	**Airborne pathogens**	**Soilborne pathogens**	**Planting time**	**Mechanization grade**
Strip cropping	Sown in more than one row of the same crop next to each other	Diversity between species	Barrier effect/ spore trapping Microclimate (induction of disease)	Distance effect Microclimate (induction of disease)	same or different planting time	fully mechanized
Relay cropping	Crops sown widespread or in rows	Diversity between species	Absence of host	Absence of host Allelopathy Inoculum reduction	delayed planting time	fully mechanized
Row intercropping	A row of one crop is at both sides accompanied by a row of the other	Diversity between species	Between rows barrier effect, within rows no effect Reduced genetic susceptibility Microclimate (Induction of disease) ISR	Barrier effect (splash dispersal) Distance effect Allelopathy ISR Microclimate (inoculum reduction and induction of disease)	same or different planting time	no mechanization to fully mechanized
Mixed cropping	One crop sown in rows or widespread, the other widespread	Diversity between species	Reduced genetic susceptibility Barrier affect/ spore trapping Microclimate (Induction of disease) ISR	Barrier effect (splash dispersal) Reduced genetic susceptibility Distance effect Reduced chemotaxis Allelopathy ISR Microclimate (inoculum reduction and induction of disease) Antagonists/competition	at the same time	no mechanization to fully mechanized

Multiline cropping	Completely random widespread or in rows	Diversity within species	Reduced genetic susceptibility	Reduced genetic susceptibility	same planting time	fully mechanized
Multi storey cropping	Crops grown widespread or in rows but having different dimensions (height, volume and size)	Diversity between height levels	Barrier effect/ spore trapping Microclimate (Induction of disease) ISR	Barrier effect (splash dispersal) Microclimate (inoculum reduction and induction of disease) Reduced chemotaxis Allelopathy Antagonists/competition	at the same time	no mechanization to fully mechanized
Natural ecosystems	Completely random, no predetermined layout	Diversity within and between species	Absence of host Reduced genetic susceptibility ISR Barrier affect/ spore trapping Microclimate (Induction of disease)	Absence of host Reduced genetic susceptibility Barrier effect (splash dispersal) Distance effect Reduced chemotaxis Allelopathy ISR Microclimate (inoculum reduction and induction of disease) Antagonists/competition	can be any time	no mechanization

non-hosts are grown (Cook, 2001). Alternations of dicotyledonous with monocotyledonous crops are effective at limiting the inoculum levels of the majority of soilborne plant pathogens. Also alternation with hosts that do not support inoculum production can be a measure to reduce the amount of pathogen inoculum. For example, sugar beet is a host to *Verticillium dahliae*, but does not contribute to inoculum build-up, as microsclerotia have not yet been produced at harvest time of the roots.

Green manure or cover crops cultivated in wintertime are part of the crop rotation. The main reason to grow a green manure crop is to protect soil from erosion and to prevent leaching of mineralized nitrogen. In narrow rotations with a high pressure of soilborne pathogens, the choice of the optimal green manure crop can be a challenge. For example, to reduce nitrate leaching in sandy soils in wintertime in the Netherlands, it is now obligatory to grow a green manure crop following maize cultivation. Due to the late harvest of maize, the choice of green manure crops is usually limited to grass or winter cereals, which to a great extent resemble maize with respect to their host status for nematodes. The single escape farmers have is to harvest earlier, so that they still can sow mustard. Several green manures are known for their capacity to reduce diseases caused by soilborne pathogens. Incorporation of several *Brassica* species has been shown to reduce disease incidence caused by *Rhizoctonia solani, Phytophthora erythroseptica, Pythium ultimum, Sclerotinia sclerotiorum,* or *Fusarium sambucinum* in potato (Larkin and Griffin, 2007). The underlying mechanism involves the production of toxic volatiles during decomposition of the organic matter. Marigold (*Tagetes* spp.) is grown as a green manure to specifically suppress *Pratylenchus penetrans* (Kimpinsky et al., 2000), which is likely due to toxic plant exudates.

The effective length of crop rotation as a method to manage specific soilborne pathogens depends on the survival of the pathogen. For example, the resting spores of *Spongospora subterranea*, the causal agent of powdery scab of potato, can survive for many years in the absence of a host (Jeger et al., 1996), while the survival of *Gaeumannomyces graminis* is limited to only a few years at most (Gerlagh, 1968). Crop rotation is therefore not suitable to manage powdery scab, but it can be a valuable measure to manage take-all disease caused by *G. graminis* (Cook, 2001). For various other soilborne pathogens, *e.g. Verticillium dahliae, Rhizoctionia solani,* root knot nematodes (*Meloidogyne* spp.) and root lesion nematodes (*Pratylenchus* spp.), the design of a proper rotation can be difficult because these pathogens are capable of infecting and/or surviving on multiple hosts.

Mixed cropping systems. Mixed cropping is defined as the cultivation of a mixture of two (or more) crops together in the same field (Trenbath, 1976; Willey, 1979). There are various types of mixed cropping (Vandermeer, 1990; Geno and Geno, 2001), each of which may affect soilborne pathogens differently (Table 1, Figure 1). Mixed cropping systems can be characterized according to the degree to which roots of different crop species interact, which is determined not only by the mixed cropping system but also by the root architecture of each of the crops in the mixture (de Kroon, 2007; Weaver, 1926).

We define here mixed cropping *sensu stricto* as the practice of growing multiple crops simultaneously without a specific spatial structure. This is used frequently in slash-and-burn fallow agriculture or ley farming, when growing multilines or species mixtures

(*e.g.* broadcast sown grass-clover mixes). In a mixed setting, distances between hosts are generally greater than when grown as single crops and disease will spread more slowly (host dilution). Also allelopathy, microclimate change, root camouflage (Gilbert et al., 1997) and microbial antagonism have been proposed as the mechanisms underlying the disease suppression induced by mixed cropping.

Strip mixed cropping is the strip-wise simultaneous cultivation of multiple crops in rows, wide enough to permit independent cultivation but still sufficiently narrow to interact agronomically (Figure 2). Typically the width of the strips is adapted to the size of the machinery to be used. Since the crops co-occur on a narrow strip, belowground interactions

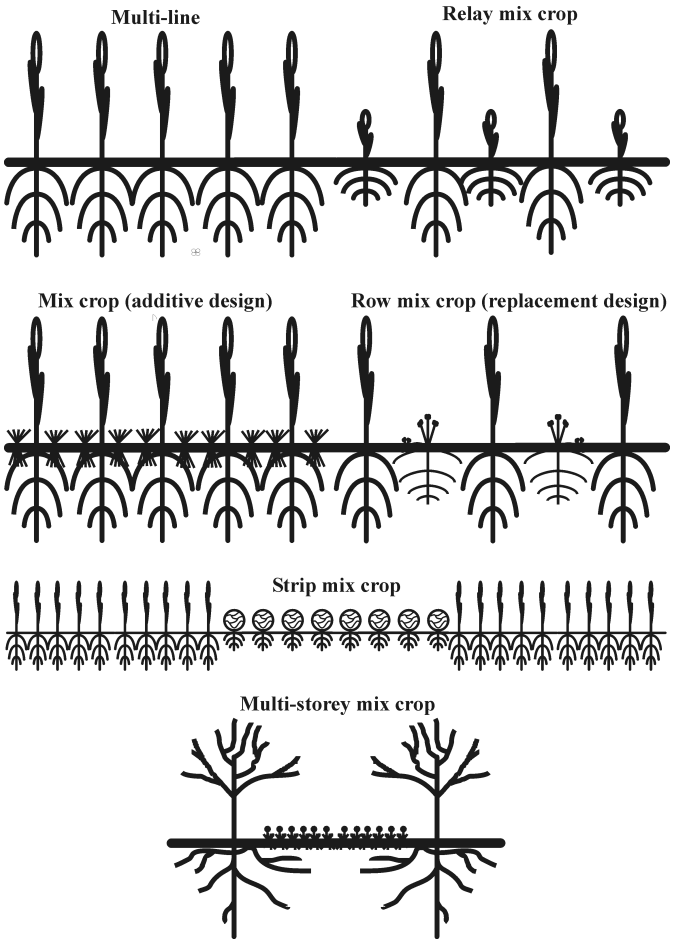

Figure 1. Mixed cropping systems.

Figure 2. Strip mix crop. (Photo courtesy of: Tim McCabe, 1999, USDA-NRCS).

Figure 3. Mixed crop, Brussels sprouts-barley, additive design (Photo: G.A. Hiddink).

between the crop species occur relatively infrequently and therefore the effects on soilborne pathogens are considered to be minor.

Relay mixed cropping is the simultaneous cultivation of multiple crops during only part of their field period. The second crop is planted at the time when the first crop reaches its reproductive stage but has not yet been harvested. When root systems of both crops overlap sufficiently, disease suppressive effects due to allelopathy, microbial antagonism or physical separation between pathogen and host may occur. Because of the time gap between sowing of both crops, (strip) tillage between rows of the standing crop can affect pathogen establishment and spread (Meynard et al., 2003).

Row mixed cropping is defined as the production of multiple crops alternately planted in rows. It can be done in an additive design, where both crops are sown at their single densities (Figure 3) or in a replacement design, where one crop is replaced by the other (Figure 4). Irrespective of plant density, disease can spread within rows like in single culture cropping systems, but between rows the alternate crop(s) can act as a barrier (Michel et al., 1997). Here, host dilution (replacement design), allelopathy, root camouflage and microbial antagonism may play a role in disease suppression.

Multi-storey mixed cropping (Figure 5) is the cultivation of tall perennials combined with shorter biannual or annual crops and is practiced in orchards and tree nurseries. The area between the rows is used to grow a cover crop to suppress weeds, fix nitrogen and reduce nutrient leaching. Allelopathy is a possible mechanism of disease suppression, but also roots can act as a physical barrier for pathogen spread, root camouflage and microbial antagonism.

Natural vegetation consists mostly of multiple species and can be considered to be closely related to (zero-tillage) mixed cropping. The disease suppressive mechanisms that

Figure 4. Mixed crop, triticale-clover, replacement design (Photo: G.A. Hiddink).

Figure 5. Multi-storey mix crop (Photo courtesy of: Gary Kramer, 2001, USDA-NRCS).

operate in natural ecosystems are probably comparable to the mixed cropping or multi-storey mixed cropping system.

Disease reduction in mixed cropping systems

In 30 out of the 37 studies where the fate of soilborne pathogens was investigated in mixed cropping systems, soilborne disease was significantly reduced in the mixtures. In the remaining 7 studies, there was no or a negative effect of mixed cropping on disease suppression (Table 2). In 10 cases, a positive effect was reported for splash-dispersed pathogens against five with no or negative effects (Table 2). The proposed modes of action for the reduction of soilborne diseases in mixed cropping are variable and depend on the pathogen and crops involved.

Host dilution. In most studies that report a reduction in soilborne diseases or pathogens in mixed cropping systems, host dilution is assumed to play a crucial role (Table 3). Host dilution is also regarded as the dominant disease reducing mechanism for airborne pathogens in mixed cropping systems (Mundt, 2002a). The effect of host dilution will likely be a reduction in disease incidence rather than disease severity of infected plants (Burdon and Chilvers, 1982). Host dilution might have direct as well as indirect effects on disease suppression in mixed crops. An increased inter-host distance reduces the spread of pathogens. In *Pythium*-garden cress experiments, a distance of 6 cm or more prevented disease spread (Burdon and Chilvers, 1975). Similarly, spread of Rhizoctonia damping-off

Table 2. Effects of mixed cropping on soilborne pathogens.

Nr	Main Crop	Second Crop	Type	Pathogen	Pathogen type[1]	Effect in mixture	Effect magnitude relative to sole crop	Proposed mechanism	Reference
1	Alfalfa	Wimmera ryegrass	Mixed crop[2]	*Pythium irregulare*	sb	reduced infection rate	13-44%	host dilution	Burdon and Chilvers 1976
2	Barley	Oats	Mixed crop[2]	*Rhizoctonia cerealis, Fusarium* spp.	sb	reduced disease incidence	*R. cerealis*: 6%; *Fusarium* spp: 23%	host dilution/physical barrier	Vilich-Meller, 1992
3	Barley	Wheat	Mixed crop[2]	*Fusarium* spp.	sb,	reduced disease incidence	50%	physical barrier/host dilution	Vilich-Meller, 1992
4	Oat	Berseem clover	Mixed crop[3]	*Fusarium* spp., *Phoma* spp., *Cercospora* spp., and black leafhopper*	sb and insect	improved plant health	12%	no mechanisms mentioned	Holland and Brummer, 1999
5	Radish	Mustard	Mixed crop[2]	*Rhizoctonia solani*	sb	reduced disease progress	12% and 38% (fraction mustard in mix resp 25% and 50%)	host dilution	Otten et al., 2005
6	Tomato	Cowpea	Mixed crop[2]	*Ralstonia solanacearum*	sb	reduced wilt	16%	physical barrier	Michel et al., 1997
7	Tomato	Soybean	Mixed crop[2]	*Ralstonia solanacearum*	sb	no significant reduction in wilt		physical barrier	Michel et al., 1997
8	Tomato	Welsh onion	Mixed crop[2]	*Ralstonia solanacearum*	sb	no wilt reduction	-	no barrier present at transplanting	Michel et al., 1997
9	Watermelon	Rice	Mixed crop[2]	*Fusarium oxysporum* f.sp. *niveum*	sb	reduced wilt	67%	allelopathy of root exudates on *Fusarium* spores	Ren et al., 2007
10	Wheat	Clover	Mixed crop[3]	*Gaeumannomyces graminis* var. *tritici*	sb	no significant effect on yield		-	Zogg, 1963
11	Wheat	Barley	Mixed crop[2]	*Rhizoctonia cerealis*	sb	reduced disease incidence	5-30%, depending on the previous crop	host dilution	Vilich, 1993
12	Wheat	Barley	Mixed[2] crop	*Gaeumannomyces graminis var. tritici*	sb	reduced disease severity	10-35%, depending on the previous crop	host dilution	Vilich, 1993
13	Wheat	Clover	Mixed crop[3]	*Gaeumannomyces graminis* var. *tritici*	sb	reduced disease rating in bioassay	42% (avg of 2 years)	reduced survival of the pathogen due to increased nitrogen uptake	Garrett and Mann, 1948
14	Wheat	Grasses	Mixed crop[3]	*Gaeumannomyces graminis* var. *tritici*	sb	reduced disease severity and incidence in bioassay passay	4-34%, depending on grass species cultivated	host root dilution or direct suppression effect	Gutteridge et al., 2006
15	Wheat	Trefoil	Mixed crop[3]	*Gaeumannomyces graminis* var. *tritici*	sb	reduced root infection	25%	increased densities of *Pseudomonas fluorescens*	Lennartsson, 1988

16	Bean	Maize	Row mix crop	*Sclerotium sclerotiorum*	sb	increased disease incidence and severity	1.8 (mono) vs 2.0 (mix)[4]	-not mentioned	Van Rheenen et al., 1981
17	Bean	Maize	Row mix crop	*Phoma exigua* var. *diversispora*	sb	reduced disease incidence and severity	3.0 (mono) vs 2.6 (mix)[4]	- not mentioned	Van Rheenen et al., 1981
18	Bean	Maize	Row mix crop	*Colletotrichum lindemuthianum*	sb	reduced disease Iincidence and severity	1.0 (mono) vs 0.8 (mix) [4]	- not mentioned	Van Rheenen et al., 1981
19	Bottle gourd	Chinese chive	Row mix crop	*Fusarium oxysporum* f.sp. *laganariae*	sb	reduced disease incidence	73%	stimulation of antagonists	Arie et al., 1987
20	Bottle gourd	Welsh Onion	Row mix crop	*Fusarium oxysporum* f.sp. *laganariae*	sb	reduced disease incidence	60%	stimulation of antagonists	Arie et al., 1987
21	Chinese Cabbage	wheat	Row mix crop	*Erwinia carotovara* ssp. *carotovora*	sb	no effect	-	-	Toshio, 1999
22	Chicken pea	Linseed	Row mix crop	*Fusarium oxysporum* f. sp. *ciceris*	sb	reduced disease incidence	18 % disease incidence in mixture[5]	-	Agrawal et al., 2002
23	Cotton	Sorghum and Moth	Row mix crop	*Macrophomina phaseoli* and *Rhizoctonia solani*	sb	reduced mortality	65%	decreased soil temperature	Luthra and Vasdeva, 1940
24	Garlic	Brassica carinta	Row mix crop	*Sclerotium cepivorum*	sb	reduced disease incidence	present in monocrops, absent in mixed crops	release of glucosinolates (biofumigation)	Zewde et al., 2007
25	Potato	Maize	Row mix crop	*Ralstonia solanacearum*	sb	reduced wilt	2.0 (NS) and 8.2% at low and high densitiy of the monocrop, resp.	spatial arrangement; host dilution	Autrique and Potts, 1987
26	Potato	Haricot beans	Row mix crop	*Ralstonia solanacearum*	sb	reduced wilt	3.5 (NS) and 9.7% at low and high density of the monocrop resp.	spatial arrangement; host dilution	Autrique and Potts, 1987
27	Pigeon Pea	Sorghum	Row mix crop	*Fusarium udum*	sb	reduced wilt incidence	30 %	delayed germination of spores due to Sorghum root exudates (allelopathy)	Natarajan et al., 1985
28	Sorghum	Pigeon Pea or Cow Pea	Row mix crop	*Macrophomina phaseoli*	sb	increased inoculum density	100% increase	doubling of host	Singh et al., 1990
29	Tomato	Chinese chive	Row mix cropping	*Ralstonia solanacearum*	sb	reduced wilt incidence	approx. 60%	allelopathic reduction of pathogen	Yu, 1999
30	Sugarbeet	Sugarbeet	Multilines	*Rhizoctonia solani*	sb	reduced crown and root rot	no data	host dilution	Halloin and Johnson, 2000

Nr	Main Crop	Second Crop	Type	Pathogen	Pathogen type[1]	Effect in mixture	Effect magnitude relative to sole crop	Proposed mechanism	Reference
31	Soja	Soja	Multilines	*Phytophthora sojae*	sb	effect depending on cultivar susceptibility	monoculture of resistant cultivar: 5% lower yield in multiline cropping (NS); monoculture of susceptible cultivar: 14% higher yield in multiline cropping	compensation of yield by resistant or tolerant variety	Wilcox and St. Martin, 1998
32	Oats	Oats	Multilines	*Helminthosporium victoria*	sb	reduction in disease incidence	23%	buffering effect of resistant plants (host dilution)	Ayanru and Browing, 1977
33	Wheat	Wheat	Multilines	*Wheat mosaic virus*(vectored by *Polymyxa graminis)*	sb	reduced virus disease incidencesymptoms	32%	host dilution with the unsusceptible host	Hariri et al., 2001
34	Wheat	Wheat	*Multilines*	*Cephalosporium gramineum*	sb	no reduction of disease incidence as measured by presence of whiteheads	-	-	Mundt, 2002b
35	Palm tree	*Pueraria javanica*	Multi-storey crop	*Fusarium* spp.	sb	increased half-life time of flax plants in bioassays	20-40 %	increased competition by non-pathogenic fusaria	Abadie et al., 1998
36	Barley	Oats	Mixed crop[2]	*Pseudocercosporella herpotrichoides*	splash	reduced disease incidence	10%	host dilution/physical barrier	Vilich-Meller, 1992a
37	Barley	Wheat	Mixed crop[2]	*Pseudocercosporella herpotrichoides*	splash	reduced disease incidence	50%	physical barrier/host dilution	Vilich-Meller, 1992a
38	Wheat	Barley	Mixed crop[2]	*Pseudocercosporella herpotrichoides*	splash	no effect	-	-	Vilich, 1993
39	Wheat	Clover	Mixed crop[3]	*Pseudocercosporella herpotrichoides*	splash	reduced spore dispersal	spore dispersal 50%	physical barrier, reduction of inoculum by increased decomposition (active microbial biomass?)	Soleimani et al., 1996

40	Wheat	Clover	Mixed crop	*Septoria tritici*	splash	reduced number of lesions per flag leaf	approx. 50%	sieving effect clover	Bannon and Cooke, 1998
41	Bean	Maize	Row mix crop	*Pseudomonas syringae pv. phaseolicola*	splash	increased disease severity	20-24%	favourable microclimate in mixed crop	Mabagala et al., 1992.
42	Pepper	Wheat	Row mix crop	*Phytophthora capsici*	splash	reduced disease incidence or severity when sown in stubble	2.5-43 %	reduction of inoculum dispersal	Ristiano et al., 1997
43	Strawberry	Sudan grass	Row mix crop	*Colletotrichum acutatum*	splash	reduced spread of C. acutatum spores	19-49% less spores depending on rain and crop density	reduction of dispersal	Ntahimpera et al., 1998
44	Strawberry	Grass	Row mix crop	*Diplocarpon earlianum*	splash	reduced spread of diplocarpon spores	-	reduction of dispersal	Newenhouse and Dana, 1989
45	Barley	Barley	Multilines	*Rhynchosporium secalis*	splash	no effect	-	-	Abbott et al., 2000
46	Wheat	Wheat	Multilines	*Pseudocercosporella herpotrichoides*	splash	no effect	-	-	Saur and Mille, 1997
47	Barley	Barley	Multilines	*Rhynchosporium secalis*	splash	reduced disease severity	Up to 50% depending on mixture composition	host dilution, morphological factors influencing dispersal	Newton et al., 1997
48	Wheat	Wheat	Multilines	*Mycosphaerella graminicola*	splash	reduced disease severity	17%	host dilution	Mundt et al., 1995
49	Wheat	Wheat	Multilines	*Pseudocercosporella herpotrichoides*	splash	no disease reduction		host dilution	Mundt et al., 1995
50	Wheat	Wheat	Multilines	*Mycosphaerella graminicola*	splash	contradictory results	-	-	Cowger and Mundt, 2002

[1] Sb is soilborne, splash is splash dispersed pathogen
[2] Crops completely widespread sown, at least not sown in rows
[3] One crop sown in rows, other crop broadcast sown
[4] Disease scores on a scale from 1 (no disease) to 5 (crop completely destroyed)
[5] No data from incidence in single crop

in radish-mustard mixtures decreased with increasing densities of the non-host mustard plants and spread halted below a host threshold density (Otten et al., 2005). Below this threshold distance, pathogen expansion is said to become invasive. The threshold distance is affected by the availability of nutrient resources and interactions with the microflora. Using the percolation theory developed in physics, Bailey et al. (2000) described the expansion of foci in microcosms of *Rhizoctonia solani* as probability events for invasive spread. This, however, is only applicable for pathogens that are able to colonize the soil environment by saprotrophic growth. It may be interesting to include a time dimension in the percolation theory that describes change in substrate quality.
With increasing densities of susceptible roots, disease spread may accelerate if secondary root infections occur as can be the case for *G. graminis* (Bailey and Gilligan, 2000) and *R. solani* (Otten et al., 2005). Such secondary infections likely occur at a lesser rate because of larger inter-root distances in mixed crop systems. For pathogens with a wide host range such as *R. solani*, low or moderately susceptible plants may also serve as nutrient source without expressing striking disease symptoms (Otten et al., 2005), thus reducing the host dilution effect. The intensity of root intermingling in mixed cropping may be an important determinant for these processes (Kroon, 2007) and the level of disease suppressiveness may therefore be determined by the crops or cultivars grown.

In contrast to pathogens capable of saprothropic growth, host dilution has hardly an effect on pathogens without a saprotrophic phase, such as powdery scab (*Spongospora subterranea*), Verticillium wilt, and clubroot (*Plasmodiophora brassicae*). For splash-dispersed pathogens in mixed cropping, the host dilution effect is comparable to airborne pathogens and is also influencing disease incidence more than severity. The non-host crop simply acts as a physical barrier, thus reducing disease spread as has been shown for *Pseudocercosporella herpotrichoides*, the causal agent of eyespot in cereals (Villich-Meller, 1992). The barrier function can reduce the impact of raindrops thus reducing dispersal, and it can intercept splashing spores that would reach the host under conditions of monoculture (Soleimani et al., 1996; Ntahimpera, et al., 1998).

Allelopathy. Allelopathy is defined as any biochemical interaction among plants, including those mediated by microorganisms, of which the result can be either detrimental or beneficial to the interacting plants (Wu et al., 2001). In five studies, it was suggested to play a role in disease suppression in mixed cropping (Table 2). When watermelon was intercropped with rice, allelopathic substances from rice roots reduced production and germination of conidia of *Fusarium oxysporum* f. sp. *melonis*, leading to a 67% reduction in wilt (Ren et al., 2007). The allelopathic exudates only reduced Fusarium conidial density in the rhizosphere and not in bulk soil indicating a relatively fast degradation of the allelopathic compound(s) or a limited diffusion. Delayed germination of spores of *F. udum*, causing wilt in pigeon pea, has been attributed to allelopathic substances exuded from sorghum roots (Natajaran et al., 1985). To be effective in inhibition of rhizosphere-inhabiting pathogens, allelopathic substances should be present at sufficiently high concentrations in the microsites where the pathogen is located, and roots of mixed crops should be in close proximity.

An interesting question is whether allelopathy causes death of the pathogen propagules (Ren et al., 2007) or only delays germination (Natajaran et al., 1985). In the

latter case, the effect would resemble fungistasis, which is the general phenomenon of restriction of germination and growth of fungal propagules in soil (Lockwoord, 1977). A high level of soil fungistasis is often assumed to be accompanied by a high level of general disease suppression (Hornby 1983, Lockwood, 1986; Janvier et al., 2007). Fungistasis can however also be regarded as a mechanism of delayed activity if conditions are unfavourable for the pathogen, which is also the case if non-lethal allelopathic substances are formed temporarily. The effect can be detrimental, but also profitable to the pathogen as germination in absence of a host plant is, generally, not a desirable trait for pathogens. Roots of non-hosts can stimulate the germination of the survival propagules of the pathogen (Mol and van Riessen, 1995) leading to a decline in the inoculum density. In relay mixed crops, this premature germination might have a disease suppressive effect, especially in combination with inoculum burial and enhanced microbial antagonism.

Biofumigation by Brassica's (Kirkegaard and Sarwar, 1998) in mixed cropping systems has been proposed as a mechanism to suppress soilborne pathogens (Haugaard Nielsen and Jensen, 2005), although with the exception of the work by Zewde et al., (2007), convincing data are not yet available. This is in contrast with studies on the biofumigation potential of Brassica crop residues (Kirkegaard and Sarwar, 1998, Smolinska et al., 2003), which showed disease suppression for various soilborne pathogens especially in controlled greenhouse experiments.

Table 3. Disease reducing mechanisms in mixed cropping systems for soilborne and splash dispersed pathogens described in literature.

Mechanism	Soilborne pathogens	Splash-dispersed pathogens	Total
Host dilution	12	5	17
Allelopathy (including biofumigation)	5	0	4
Antagonists	4	0	4
Inoculum reduction	2	0	2
Unfavorable microclimate	1	1[1]	1
Compensation (yield)	1	0	1
Physical barrier	0	5	5
Not mentioned	5	0	5
Total positive effects	29	10	39
Negative or no effects	6	5	11
Total	35	15	50

[1] Both physical barrier and unfavorable microclimate are mentioned for disease suppression, in totals therefore only taken up once (as physical barrier).

Microbial antagonists. In four of the cropping systems listed in Table 2, enhanced antagonistic populations were proposed as a main mechanism for disease reduction in mixed cropping systems. In three cases, pseudomonads and probably antibiotics were involved. For example, wheat root infection by *G. graminis* var. *tritici* was reduced by 25% in wheat-trefoil (*Medicago lupulina*) mixes (Lennartsson, 1988). Also an increased occupation of available niches by non-pathogenic Fusaria was held responsible for an increased disease suppression in oil palm-legume mixed cropping (Abadie et al., 1998). The build-up of populations of antagonistic microorganisms has been studied mostly in single crop systems. It seems that the natural build-up of antagonists to levels where they are effective takes place mostly due to selection or even co-evolution, *i.e.*, continuous cultivation of the same single crop in presence of the pathogen (Schneider, 1982; Weller et

al., 2002). Nevertheless, also in these agro-ecosystems the fate of introduced antagonistic microorganisms is often inconsistent (Whipps, 2001). Rhizosphere microbial communities, including pathogens, antagonists and plant growth promoting bacteria are crop- and cultivar specific (Smith et al., 1999; Germida and Siciliano, 2001) and it might be worthwhile to investigate if these communities can be manipulated by the choice of cultivars in a mixed cropping setting. Crop or cultivar specific resistance against races of pathogens is widely known and is often applied, even in mixed crops (Mundt, 2002a). Mazzola and Gu (2002) used wheat to stimulate the natural antagonistic populations of fluorescent pseudomonads, which led to control of apple replant disease. The rhizospheres of old wheat cultivars were less aggressively colonized by fluorescent pseudomonads than those of modern ones (Germida and Siciliano, 2001). Among tomato lines, genetic differences correlated with *Pythium* suppression by *Bacillus cereus* and growth of this biocontrol agent on seeds (Smith et al., 1999). Also legumes may stimulate and support antagonistic *Rhizobium* bacteria in the rhizosphere (Dakora, 2003; Simpfendorfer et al., 1999), which might result in increased pathogen suppression in mixed crops. When growing white clover together with triticale, take-all disease was reduced (Hiddink et al., 2004), although the exact disease suppressive mechanism remains elusive.

In mixed crops, increased plant diversity leads to more diverse root exudates and consequently to a more diverse rhizosphere-inhabiting microbial community (Westover et al., 1997; Kowalchuck et al., 2002). Rhizospheres of mixed crops support different bacterial and fungal microbial communities compared to the corresponding single crop rhizospheres (Song et al., 2007; Hiddink et al., 2004). On the other hand, the effect of mixed cropping on the bulk soil microbial community has not been shown (Kowalchuck et al., 2002; Hiddink et al., 2005a). In a more biodiverse setting, the likelihood to encounter microorganisms with antagonistic properties is higher, but at the same time their densities are expected to be lower under these conditions. However, if a higher biodiversity would mean a higher diversity in functions, a higher rate of consumption of root exudates could be expected, which relates to the root camouflage concept proposed by Gilbert et al., (1997). Although increased microbiological diversity is often referred to as an important indicator for soil health (Doran and Zeiss, 2000; Van Elsas et al., 2002; Mäder et al., 2002), its effects can be both positive (more consumption of root exudates, more antagonists) and negative (potentially effective antagonists suffer more from competition and fail to establish and be active).

For bulk soil, an increased microbial diversity seems to be related to increased disease suppression. Hiddink et al. (2005a) reported that higher diversity indices were correlated with a lower disease severity. Suppression of corky root of tomato, caused by *Pyrenochaeta lycopersici*, was related to a more diverse actinomycete community in bulk soil (Workneh and van Bruggen, 1994). Although mixed cropping does increase rhizosphere microbial diversity, the effect on bulk soil biodiversity seems limited (Hiddink et al., 2005a).

Discussing the effect of microbial diversity on disease suppression is complicated since proper methods to quantify diversity are still under development. Cultivation-based approaches do not take into account the unculturable species, whereas cultivation-independent approaches such as analysis by Denaturing Gradient Gel Electrophoresis (DGGE) underestimate the microbial diversity in soil as only the more abundant species

(approx. 0.1-1% of the microorganisms present) are detected (Muyzer et al., 1993). One may assume, however, that the abundant species will also harbor species that contribute to competition for nutrients and space. Another challenge is linking microbial diversity to ecological function (Nannipieri et al., 2003, Hiddink et al., 2005a). The degree of functional redundancy (here: with respect to disease suppression) could perhaps be regarded as a reliable measure for disease suppression, but how this redundancy could be measured is yet unclear (Giller et al., 1997; Nannipieri et al., 2003). This could explain why a high biodiversity can be considered a desirable trait, but until indicators quantifying functional redundancy have developed this topic will remain largely speculative.

There clearly is a contradiction between desiring a high functional diversity on the one hand and a high establishment of a given antagonist on the other hand. In soils with a high microbial diversity, a low conduciveness for establishment and growth of an introduced antagonist or pathogen is to be expected. If disease suppression would be controlled by a single antagonist, a high microbial diversity would then be an undesirable trait of soils. This is in line with the observation that establishment of pseudomonads in organic soils (which showed a higher microbial diversity) is more limited than in conventional soils (Hiddink et al., 2005b).

Microclimate. Mixed cropping generally changes the microclimate. Higher soil coverage leads to lower soil temperatures which have been associated with lower disease incidence of *Macrophomina phaseolina* and *Rhizoctonia solani* in cotton-sorghum mixtures (Luthra and Vasdeva, 1940). The lower level of disease severity of the splash-dispersed *Pseudocercosporella herpotrichoides* in wheat-clover systems was attributed to a higher decomposition rate of organic material that serves as a base for survival of the pathogen spores (Soleimani et al., 1996). However, increased moisture content in the mixed crop can increase airborne diseases, and also several soilborne pathogens such as Pythium spp. can be expected to survive and disperse easier in moist soils. Likewise, halo blight caused by *Pseudomonas syringae* pv. *phaseolicola*, was more severe in mixed bean/maize than in a single bean crop (Mabagala and Saettler, 1992).

Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR). Mixed cropping can bring about ISR (induced by non-pathogenic microorganisms) or SAR (other stress inducers like water stress, salinity, allelopathic substances or pathogens) if one crop creates the right condition for ISR/SAR inducers for which the alternate crop is sensitive (Hammerschmidt et al., 2001). Such a mechanism has however not been suggested (Table 2), probably because of difficulties in proving it experimentally. Induced resistance can be due to direct effects of the root exudates or indirect effects via root exudate-affected microbial populations (Kloepper et al., 1992). ISR has been mentioned as a mechanism for reduction of several airborne pathogens such as powdery mildew in barley cultivar mixtures (Chin and Wolfe, 1984).

Nutrients and disease development. Nutrients can affect disease development, above and belowground (Walters and Bingham, 2007). In mixed crops, uptake of nitrogen from undersown clover reduced take-all disease severity in barley (Garrett and Mann, 1948). Not only amount but also the form of nitrogen is important. Exudation of ammonium from

clover roots (Paynel and Cliquet, 2003) may lead to a reduction of the rhizosphere pH in cereal roots, thereby influencing the antagonistic microbial population and decreasing infection by G. graminis (Smiley, 1978; Sarniquet et al., 1992). Also availability of several other elements such as potassium, phosphorus, sulphur and silicon will influence disease development direct or indirectly (e.g. Walters and Bingham, 2007) in mixed crops but are not further discussed in this review.

Practical feasibility of mixed cropping

Although it is clear that mixed cropping can reduce soilborne diseases, it also has an inherent weakness: the presence of multiple crop species may also bring about a greater variety of soilborne pathogens albeit likely at lower densities for each of the crops. Another complication is whether and how mixed crops should be rotated and what the choice of rotation crops in time should be. When rotated, mixtures of wheat or barley containing oats resulted in lower disease levels in the crops the following year than mixtures of barley and wheat (Vilich, 1993). An additional question that should be addressed is: does mixed cropping of two crops continuously for two (or more) years lead to less disease than growing those same two crops in rotation? It is surprising that, to the best of our knowledge, this question cannot be answered based on the data available in the current literature. The situation can be complex, as was shown by Hiddink et al. (unpublished). In their study, take-all disease was lower during three consecutive years in a triticale-white clover field compared to single cropping triticale. However, in the fourth year, *Fusarium* infected white clover and reduced its stand, which in turn caused an increase in take-all in triticale in the mixture to a disease level above that obtained in the single cropped triticale. Soilborne pathogens with broader host ranges or long-term survival structures are likely to be less suppressed in mixed crops grown repeatedly. If pathogens like the *Fusarium* in clover (Hiddink et al. unpublished), are not actively suppressed by the co-ocurring crop, inoculum will continue to build up and rotating the crops in the mixture would have been a better tool to suppress the pathogens. To manage mixed crops to suppress soilborne diseases requires advanced skills of the farmer and knowledge of the diseases that might cause diseases in both mixed crop components. It can be more labor-intensive and not suitable for mechanized production in all crops. Certain crops are not suitable to grow in mixed crops because of their weak competiveness and the degree of intercrop competition is decisive whether a certain combination can be grown. Thus, although clubroot, caused by *Plasmodiophora brassicae*, was reduced in a barley-Brussels sprouts mixed crop, yield of Brussels sprouts was reduced by nearly 50% because of competition (Hiddink et al., unpublished). However often an overall yield increase is observed in mixed crops. This effect is generally expressed as the Land Equivalent Ratio (LER, Vandermeer 1990). The LER is the sum of the yields of both components per unit of land area combined divided by the area of land needed to obtain the same yields when both components are grown as single crops (Vandermeer 1990). Mixed crops have been grown for ages, because of their yield stability and they are still practiced for this reason in tropical regions (Vandermeer, 1990). Co-occurring crops compensate for failure of one of the crops due to soil- and airborne pathogens, weeds, temperature- and water stress (Vandermeer, 1990). Furthermore, growth compensation is an important effect of mixed cropping.

Conclusions

All three cropping systems discussed can contribute to the management of soilborne pathogens. Crop rotation is the most generally applied method to manage soilborne pathogens. However, while well-defined rotation schemes to reduce specific soilborne pathogens exist, for several pathogens these solutions are not available. Continuous cultivation of the same crop can result in a persistent decline of the pathogen, as is the case for take-all disease of cereal crops. However, continuous cultivation of the same crop has not been 'invented' as a management tool for soilborne pathogens *per se*, but induction of disease suppression is a complementary benefit in situations where no options other than continuous cultivation of single crops are available. This specific suppression usually is only active against a single pathogen leaving opportunities for other soilborne pathogens to develop and cause disease. Mixed cropping has been practiced for ages in all sorts of combinations, although not specifically designed for suppression of soilborne pathogens, but rather as an insurance against crop failures and soil erosion.

In all three types of cropping systems, multiple disease reducing mechanisms are active, but mixed cropping offers the most pluriform types of disease suppression because root systems of different crop species interact. In mixed cropping systems, the most important disease reducing mechanism appears to be host dilution. The magnitude of the effect depends on the planting density, the type of mixed cropping and root architecture of the crops grown. Competition will affect the distribution of roots in mixed crops (reviewed by Hauggaard-Nielsen and Jensen, 2005; de Kroon, 2007). Allelopathic effects, nutrient concentrations and water flow will determine how the roots interact and the diversity of (microbial) interactions in the rhizosphere (Bowen and Rovira, 1976). Furthermore, as long as host species are mix-cropped with non-hosts in lower densities, host dilution will inevitably lead to a reduction of the number of diseased plants per area.

Other factors that result in disease suppression like allelopathy and antagonism induced by the non-host crop depend on characteristics of all crops present in the mix. Biofumigation using Brassica species in mixed cultivation has received attention recently, but its effectiveness is still limited (Hiddink et al., 2005a). Breeding for Brassica species exhibiting higher glucosinolate contents is an option to increase their effectiveness (Matthiessen and Kirkegaard, 2006). More effective suppression can be expected from legumes, which can excrete allelopathic root exudates and support potentially antagonistic microorganisms (Dakora, 2003). Also use of specific crops and cultivars that support antagonistic microorganisms (Smith et al., 1999; Mazzola and Gu, 2002) can be a valuable tool to create mixtures that actively suppress soilborne pathogens.

In spite of the prospects of mixed cropping for management of soilborne pathogens, it will not be a panacea for combating pathogens, but it can contribute substantially in some cases. However, design of mixed cropping as a tool for suppressing plant pathogens is still in its infancy and much can be done to optimize the disease suppressive effects based on allelopathy, antagonism and other factors. Effects of mixed cropping beyond the ones described here for pathogen control should not be overlooked. Reduction in plant pests and weeds has been reported widely (Bukovinszky, 2004; Baumann et al., 2001). Failure or stress of one crop results in lower competition and can increase the production of the accompanying crop and thus increase yield stability. Another important benefit is the higher possible total yields per area of cultivated land. This would

reduce the plant production acreage needed to produce a certain amount thus using more efficiently the available production factors and reducing nutrient leaching, water run-off, and soil erosion per unit of yield. More production per area of land also means that tension between the area of land needed for human and animal consumption on the one hand and bio-fuels on the other hand can be loosened to a certain extent if they can be grown on the same area of land at the same time.

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Chapter 4

Effect of mixed and single crops on disease suppressiveness of soils

Hiddink G.A., Termorshuizen A.J., Raaijmakers J.M., and van Bruggen A.H.C.
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Abstract

The effect of mixed cropping on disease suppressiveness of soils was tested for two cropping systems, Brussels sprouts – barley, and triticale – white clover. Disease suppressiveness of field soils was evaluated in bioassays for the soilborne pathogens: *Rhizoctonia solani (*Rs*)*, *Fusarium oxysporum* f.sp. *lini (*Fol*)*, and *Gaeumannomyces graminis* var. *tritici (*Ggt*)*. For both cropping systems, mixed cropping did not enhance disease suppressiveness of the soils. In some cases, soil cropped to barley alone was significantly more suppressive to Fol than soils cropped to Brussels sprouts or the mixture of Brussels sprouts and barley. Analyses of the diversity of the indigenous bacterial and fungal microflora by denaturing gradient gel electrophoresis (DGGE) of amplified 16S- and 18S-rDNA fragments, respectively, revealed, in most cases, no significant differences between mixed and monocropped soils. In conclusion, in this study mixed cropping of soils with Brussels sprouts and barley or with triticale and white clover, did not enhance microbial diversity or disease suppressiveness of soils to three different soilborne plant pathogens.

Introduction

Intensification of agriculture has contributed substantially to the increase in world food production over the past 50 years (Matson et al., 1997). However the ecological side effects, including soil erosion, water pollution, eutrophication and reduced soil fertility may be as profound as the increase in food production. Concerns have developed over the long-term sustainability and environmental consequences of the intensification of the agricultural system (Matson et al., 1997). Therefore a change to a more sustainable way of food production has been advocated over the past decade.

One of the constraints to future food production might be the occurrence of soilborne pathogens as serious threats of crops. Most soilborne pathogens are difficult to control and resistance in plants to many soilborne pathogens is lacking or has not been fully explored (Whipps, 1997). Crop management strategies, including mixed cropping, might provide a way to reduce deleterious effects from soilborne pathogens.

There are some examples that show that monoculture can lead to specific disease decline in some pathosystems when a susceptible crop is grown continually in the absence of rotation (Whipps, 1997). In general, however, continuous cropping of one specific crop leads to an increase in disease incidence caused by soilborne and other plant pathogens. Most natural and agricultural soils exhibit some degree of suppressiveness to soilborne plant pathogens. This phenomenon is referred to as general suppression or general

antagonism (Gerlagh, 1968; Hornby, 1983; Weller et al., 2002), which appears to be non-specific and active against a wide range of soilborne pathogens. Mixed cropping might stimulate these general disease suppressive properties of soils.

Mixed cropping is a way of farming practiced by many traditional farmers (Geno and Geno, 2001; Vandermeer, 1989). In contrast to single cropping, two or more crops are grown in the same field at the same time (Vandermeer, 1989). Farmers using mixed crops could be less dependent on the input of pesticides because of lower pest and disease pressure and fertilizer inputs due to higher efficiency of nutrient use (Geno and Geno, 2001). Therefore, mixed cropping has been proposed to lead to a more sustainable farming system (Vandermeer, 1989).

Much effort has been made to investigate mixed cropping systems, especially with respect to the ecological processes involved in crop performance (Geno and Geno, 2001). The relation between crop or cultivar mixtures and disease severity has been investigated extensively, but has mostly focused on disease caused by aboveground pathogens like rice blast (Zhu et al., 2000) and foliar pathogens of small grains (Mundt, 2002). Effects of mixed cropping on soilborne pathogens have been described less frequently (Abadie et al., 1998; Autrique and Pots, 1987; Burdon and Chilvers, 1976; Vilich, 1993). For example, Burdon and Chilvers (1976) showed reduced spread of *Pythium* in a garden cress–ryegrass mixture and Villich (1993) reported a reduction in disease severity of soilborne pathogens in barley – wheat mixtures. In these experiments, disease suppressiveness was investigated for pathogens specific to one or both of the crops included in the mixed cropping. It is not known, however, if mixed cropping also stimulates the general (non-specific) disease suppressiveness of soils to a variety of soilborne pathogens.

Soil biological processes underlying suppressiveness to soilborne pathogens have not been investigated extensively for soils with mixed crops. Suppressiveness to soilborne pathogens is affected by biological, chemical and physical characteristics of the soil (Whipps, 1997). Soil biological characteristics are strongly influenced by plant species composition, diversity and sequence. Plant species composition and specific plant traits have more important effects on soil ecological processes than species richness of crop mixtures Hooper and Vitousek, 1997; Wardle et al., 1999). Increased plant diversity can stimulate microbial community biomass, respiration and fungal abundance (Zak et al., 2003), and removal of particular plant species from mixed plant communities can influence soil microbial community composition and diversity, the associated food webs, and as a result ecosystem properties and processes (Wardle et al., 1997) in bulk soil. Some of these processes, including microbial activity, are known to affect soilborne pathogen behavior (Hornby, 1983; Weller et al., 2002; Workneh and van Bruggen, 1994). In a limited number of cases soil microbial diversity has been directly linked to root disease suppression (Nitta, 1991; Workneh and van Bruggen, 1994). For example, corky root of tomato was more suppressed in organic soils containing a more diverse population of actinomycetes than conventional soils containing a less diverse population (Workneh and van Bruggen, 1994). More diverse microbial populations have a higher chance to harbor particular species that can suppress soilborne plant pathogens.

The objectives of the present study were to determine the effect of mixed cropping on 1) general disease suppressiveness of bulk soils to three different soilborne pathogens, 2) microbial diversity and activity in bulk soils, and 3) the relation between microbial

diversity, activity and general disease suppressiveness in those soils. Field soils cropped to Brussels sprouts, barley, triticale, white clover and the mixtures Brussels sprouts - barley and triticale – white clover were evaluated in greenhouse assays for their suppressiveness to *Rhizoctonia solani (*RS*), Fusarium oxysporum* f.sp. *lini (*FOL*),* and *Gaeumannomyces graminis* var. *tritici (*GGT*).* Three different pathosystems were used because different pathogens generally react differently to biotic and abiotic properties of a soil (Grünwald et al., 2000). We used two pathosystems with crops (carrot with *R. solani* and flax with *F. oxysporum* fsp. *lini*) not grown in the field to circumvent the effect of adaptation of the microbial community, including soilborne pathogens, to a certain host. Results of tests for general disease suppressiveness could otherwise be confused with specific disease suppression phenomena. Diversity of the indigenous bacterial and fungal microflora was determined with denaturing gradient gel electrophoresis (DGGE) of amplified 16S- and 18S-rDNA fragments, respectively, and related to disease severity determined in the different bioassays.

Materials and methods

General set-up. At two locations near Wageningen (The Netherlands), Wageningen-Hoog (WH) and Achterberg (AB), barley, Brussels sprouts and their mix were grown for two consecutive years. At another location in Wageningen, Bornsesteeg (BSW), triticale, clover and their mix were grown for three consecutive years. Crops were grown organically (for details, see below). Soil was sampled from the field at various points in time and bioassays were performed to determine disease suppressiveness of the soils against the plant pathogens *Rhizoctonia solani* Kühn (AG1) (using carrot as test plant), *Fusarium oxysporum* Schlecht.: Fr f.sp. *lini* [(Bolley) Snyd.&Hans.] (flax as test plant), and *Gaeumannomyces graminis* (Sacc) v. Arx & Olivier [senso lato] var. *tritici* J. Walker (triticale or barley as test plant) (Table 1).

Table 1. Host-pathogen systems used to test disease suppressiveness in soil samples collected from fields with mixed and single crops.

Pathogen	Host	Bioassay[w]	Mixed crops	Date[x]	Years of crop growth[y]
Rhizoctonia solani (AG1)	Carrot	RS1	B. sprouts - barley[z]	05-11-01	1
		RS2	Triticale - white clover	06-12-01	1
Fusarium oxysporum f.sp. *lini*	Flax	FOL1	B. sprouts - barley	07-16-01	2
		FOL2	Triticale - white clover	06-23-03	3
Gaeumannomyces graminis var. *tritici*	Barley	GGT1	B. sprouts - barley	02-22-02	2
	Triticale	GGT2	Triticale - white clover	06-25-02	2

[w] Bioassays with soils from different locations and cropping history were performed with *Rhizoctonia solani* on carrots (RS1 and RS2), *Fusarium oxysporum* f.sp. *lini* on flax (FOL1 and FOL2) and *Gaeumannomyces graminis* var. *tritici* on barley or triticale (GGT1 and GGT2).
[x] Starting date of the bioassay.
[y] Number of years of the cropping history at the start of the bioassay.
[z] Brussels sprouts.

Table 2. Properties of the soil samples used in the bioassays.

Location[x]	Soil type	OM[y] %	N %	C %	P %	K %	C/N[z]	Clay %	Silt %	Sand %
Wageningen Hoog (WH)	sand	3.4	0.11	1.68	0.09	0.12	14.7	1.4	7.5	91.1
Achterberg (AB)	Sand	3.5	0.12	2.01	0.05	0.14	16.6	1.3	6.1	92.6
Bornsesteeg Wageningen (BSW)	Sand	4.9	0.18	2.63	0.07	0.21	14.9	2.1	11.9	86.0

[x] Locations are all within 10 km distance from the city of Wageningen, The Netherlands.
[y] Organic matter content, determined by glow loss method (Ball, 1964).
[z] C to N ratio.

Field experiments. Barley (*Hordeum aestivum* L., cv. Video, Samenwerkende Graankweekbedrijven Wiersum-Zelder, The Netherlands), Brussels sprouts (*Brassica oleracea* L., cv. Maximus, Syngenta, Basel, Switzerland) and their mix were grown at WH and AB for two consecutive years (2000 and 2001) in two replicates at each location. Plots measured 25 × 25 m. Fields were fertilized annually in spring with 25 ton ha^{-1} pig manure (NPK kg ha^{-1}, 180:105:180) and plowed to a depth of 30 cm. Organically grown, six-week-old Brussels sprouts plants obtained from a plant nursery, 'West Plant Group' (Venlo, the Netherlands), were planted in May at a distance of 50 and 75 cm within and between rows, respectively. Barley was sown the same day at an inter-row spacing of 12.5 cm, an intra-row spacing of approximately 2.5 cm, and a quantity of 125 kg seed ha^{-1}. In the crop mixture, five rows of Brussels sprouts were alternated with five rows of barley, each at the same inter- and intra-row distances as the respective single crops. Mechanical weed control was performed in the single crops two to three times during the growing season. Mechanical weeding was not possible in the mixed crops but was done manually when necessary. Later in summer both single and mixed crops were hand-weeded.

Triticale (X *Triticosecale* Wittm. cv. Galtjo, Svalöf–Weibull B.V., Emmeloord, The Netherlands), white clover (*Trifolium repens* L., cv. Pertina, Cebeco Zaden, Vlijmen, The Netherlands) and their mix were grown at BSW in three replications during three growing seasons (2000 – 2003). Fields were fertilized with 30 ton ha^{-1} farmyard manure (NPK kg ha^{-1}, 207:114:222) incorporated with a rotary spade digger to a depth of 30 cm. Triticale and white clover were sown on the same day between the 28th of September and the 11th of October. Triticale and white clover single crops were each sown at an inter-row distance of 25 cm and intra-row distance of 2.5 and 0.5 cm, respectively, (125 kg ha^{-1} for triticale and 15 kg ha^{-1} for white clover). For the mixed crops, triticale and white clover were sown additively in alternating rows at the same distances and amounts as the single crops. Triticale plots were harrowed several times to remove weeds. White clover and the mixed crop were not harrowed to prevent damage to the white clover. In late spring hand weeding was performed in all plots.

Soil sampling. At various times (Table 1) and from 10 to15 random sampling points in each plot soil was sampled from the upper 15 cm and pooled per plot. A small spade was used to sample the soil (200 to 300 g soil per subsample) and collected in plastic bags. The spade was cleaned between plots. The same day, the soils were sieved through a 4 mm mesh sieve, thoroughly mixed and used for one bioassay. From this batch, subsamples were taken, pooled and stored at –20˚C until DNA-extraction. Soil samples for general soil and nutrient analyses (approx. 100 g) were air-dried at room temperature for 7 days and stored

in closed containers until further analysis.General soil characteristics (Table 2) were determined by the Department of Soil Quality, Wageningen University, (Wageningen, The Netherlands) and were similar for all three locations.

Rhizoctonia solani bioassays (RS1 and RS2). Pipes (PVC, polyvinyl chloride) were longitudinally cut in half (4.4 × 2.2 × 40 cm) and holes were made at every 10 cm in the bottom to allow water–uptake when necessary. The pipes were placed horizontally on a greenhouse bench and filled with field soil. The greenhouse temperature was maintained at ± 20°C. Carrot seeds (*Daucus carotas* L. cv. Amsterdamse Bak, Pieterpikzonen, Heerenveen, The Netherlands) were surface-sterilized for 1 min in 1% NaOCl, rinsed with running tap water for 5 min and sown in a 35-cm-long row with 5 to10 carrot seeds every 2.5 cm. Pipes were placed on a bench covered with a moist irrigation mat (BWM 300 plus (water holding capacity 300 g m^{-1}), Henofa bv, The Netherlands) in the greenhouse in a randomized complete block design with 4 replications and covered with plastic to maintain a relative humidity of 100%. *Rhizoctonia solani* (AG1, isolate 55, originally isolated from *Cotoneaster* sp.) (Tuitert et al., 1998) was grown on malt extract agar (MEA) for 2 to 3 days at 25°C in darkness. Immediately after seedling emergence (approx. 5 days) an agar plug (5–mm-diam.) with actively growing mycelium of a 2 to 3-day-old colony of *R. solani* was placed, 1 cm below the soil surface, at one end of the row carrots in each pipe. Pipes that were not infested served as controls. When necessary the growth blanket was rewetted with water, no fertilizer was added during the experiment. Progress of the disease was determined by counting the infected seedlings twice per week. The experiment was terminated when the pathogen reached the end of a row carrots in one of the treatments.

Fusarium oxysporum bioassays (FOL1 and FOL2). Fusarium oxysporum fsp. *lini* (isolate Foln3, kindly provided by P. Lemanceau, INRA, Dijon, France) inoculum was prepared by growing the fungus in malt extract broth (Oxoid, Ltd, Basingstoke, Hampshire, England) for 3 weeks at 25°C on a rotary shaker. The conidial suspensions were centrifuged (4000 rpm) and washed three times with sterile deionized water. The conidia were dried for two weeks in talc (Merck KgaA, Darmstadt Germany) in a container with a sterile airflow. Conidial density was determined by dilution plating on Komada medium (Komada, 1975) with 0.15 g $liter^{-1}$ oxytetracycline instead of streptomycin sulfate. Soils were mixed with talc inoculum to a final density of 2×10^5 conidia g^{-1} soil. Control soils were mixed with talc only. Plastic pots (7 × 7 × 8 cm) were filled with 150 g (dry weight) of infested or control soil and placed in the greenhouse (20°C, RH 70%) in a randomized complete block design with 6 replicates. Flax seeds (*Linum usitatissimum* L. cv. Opaline, kindly provided by P. Lemanceau, INRA, Dijon, France) were surface-sterilized as described above. Seeds were left overnight at 20°C in a sterile petri dish on moistened sterile filter paper to germinate. The next day, 10 germinated seeds were sown per pot and covered with a layer of 25 g of uninfested soil. Plants were watered on dishes when necessary and 10 ml of full strength Hoagland solution (0.005 M $CaNO_3$; 0.005 M KNO_3; 0.001 M KH_2PO_4; 0.002 M $MgSO_4\times7H_2O$; 0.5% Fe tartrate and 1 ml micronutrient solution (2.86 g l^{-1} H_3BO_3, 1.81 g l^{-1} $MnCl_2 \times 4\ H_2O$, 0.22 g l^{-1} $ZnSO_4 \times 7H_2O$, 0.08 g l^{-1} $CuSO_4 \times 5H_2O$, 0.02 g l^{-1} $H_2MoO_4 \times H_2O$) was added once a week. The number of diseased plants per pot, scored once a week, was used to calculate the area under the disease progress curves (AUDPC) (Campbell and

Madden, 1990). The AUDPCs from the non-infested pots were subtracted from AUDPCs calculated for the infested pots.

Gaeumannomyces graminis bioassays (GGT1 and GGT2). Gaeumannomyces graminis var. *tritici* (isolate R3-111-a-1) (Raaijmakers and Weller, 1998) inoculum (ground oats, particle size 0.2 to 0.5 mm) was mixed with the soils to a final concentration of 0.5% (w/w). Control pots were infested with autoclaved inoculum at the same concentration. Pots were filled with 150 g (dry weight) infested soil and covered with 25 g of uninfested soil. In every pot five, surface-sterilized (1% NaOCl for 1 min and rinsed 5 min in running tap water), pregerminated seeds of barley or triticale were sown and covered with 25 g uninfested soil (in total 50 g uninfested soil). Pots were placed in the greenhouse in a completely randomized block design with four replicates. Plants were watered when necessary and 10 ml of full strength Hoagland solution was added once per week. Root disease severity was scored after 4 weeks of plant growth on a scale from 0 to 8 (0 = healthy, 1 = small lesions on 1 root, 2 = larger lesions on 2 or more roots, 3 = 1 lesion reaching the stem base, 4 = 2 or more lesions reaching the stem base, 5 = stem base infected, 6 = infection reaching the stem, 7 = plants yellowing and wilting, 8 = dead plant) (Thomashow and Weller, 1988).

DNA-extraction. From the soil samples stored at –20˚C, DNA was extracted using the FastDNA SPIN Kit for soils (Bio101 systems, Carlsbad, CA), according to manufacturer's protocol with some small adaptations. All buffers used, were provided by the manufacturer in the kit. In short, 500 mg soil was added to 978 µl Phosphate-buffer (pH 8.0) and 122 µl MT-buffer; the mix was subjected to a bead-beater (Braun Celhomogenisator model MSK, B.Braun Biotech International, Germany) three times for 90 s and centrifuged 5 min at 14,000 × *g*. The supernatant was added to 250 µl PPS-reagent (Protein Precipitation solution), mixed 10 times and centrifuged again for 5 min at 14,000 × *g*. The supernatant was transferred to 1 ml binding matrix, shaken for 2 min on a rotator (Heidolph unimax 2010, 60 rpm), and centrifuged for 1 min at 14,000 × *g*. The supernatant was discarded except for approx. 500 µl that was used to dissolve the matrix again. The solution was transferred to a spin filter and centrifuged for 1 min at 14,000 × *g*. To the filter containing the matrix, 500 µl SEWS-M (salt ethanol washing solution) was added and centrifuged for 1 min. After discarding the flow-through, the filter was centrifuged for 2 min, put in a new tube and left to air dry for 5 min at room temperature. To the dry matrix 100 µl DES (Dnase/Pyrogen free, ultrapure water, 65°C) was added, vortexed, left at 65°C for 20 min in a water bath and centrifuged for 1 min at 14,000 × *g*. Quality and concentration of DNA in the flow-through was determined on a 1.2% agarose gel using diluted Lambda-DNA (Roche Diagnostics GmbH, Mannheim, Germany). Samples were diluted to a final concentration of approximately 1 ng μl^{-1} before PCR.

PCR and DGGE. The PCR amplifications were performed using the U968 (40-bp GC clamp) and L1401 universal eubacterial primers (Heuer and Smalla, 1997) and primers FR1 (58-bp GC-clamp) and FF390 for amplification of fungal SSU rDNA (Vainio and Hantulla, 2000). For both primer sets, each PCR reaction was performed as described by Rosado et al. (1998) with small adjustments. Each reaction contained 31.34 µl Deionized water, 5 µl

10 × Stoffel-buffer, 1 µl 10 µM DNTP mix, 7.5 µl 25 mM $MgCl_2$, 1 µl 10 µM U968 (or FR1), 1 µl 10 µM L1401 (or FF390), 0.5 µl formamide 100%, 0.16 µl T4 gene 32 protein (1.19 µg μl^{-1}, USB Corporation, Cleveland, Oh), 0.5 µl Stoffel-Taq 10 U μl^{-1} (Applied Biosystems, Foster City, CA) and 2 µl diluted DNA extract (1 ng μl^{-1}). Reactions were carried out in a PTC-200 (MJ research, Waltham, Ma) using the following program for the eubacterial primers: 94°C (3 min), 94°C (1 min), 60°C (1 min) and 72°C (2 min) (2 cycles). The annealing temperature dropped 1°C per two cycles until 55°C was reached, then the program was repeated for 29 cycles followed by a final extension step of 72°C for 10 min. The PCR program for the fungal SSU rDNA primers was: 94°C (4 min), 92°C (0.5 min), 55°C (1 min), 68°C (2 min) (2 cycles), then the temperature dropped 2°C every two cycles until 47°C was reached. The program was run for an additional 29 cycles: 92°C 0.5 min, 47°C 1 min, 68°C 45 s + 1 s $cycle^{-1}$ and a final extension step at 68°C 10 min. The amount and quality of the obtained PCR products were checked on 1.2% agarose gel prior to DGGE analysis.

The DGGE analysis was carried out using the DCode universal mutation detection system (Bio-Rad Laboratories, Hercules, CA). Vertical denaturing gradients 45 to 60% (16S-rDNA) and 30 to 60% (18S-rDNA) (100% denaturant is defined as 7 M urea plus 40% formamide) were used. Samples (20 µl) were loaded in duplicate gels in a random order. Gels were run at 60°C at 100 V for 16 h and bands were visualized using the Bio-Rad Silver Stain Kit (Bio-Rad laboratories, Hercules, CA) according to manufactures protocol. Gels were analyzed with Phoretix 1D Advanced version 4.00 (Nonlinear dynamics ltd, Newcastle upon Tyne, UK). Background was subtracted using the rolling disc method (radius 40 pixels). Minimum peak height was set at 2 pixels and width was fixed at 5 pixels. Shannon-Weaver 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 = peak intensity of the i^{th} band and N the sum of the intensities of all peaks in one lane profile (Eichner et al., 1999). Similarity indices were calculated as described by McGaig et al. (2001).

Nitrogen and pH. A portion of each soil sample (100 g) collected for the use in bioassays was air dried for 7 days, sieved over a 1-mm-mesh sieve, and stored in plastic containers until further processing. For nitrogen analysis and pH measurement, 2 g soil was added to 100 ml 0.01 M $CaCl_2$ and shaken for 2 h. The pH was measured in this solution with an Inolab pH level 1 (WTW GmbH, Weilheim, Germany). Nitrate and ammonium concentrations were determined with an Autoanalyzer II (Technicon Instrument Corp., Tarreytown, NY) according to Houba and Novozamsky (1998).

Microbial respiration. Soil samples were stored for 24 h at room temperature before further processing. Microbial respiration was performed according to the protocol described by Heinemeyer *et al.* (1989) for an infrared gas analyzer. Fifty grams of moist soil was placed in closed containers 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). Production of CO_2 was calculated by subtracting CO_2 concentration of an unfilled control tube from the tubes filled with soil. Microbial respiration was only measured in soils used for the FOL2 and GGT2 bioassays from the

triticale – white clover system.

Statistical analysis. All data were analyzed using the SAS system for Windows V8.00 (SAS institute Inc, Cary, NC). Disease scores from the bioassays (average AUDPCs for FOL1, FOL2, RS1 and RS2 and disease indices for GGT1 and GGT2) were analyzed with split-plot ANOVA (PROC MIXED, Brussels sprouts - barley) (Schabenberger and Pierce, 2002) for FOL1, GGT1 and RS1 or one-way ANOVA (PROC GLM, triticale – white clover) for FOL2, GGT2 and RS2 (pooled samples). Crop effects on disease scores were tested using contrast analysis, and crop × location interaction effects were compared using Least square means. Residuals were tested for normality. Shannon-Weaver indices obtained from DGGE analyses also were analyzed with one-way ANOVA or split-plot analyses. Log-transformed band intensities were subjected to principal components analysis (McGaig et al., 2001) to distinguish between soils grown to the different crops. Relative disease scores, diversity indices and nitrogen concentrations were calculated as a fraction of the highest value to be able to compare crop effects within a cropping system using all bioassays as replicates. Fractions were square root-arcsine transformed to obtain normality for both disease scores and diversity indices. Simple and multiple linear regressions were used to describe the relation between disease scores, nitrogen data and diversity indices.

Results

Effect of mixed cropping on disease suppressiveness.

Rhizoctonia solani. In containers not infested with *R. solani*, no carrot plants were infected during the course of the bioassay. In infested containers, the rate of disease spread (expressed as AUDPC, Table 3) was not significantly different between the soils with the mono- and mixed crop treatments for both the Brussels sprouts – barley and the triticale – white clover systems. Disease severity was neither significantly different between the locations AB and WH nor for the crop × location interaction effect. The bioassay was terminated after 17 days when carrot plants at the end of the row showed damping-off and the fungus had spread 35-cm from the point of inoculation.

Fusarium oxysporum. The AUDPCs for the non-infested pots were low and flax plants had no wilting. In infested pots, the AUDPCs for Fusarium wilt were significantly affected by the cropping history of the soil collected at locations AB and WH (Table 3). Soils cropped to barley showed on average 16% lower AUDPC values than soils cropped to Brussels sprouts alone (P = 0.01) or to the mixed crop (P = 0.01). Location was not important for the AUDPC in this bioassay and there was also no interaction effect of crop × location. For the soils from the BSW location, grown to triticale, clover or the mix, no significant crop effects were found on disease development caused by F. oxysporum.

Gaeumannomyces graminis var. *tritici*. In the non-infested soils, Ggt-like infections were low. In artificially infested soils, disease scores were lowest in the barley soils for both locations (Table 3) but crop effects were not significant (P = 0.16). Location had no significant effect on take-all disease severity in the GGT1 bioassay and there was no crop × location effect. No differences were observed between single crops and the mixed triticale – white clover crop at the BSW location.

Table 3. Disease scores in bioassays used to test disease suppressiveness of soils cropped to Brussels sprouts (B. sprouts), barley or their mix, or triticale, white clover or their mix.

		Bioassay[u]		
Location[v]	**Crop**	**RS**	**FOL**	**GGT**
AB	B. sprouts[w]	75.3[x]	11.1 ab	4.7
	Barley	57.1	9.5 a	3.9
	Mix	56.4	11.3 b	4.2
WH	B. sprouts	104.9	10.6 a	3.7
	Barley	109.0	8.8 b	1.6
	Mix	86.2	10.5 a	3.3
AB and WH[y]	B. sprouts	90.1	10.8 a	4.2
	Barley	83.0	9.2 b	2.8
	Mix	71.3	10.9 a	3.7
BSW	Triticale	46.8[z]	10.1	3.6
	White clover	56.3	9.9	4.2
	Mix	42.7	10.6	3.3

[u] Disease scores for the bioassays with *Rhizoctonia solani* on carrots (RS) and *Fusarium oxysporum* f.sp. *lini* on flax (FOL) are averaged AUDPCs, and those for the bioassays with *Gaeumannomyces graminis* var. *tritici* on barley or triticale (GGT) are averaged disease indices (0 = healthy, 1 = small lesions on 1 root, 2 = bigger lesions on 2 or more roots, 3 = 1 lesion reaching the stem base, 4 = 2 or more lesions reaching the stem base, 5 = stem base infected, 6 = infection reaching the stem, 7 = plants yellowing and wilting, 8 = dead plant).

[v] Disease suppressiveness bioassays were executed with soils from different locations and crops. AB = Achterberg, WH = Wageningen Hoog, and BSW = Bornsesteeg Wageningen.

[w] Brussels sprouts are abbreviated as B. sprouts.

[x] Numbers followed by different letters within the same columns per location differ at $P = 0.05$. No letter means not significantly different at $P = 0.05$.

[y] Data from the locations AB and WH were pooled before analysis.

[z] Three field plot samples were pooled. The P-value was calculated from four blocks in the bioassay.

When relative disease scores were pooled and analyzed for all three bioassays together at the locations AB and WH, a significant crop effect was obtained ($P = 0.04$, data not shown). Contrast analyses indicated that soils grown to barley were more disease suppressive than soils grown to Brussels sprouts ($P = 0.013$). Soils grown to the mixed crop did not differ in disease suppressiveness from those grown to Brussels sprouts or barley. Disease suppressiveness was not different between the two locations, however, a significant bioassay × soil effect was present ($P = 0.04$). No crop × location effect was present when all bioassays were analyzed together. Analyses of the relative disease scores of all bioassays together from the BSW location (triticale - white clover), indicated no differences between the single and mixed cropped soils with respect to disease suppression.

Effect of mixed cropping on microbial diversity. Shannon-Weaver indices (SW-indices) of 16S- (bacterial) and 18S-rDNA (fungal) DGGE bands were, in general, similar for most bulk soils tested (Table 4). The bacterial SW-indices were higher than fungal SW-indices in all bioassays. The number of detectable bands was 38 ± 7 bands per lane for the 'bacterial gels' in the Brussels sprouts - barley system and 34 ± 6 in the triticale – white clover system. Brussels sprouts - barley had 21 ± 6 bands on the fungal gels and triticale – white clover had 27 ± 6 bands. There was no indication that the mixed cropped soils had a greater number of bands or higher diversity indices than the mono-cropped soils for both bacterial

Table 4. Shannon-Weaver indices of 16S-rDNA DGGE profiles of soils used to test disease suppression. The soils originated from fields grown to Brussels sprouts, barley and their mix, or triticale, white clover and their mix.

			Bioassay[u]	
Location[v]	**Crop**	**RS**	**FOL**	**GGT**
AB	B. sprouts[w]	1.48[x]	1.20 a	1.27
	Barley	1.47	1.33 b	1.28
	Mix	1.55	1.26 ab	1.35
WH	B. sprouts	1.48	1.30	1.32
	Barley	1.44	1.34	1.43
	Mix	1.52	1.29	1.43
AB and WH[y]	B. sprouts	1.45	1.25	1.29
	Barley	1.48	1.33	1.36
	Mix	1.54	1.28	1.39
BSW	Triticale	1.38[z]	1.45	1.52
	White clover	1.41	1.47	1.41
	Mix	1.09	1.46	1.45

[u] Shannon Weaver indices belonging to the bulk soil samples from disease suppressiveness bioassays with soils from different locations and crops. Bioassays were executed with *Rhizoctonia solani* on carrots (RS), *Fusarium oxysporum* f.sp. *lini* on flax (FOL) and *Gaeumannomyces graminis* var. *tritici* on barley or triticale (GGT). Indices were calculated as $H' = -\Sigma\, p_i (\log p_i)$, where $p_i = n_i/N$ and n_i = the area of a peak in intensity and N = sum of all peaks in a lane profile (Eichner et al., 1999).

[v] AB = Achterberg, WH = Wageningen Hoog, and BSW = Bornsesteeg Wageningen.

[w] Brussels sprouts are abbreviated as B. sprouts.

[x] Shannon weaver indices with the same letter within columns per soil type are not significantly different at P = 0.05. No letter means not significantly different at P = 0.05.

[y] Data from the locations AB and WH were pooled before analysis.

[z] Pooled samples, only one value.

and fungal communities when analyzed per bioassay. Overall, there was no effect of crop or location based on the bacterial SW-indices for soils used in the FOL1 bioassay. Similarly, in the soils used for the GGT1 or RS1 bioassays, crop or location effects were not significant. The SW-indices of soils collected from the BSW location and used in the bioassays FOL2, GGT2 and RS2 were not significantly different for both the bacterial and fungal community.

Analyses of relative bacterial SW-indices for all bioassays per cropping system together (Brussels sprouts – barley or triticale – white clover), indicated a significant crop effect (P = 0.04) in the Brussels sprouts - barley system. Mixed cropped soil had a higher relative SW-index than soil cropped to Brussels sprouts (P = 0.02). Differences between barley and Brussels sprouts and the mixed cropped soil were not significant. Also the comparison between locations AB and WH showed no differences. Relative SW-indices of fungal communities were not significantly different between crops and between soil types in the Brussels sprouts - barley system. Overall analyses of relative SW-indices showed no crop effect in the triticale – white clover system. A bioassay × crop interaction was near significant (P = 0.06) indicating that time of sampling might have an effect on the soil microbial diversity.

In all bioassays, soils from locations AB and WH could be separated by principal component analyses (PCA) based on band number and intensity of bacterial DGGE profiles

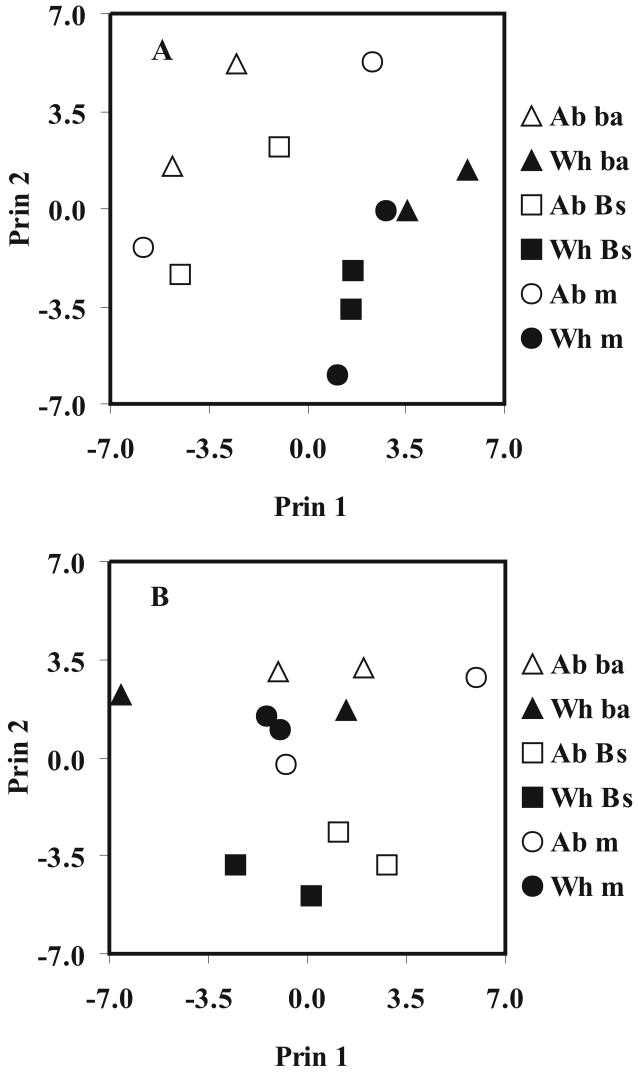

Figure 1. Principal component analysis on similarity between lanes of 16S-rDNA (A) or 18S-rDNA (B) PCR-DGGE profiles from bulk soil samples used in the GGT1 bioassay. Prin 1 represents 20.6 % (A) and 16.0 % (B) of the variation and Prin 2, 17.5 % (A) and 16.1 % (B). (ba = soil grown to barley (triangle), Bs = soil grown to Brussels sprouts (squares), m = soil grown to the mix crop (circles); Ab = location Achterberg, Wh = location Wageningen Hoog).

(data only shown for the GGT1 bioassay, Figure 1A). After PCA analysis of the fungal community, locations AB and WH could also be separated, except for those used in the GGT1 bioassay, where the soils grown to Brussels sprouts clustered together more than soil type (Figure 1B). Barley probably induced the observed effect, as the mixed cropped bulk soil was more similar to the mono-cropped barley soil than to the mono-cropped B. sprouts soil (Figure 1B). This separation only occurred in the GGT1 bioassay and the moment of sampling might be the reason for this. In general, closest neighboring field plots clustered more together than the crop treatments designated to those plots.

Effect of mixed cropping on microbial activity. There were no significant differences for microbial respiration, measured in soils used for the bioassays FOL2 and GGT2 between single and mixed cropped soils (data not shown). No significant correlation was found between microbial respiration of the sampled soils and final disease severity.

Relationships between disease suppressiveness, microbial diversity and microbial activity. In the GGT1 (Figure 2) and RS1 bioassays, relative disease score and relative bacterial (16S-rDNA) SW-indices were negatively correlated (Table 5). Slopes were negative but not significant in the bioassays FOL1, FOL2, GGT2. The fungal (18S-rDNA) SW-indices were positively correlated with disease scores in the FOL1 and in the GGT1 bioassays (Table 5), while they were negatively correlated with disease score in the GGT2 bioassay. Microbial respiration was not significantly correlated with the disease scores or SW-indices in both bioassays.

When relative disease scores and relative SW-scores from the crops were pooled, disease scores were correlated to SW-indices in the Brussels sprouts – barley experiments. A negative correlation between 16S-rDNA diversity and disease for the Location AB (slope = -1.72, P = 0.0032) was found, however for the WH location there was no significant correlation. A positive correlation was detected between 18S-rDNA SW-indices and disease (slope = 1.48, P = 0.0135) for location WH but the relation was not significant for the AB location.

Overall regression analysis for the Brussels sprouts - barley system indicated a negative correlation between 16S-rDNA SW-indices and disease score (slope = -0.96, P = 0.005) and a positive correlation between 18S-rDNA SW-indices and disease scores (slope = 0.68, P = 0.007). In the triticale – white clover bioassays no correlations were significant.

Effect of mixed cropping on available nitrogen. Available nitrogen content of the soils used in the bioassays was generally low. Nitrate concentration ranged from 7.7 to 41.7 mg NO_3 kg^{-1} soil, and ammonium concentration ranged from 0.5 to 3.48 mg NH_4 kg^{-1} soil. No differences were observed among ammonium concentrations in soils from the different locations grown to various crops. Nitrate content of soils sampled at location AB for the FOL1 bioassay was significantly higher in soils cropped to Brussels sprouts than in soils with barley alone or with the mixed crop (P = 0.0014 and P = 0.0032, respectively). Nitrate concentration in soils collected for the other bioassays or from the WH location did not differ among the crops grown in the Brussels sprouts - barley systems. Also soils from the triticale – white clover system did not differ in available nitrogen concentrations among the three crops. No significant correlations were observed between nitrate or ammonium

Table 5. Simple linear regression analysis of relative disease severity (as a fraction of the highest value per test) and relative 16S-rDNA or 18S-rDNA diversity indices per bioassay. Fractions were square root arcsine transformed prior to statistical analysis.

Bioassay[y]	16SrDNA				18SrDNA			
	Intercept	Slope	R^2	$P < r$ (slope)	Intercept	Slope	R^2	$P < r$ (slope)
FOL1	1.65	-0.30	0.04	0.53	0.37	0.66	0.31	0.06
FOL2	1.62	-0.41	0.04	0.63	- 0.50	0.42	0.06	0.54
GGT1	3.06	-1.44	0.31	0.06	-0.58	1.31	0.42	0.02
GGT2	2.44	-0.96	0.22	0.21	3.86	-1.89	0.70	0.01
RS1	3.44	-1.60	0.54	0.01	0.86	0.27	0.04	0.54
RS2[z]	0.47	0.36	0.73	0.35	1.45	-0.34	0.09	0.81

[y] Bioassays with soils from different locations and crops were performed with *Rhizoctonia solani* on carrots (RS), *Fusarium oxysporum* fsp. *lini* on flax (FOL) and *Gaeumannomyces graminis* var. *tritici* on barley or triticale (GGT).

[z] Three data points only.

contents and relative disease scores (as fractions of the highest values per bioassay per soil type) in either cropping system (data not shown).

Discussion

In this study, no significant effects of mixed cropping on general disease suppressiveness of three different soils to three different plant pathogens were found. Except in one bioassay (FOL1), an adverse effect of mixed cropping on disease suppression to *F. oxysporum* was found. For Fusarium wilt, soils cropped with barley had the highest level of disease suppressiveness compared to soils cropped with Brussels sprouts and the mixed crop. This result was supported by the results from the GGT1 bioassay, in which a weak but similar trend for suppressiveness against take-all disease of barley was noted. Relative disease scores were significantly different among crops when bioassays were analyzed together. Again, soils collected from barley plots had the lowest relative disease severity compared to soils from plots grown to Brussels sprouts or the mixed crop. In the triticale – white clover system, differences among disease scores in any of the bioassays were too small to be significant. Collectively these results indicate that mixed cropping does not increase the general disease suppressiveness of soils, at least not for the cropping systems and soilborne pathogens tested in this study. In our experiments, we observed a trend towards higher levels of disease suppression in soils grown to barley, but not to triticale. This was true for bioassays with both dicotyledonous and monocotyledonous test plants. This suggests that the lower disease severity in soils grown first to a barley crop was not simply a rotation effect.

Three pathogens were tested in our bioassays because the mechanisms of attack and suppression differ for these fungi, so that results could be generalized with respect to disease suppression induced by mixed crops. *Rhizoctonia solani* is primarily affected by general microbial antagonism (Tuitert et al., 1998). Suppression of *F. oxysporum* has mainly been attributed to the activity of nonpathogenic *F. oxysporum* species and *Pseudomonas* spp., which act primarily by competition and induced systemic resistance (Weller et al., 2002). *Gaeumannomyces graminis* var. *tritici* is sensitive to general and specific microbial activity in the soil and its full pathogenic potential is not reached in

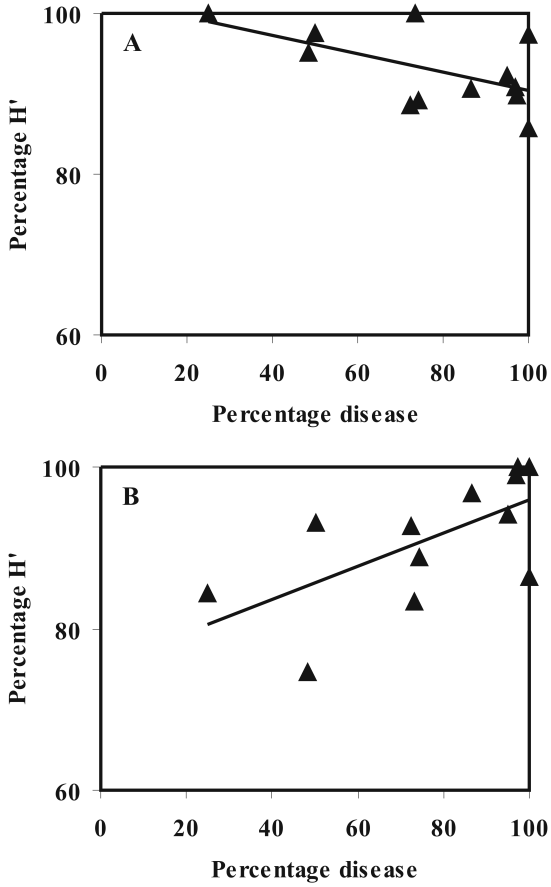

Figure 2. Percentage disease plotted against the percentage Shannon-Weaver index 16S-rDNA (A) and 18S-rDNA (B) from the GGT1 bioassay. Percentages were calculated for both Shannon-Weaver indices and disease scores as fractions of the highest value within one location × 100. Corresponding R-squares are 0.31 ($P = 0.06$) (A) and 0.42 ($P = 0.02$) (B).

natural soils because of competition and antibiosis (Gerlagh, 1968; Weller et al., 2002). In our experiments, no relationship was found between soil microbial activity and disease severity of any of the tested pathogens including *Ggt*. Besides general suppression, specific disease suppression of take-all by *Pseudomonas fluorescens* has been described many times (Weller et al., 2002). From soils used in both systems, *P. fluorescens* colonies were enumerated, but no relation was found between colony numbers and take-all severity (data not shown). Qualitative differences and more specifically the effects of mixed cropping on specific antagonists, including antibiotic-producing *Pseudomonas*, are currently under investigation. Pathogen behavior can also be affected directly or indirectly by the nitrogen

form and availability. This has been described for *R. solani* (van Bruggen et al., 1996) and *G. graminis* var. *tritici* (Huber et al., 1969; Sarniquet et al., 1992; Trolldenier, 1981). However, in our study no relationship was found between available nitrogen content in soil and disease severity.

As plants have a profound effect on soil microbial communities and their processes (Insam and Domsch, 1988; Wardle et al., 1997), increased root diversity and density by mixed cropping were thought to result in increased microbial activity and diversity. However, Shannon-Weaver diversity indices based on 16S- and 18S-rDNA were hardly affected by the crops that had been grown. As crop-induced microbial shifts are mainly present in the rhizosphere (Grayston et al., 1998; Kowalchuk et al., 2002; Wardle et al., 1997; Zak et al., 2003), the effects of sampling and mixing bulk and rhizosphere soil probably diluted or masked these shifts, so that specific rhizosphere effects were not discernable and influences on general disease suppression were relatively small or not detectable. However, disease suppressiveness of soils has been demonstrated in bioassays using field collected bulk soil, even if the site of suppression appeared to be in the rhizosphere as in the case of suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* (Raaijmakers and Weller, 1998). Furthermore, differences in disease scores may have been the result of small population changes not detectable by PCR-DGGE profiles (Boon et al., 2001; Fromin et al., 2002; Muyzer et al., 1993) generated by the general primers we used.

Similarity coefficients of DGGE-gels of bacterial communities from soils grown to Brussels sprouts were not different from those grown to barley or the mixed crop. Nevertheless, barley or the mix with barley resulted in slightly higher SW-indices than Brussels sprouts. This may have been due to the release of isothiocyanates (ITCs) by this crop (Bending and Lincoln, 2000). Brussels sprouts are known to produce ITCs that are released during decomposition of residues and are known to exhibit fumigating effects (Bending and Lincoln, 1999; Drobnica et al., 1967; Smolinska and Horbowicz, 1999) affecting microbial communities including soilborne plant pathogens (Abadie et al., 1998; Sarwar and Kirkegaard, 1998). Consequently they may also affect beneficial organisms, which may adversely affect disease suppressiveness of the soil. In two of our bioassays the relative SW-index based on 16S-rDNA was negatively correlated with relative disease severity and in three bioassays this correlation was insignificant. This indicates that disease suppression is to some extent related to bacterial diversity. Similarly, a negative correlation between actinomycete diversity and disease severity was found for corky root of tomato (Workneh and van Bruggen, 1994). No consistent relationship was found between fungal diversity based on 18S-rDNA and disease severity in our bioassays.

In several studies, total microbial activity affected disease suppression (Hoitink and Boehm, 1999) significantly more than the effects of individual species or select microbial groups (Baker and Cook, 1985). However, in our study there were no correlations between respiration measurements, diversity indices, disease scores and there were no significant differences among the soils grown to different crops. Similarly, Wardle et al. (1997) showed that removal of a plant species did not result in a decrease in microbial respiration except when all plants were removed. These findings support our observations that crop mixtures do not have a substantial influence on bulk soil microbial communities and their ecological processes.

We conclude that mixed cropping does not increase the disease suppressiveness of the soil or the diversity of the soil microbial community. Effects of growing crops on a soil remain apparently limited to the rhizospheres. Cross-over effects of mixed crops, due to root-root interactions, might be small but have not yet been thoroughly investigated. It should be emphasized that our study did not consider direct effects of mixed cropping on disease incidence in the respective crops in the mix, but focused primarily on the effects of mixed cropping on general suppressiveness of bulk soils. It would therefore be too simple to state that mixed cropping has no advantages in suppression of soilborne diseases compared to single crops. On the contrary, mixed cropping has shown promising results by reducing disease severity of soilborne pathogens within the field where the crops are grown (Abadie et al., 1998; Autrique and Pots, 1987; Burdon and Chilvers, 1976; Vilich-Meller, 1992; Yu, 1999). Beside reduction in disease severity, other factors may be important for deciding to grow crop mixtures, for example reduced damage by airborne pathogens (Mundt, 2002), weed suppression, pest suppression (Fromin et al., 2002; Vandermeer, 1989) and possibly increased productivity.

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Chapter 5

The role of clover in the suppression of *Gaeumannomyces graminis* var. *tritici* in mix-cropped triticale-clover

G.A. Hiddink, J.M. Raaijmakers, A.J. Termorshuizen, and A.H.C. van Bruggen.
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Abstract

The effect of mixed cropping triticale with white clover on disease incidence and severity of *Gaeumannomyces graminis* var. *tritici* was studied both in a field and under greenhouse conditions over multiple cropping cycles. In a field experiment, disease severity on triticale roots increased in both mono- and mix-cropped triticale during the first three years but to a slightly lesser extend in the mix-cropped triticale. After three growing seasons the percentage infected triticale roots was lower in the mix-cropped triticale. Due to bad clover establishment, the effect was not significant in the fourth growing season. In the greenhouse experiment, *Ggt* disease severity on triticale roots declined significantly in mix-cropped triticale-white clover during 5 successive cycles compared to the mono-cropped triticale. Several known mechanisms that can affect *Gaeumannomyces* graminis var. *tritici* were investigated, including microbial activity, nitrogen concentration and population density of *phlD*$^+$-pseudomonads. In the greenhouse experiment, microbial respiration was significantly higher in the mix-cropped treatment, positively correlated with clover biomass, and negatively correlated with the fraction of *Ggt*-infected roots. Also in the field experiment, a decreasing clover biomass correlated significantly with increasing Ggt-disease incidence over the years. The ammonium concentrations were significantly higher in mixed triticale-clover crops than in triticale mono-crops, but did not correlate directly with disease severity. We conclude that clover biomass in mixed triticale-clover crops can directly reduce take-all disease by increasing the distance between host roots or indirectly by enhancing microbial activity and possibly by increasing ammonium concentrations in soil.

Introduction

Soilborne pathogens are difficult to manage since effective chemical control measures such as methyl bromide are globally banned (Schneider et al., 2003) and resistance in plants to many soilborne pathogens is insufficiently explored (Whipps, 1997). Crop management strategies, like organic farming and mixed cropping, might provide a way to reduce detrimental effects from soilborne pathogens on crops (Hiddink et al., 2005a) using ecologically sound measures. Farmers using mixed crops can be less dependent on the input of pesticides because of reduced pressure of airborne disease and pest organisms, while available nutrients are used more efficiently, resulting in a higher productivity per unit area (reviewed by Geno and Geno, 2001).

Under-sowing or mix-cropping legumes with cereals or grass is practiced regularly in low input agroecosystems and in organic farming where legumes are mainly used for

nitrogen fixing properties. In addition to nitrogen fixation, legumes used in a crop mixture have the potential to reduce nitrate leaching, control weeds and suppress pests and diseases (Wolfe, 1985). Legumes have been shown to be able to reduce soilborne pathogens (reviewed by Dakora and Phillips, 1996). Direct phytotoxic effects of root exudates from e.g. red clover against *Rhizoctionia solani* and *Sclerotium rolfsii* have been shown (Weidenborner et al., 1990).

Soilborne disease severity was reduced in several crop mixtures compared to the same crops in monoculture. *Gaeumannomyces graminis* var. *tritici* (Ggt) disease severity was lower in wheat intercopped with trefoil (*Medicago lupulina*) (Lennartson, 1988). *Pythium irregulare* spread more slowly in a garden cress–ryegrass mixture (Burdon and Chilvers, 1976). Fusarium root and foot rot was less severe in barley-wheat mixtures (Villich-Meller, 1992), *Ralstonia solanacearum* wilt was reduced in tomato-Chinese chive (Yu, 1999), potato-maize and potato-haricot beans (Autrique and Pots, 1987), and Fusarium wilt was less severe in oil-palm-*Pueraria javanica* (Abadie et al., 1998). All these investigators found different (general and specific) mechanisms for disease reduction like dilution of host plants (Burdon and Chilvers, 1976), physical barriers (Autrique and Pots, 1987), stimulation of antagonistic groups (Abadie et al., 1998) and probably enhanced microbial activity.

Gaeumannomyces graminis var. *tritici* is a soilborne pathogen that is sensitive to both general and specific disease suppression (Gerlagh, 1968; reviewed by Weller, 2002, Cook, 2003). High microbial activity and competition (general suppressiveness) reduces saprotrophic growth (Gerlagh, 1968). Specific suppression of Ggt is induced by successive cropping of wheat resulting in increased levels of 2,4-DAPG producing pseudomonads (Raaijmakers and Weller, 1998) and lower disease levels.

Mixed cropping might induce microbial changes in the rhizosphere that can reduce the contrast between bulk soil and the rhizosphere decreasing the rhizosphere effect (Gilbert et al., 1994) or even influence rhizospheres of accompanying crops (Kowalchuk et al., 2002).

The goal of our study was to examine the effect of mixing triticale with white clover on *Ggt.* We carried out repeated cropping experiments in the same soil both in the field and greenhouse. The objectives were to determine:

1) If mixed cropping of triticale with clover would result in reduced take-all disease severity compared to mono-cropping triticale.
2) If repeated cropping of triticale (in mono- and mixed culture) would result in reduced take-all disease severity in successive cropping cycles.
3) The mechanisms underlying take-all disease suppression in mixed compared to monoculture and in successive cropping cycles, including differences in nitrogen availability, microbial activity and composition, populations of *Pseudomonas fluorescens*, and distance between host plants.

Materials and Methods

Experimental design. In a field and a greenhouse experiment, mixed cropping of triticale with white clover was compared with triticale mono-cropping. In the field experiment, crops were grown at one location with a natural infestation of *Gaeumannomyces graminis* var. *tritici*. Treatments were carried out in triplicate in a randomized complete block design

and the field was re-sown in four subsequent seasons (2000-2004) without crop rotation. In a greenhouse experiment, three field soils were collected and infested with Ggt and cropped for four weeks as mono and mix crops, in five-fold, in a randomized complete block design. Soil and plants, including the roots, were sampled, investigated as described in detail below, and the remaining soil was returned to the pots, the soil was re-infested with Ggt, and again the same crops were sown. In total, a crop or crop mixture was grown five times.

Soils used in the greenhouse experiment. Three soils were used (Table 1), (1) an organically treated sandy soil (code OtS, meaning sand with organic treatment) collected from a triticale plot in September 2001 from our experimental field in Wageningen, The Netherlands, (2) an organically managed sandy soil (code OS, meaning sand with long-term organic management) collected from a rye field under rotation in September 2001 from an organic farm in Blokzijl, The Netherlands, and (3) a conventionally managed silty loam (code CsL, meaning silty loam with conventional management) collected from mono-cropped (14 years monoculture) wheat fields in December 1997 from an agricultural polder field (Woensdrecht, The Netherlands). Soils were air-dried at 25°C, sieved (2 mm) and stored until further use in closed containers at room temperature. Before the actual start of the experiment, 10 triticale plants were grown in the rewetted soils for six weeks to revitalize the microbial community.

Table 1. Characteristics of soils used for the greenhouse experiments (CsL = conventional silty loam; OS = organic sandy soil; OtS = sandy soil with organic treatment).

Code	Soil type	pH	NO_3	NH_4	Clay %[1]	Silt %	Sand %	O.M. %[2]
OtS3	Sandy	5.4	1.2	6.6	2	12	86	4.8
OS	Sandy	7.3	2.4	5.9	2	22	76	3.0
CsL	Sandy loam	7.6	3.2	5.3	6	60	34	3.6

[1] Textures are size fractions. Organic matter is incorporated in these percentages.
[2] Organic matter determined by the glow loss method (Houba et al., 1989).
[3] The OtS-soil was sampled from our experimental field, therefore the soil characteristics of the experimental field are not seperately mentioned here.

Inoculum preparation. In Erlenmeyers, sterile oat grains were inoculated with five agar plugs (1/5 potato dextrose agar (Merck KgaA, Darmstadt, Germany) and 4/5 technical Agar nr 3 (Oxoid limited, Basingstoke, England)) with actively growing mycelium of *Gaeumannomyces graminis* (Sacc) v. Arx & Olivier var. *tritici* J. Walker; isolate R3-111-a-1. This isolate originated from wheat grown near Moses Lake, Washington State, USA (de Souza et al, 2003). The Erlenmeyers were incubated for four weeks at 20˚C in the dark and shaken weekly. Completely colonized oats kernels were dried for 72 h in a laminar flow cabinet, and stored at 4°C until further use.

Soils were amended with the ground oat inoculum (0.5% w/w, particle size 200-500µm, Wilkinson et al., 1985). Autoclaved inoculum served as control after the autoclaved grains were checked for sterility by plating on 1/5 PDA. Pots were filled with 150 g (dw) infested (viable or autoclaved inoculum) soil and this was covered with 25 g original non-infested soil to prevent seeds from being sown directly in the infested soil. After sowing, the seeds were covered with another 25 g of non-infested soil.

Plant cultivation in the field experiment. Triticale (*X Triticosecale* Wittm. cv. Galtjo,

Svalöf–Weibull B.V., Emmeloord, The Netherlands), white clover (*Trifolium repens* L. cv. Pertina, Cebeco Zaden, Vlijmen, The Netherlands) and their mix were organically grown at an experimental field close to Wageningen (OtS-soil). Fields were fertilized annually with 30 ton ha^{-1} farmyard manure (approx. N:P:K, 207:114:222 kg ha^{-1}) which was incorporated with a rotary spade digger to a depth of 30 cm. Triticale and white clover were sown on the same day between 28th of September and 11th of October. Mono crops were each sown at an inter-row distance of 25 cm (in 2000 – 2003) and at 12.5 cm (2004, to reduce weeds) and an intra-row distance of 2.5 and 0.5 cm, respectively (125 kg ha^{-1} for triticale and 15 kg ha^{-1} for white clover). For the mixed crops, triticale and white clover were sown additively in alternating rows at the same distances and amounts as the mono crops. White clover plots were re-sown in March 2003 because of night frost in fall 2003 so that only few young white clover plants survived. The white clover sown in September 2003 germinated well but grew slowly, and all plots were re-sown in March 2004. However, white clover did not establish well that year in all plots except in one mix-cropped plot. Triticale plots were harrowed several times to remove weeds. White clover and the mixed crops were not harrowed to prevent damage to the white clover. In late spring (May) hand weeding was performed in all plots to remove tall weeds (mainly dicots).

Disease and biomass measurements in the field experiment. Take all root disease was scored from June 2001 onwards in the mono- and mixed-cropped triticale. Ten randomly selected plants per plot were dug out and stored at 4˚C until disease was scored (percentage infected roots) the same day or one day later. Diseased root parts were plated out onto semi-selective medium (Duffy and Weller, 1994). DNA extracted from some of the growing fungal colonies was tested with PCR to confirm the identity of isolated Ggt (primer pair pGt1 and pGt2; Bryan et al., 1995). Aboveground disease symptoms (whiteheads) were scored at the end of the growing season (mid July, growth stage 11.1 Feeke's scale, Large, 1954). At this time also biomass was determined. A wooden square was used to select randomly 0.25 m^2 for destructive harvesting. Plants were dug from the soil completely and transported to the lab were they were stored at 4˚C until the next day. The biomass was sorted by crop and weighed (aboveground parts only). The biomass was dried at 70˚C for 48 h and weighed again. Triticale seeds were harvested with a combine harvester and yield was determined after air-drying (25˚C for 72 h) the harvested and cleaned seeds.

Plant cultivation in the greenhouse bioassay. Triticale seeds (*X Triticosecale* Wittm. cv. Galtjo, Svalöf–Weibull B.V., Emmeloord, The Netherlands) were surface-sterilized with 1% NaOCl for 1 min and rinsed with running tap water for 5 min. Seeds were pregerminated under sterile conditions in petri-dishes on moist filter paper for three days. White clover seeds (*Trifolium repens* L. cv. Pertina, Cebeco Zaden, Vlijmen, The Netherlands) were treated in the same way except that they were pre-germinated for two days. Ten pre-germinated triticale seeds were sown per pot with infested soil as both mono- and mix-cropped triticale, and 0.5 g white clover seeds were sown in mono- and mix-cropped white clover pots. Pots were watered when needed and fertilized weekly with organic manure (7.10^{-3} g N ml^{-1}, 1 ml pot^{-1}, 'ECOstyle organische tuinmest', NPK 7-3-5, ECOstyle bvba-sprl, Geetbets, Belgium) suspended in water on saucers. After four weeks of plant growth, plants were harvested including all the roots. Root samples (1 g) were

collected and stored immediately at -20˚C for DNA-extraction and PCR-DGGE. On the remaining roots (10 plants), disease severity was scored.
At harvest, plants were separated from the soil and both soil and plants were stored in plastic bags until further handling. A soil sample (25 g) was taken and air-dried at room temperature (25˚C, one wk) and stored for NO_3 and NH_4 analyses. At the day of harvest, from the remaining soil (150 g) was amended again with Ggt inoculum (0.5% w/w), returned to the same pot, covered with non-infested soil (25 g), moistened, replanted with germinated seeds and covered with non-infested soil (25 g) as described above. Five successive cycles were performed in this way.

Disease and biomass measurement in the greenhouse experiment. Disease severity was estimated as fraction of diseased roots, and using a disease rating scale from 0-8, where 0 = no symptoms, 1 = small lesions on one root, 2 = larger lesions on two or more roots, 3 = one lesion reaching the stem base, 4 = two or more lesions reaching the stem base, 5 = stem base infected, 6 = infection reaching the stem, 7 = plants yellowing and wilting, 8 = dead plant (Thomashow and Weller, 1988). Roots with take-all disease symptoms were surface-sterilized in 1% $AgNO_3$ (15 s), washed 2 x 1 min in sterile demi-water and plated out on a semi-selective medium for Ggt (Duffy and Weller, 1994). Shoot height and fresh shoot weight were determined and aboveground plant parts were dried at 70˚C (48 hrs), weighed and stored at room temperature.

Nitrogen and pH. Part of the soil samples (25 g) were air-dried for seven days immediately after collection, sieved over a 1-mm mesh sieve and stored in plastic containers until further processing. For nitrogen analysis and pH measurement, 2 g soil was added to 100 ml 0.01 M $CaCl_2$ and shaken for 2 h (Houba and Novozamsky, 1998). The pH was measured in this solution with an Inolab pH level 1 (WTW GmbH, Weilheim, Germany). Nitrate and ammonium concentrations were determined with an Autoanalyzer II (Technicon Instrument Corporation, Tarreytown, NY).

Pseudomonas fluorescens densities in rhizospheres of triticale and clover. Densities of *P. fluorescens* in rhizospheres of triticale and clover were determined by dilution plating of rhizosphere samples from the field experiments in 2003 (six months after collection, stored at -20˚C) and 2004 (plated within 6 h after collection). Of each sample, 1.0 g triticale or white clover roots with adhering soil in 5 ml sterile demi-water, were vortexed (1 min) and sonicated (1 min) in an ultrasonic cleaner (Bransonic 12, Branson Ultrasonics Corp., Geneva, Switzerland) and serially diluted in sterile demi-water. Fifty µl (dilutions 10^{-4} and 10^{-5}) was plated in three-fold on Kings medium B amended with 200 mg l^{-1} Delvocid (50 % a.i. Natamycine (in stead of 100 mg l^{-1} cycloheximide), DSM Food Specialties, Delft, The Netherlands) before autoclaving, and with ampicilline (40 mg l^{-1}, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and chloramphenicol (13 mg l^{-1}, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) (Simon and Ridge, 1974) added to the cooled medium. After 48 h incubation at 25°C in the dark, CFU were counted. Plates were stored at 4°C until they were assayed for the presence of the *phlD*$^+$-gene (start of the bioassay only).

To estimate the density of 2,4-DAPG-producing *P.fluorescens* in the rhizospheres, the number of *phlD*$^+$-*P. fluorescens* colonies was determined on rhizosphere samples

collected in 2004 by colony hybridization followed by PCR (Raaijmakers *et al.*, 1997). Bacterial colonies were transferred to Hybond-N^+ nylon membranes (Amersham International, Little Chalfont, United Kingdom). The *phlD*$^+$ probes were produced using Dig-labeled nucleic acids (Roche Diagnostics, Basel, Switzerland) and detected with the DIG Nucleic Acid Detection Kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol.

In the greenhouse experiments, *P. fluorescens* densities were determined in bulk soil and triticale rhizospheres as described for the field experiment. This was done before the actual start of the greenhouse experiment (five repetitions) when triticale was sown in dried soils to revitalize them. At the end of the fifth cycle *P. fluorescens* densities were determined in triticale and clover rhizospheres (three blocks only).

Microbial respiration. Microbial respiration of collected field soils from the experimental field was measured at the end of the growing seasons in 2002, 2003 and 2004 (growth stage 11, Feeke's scale, Large, 1954). Microbial respiration of soil samples from the greenhouse experiments was measured after cultivation cycles two, three and four within one wk after harvesting the plants. The amount of released CO_2 was measured with an infrared gas analyzer (ADC 7000 gas analyzer, Hoddesdon, England) (Heinemeyer et al. (1989). Closed containers filled with soil (25 g) were connected to a continuous flow system where moisture saturated air was blown over the soil at 20˚C. The released CO_2 was calculated by subtracting the CO_2- concentration in control tubes (air) from the tubes filled with soil.

DNA-extraction. Soil samples were stored immediately after collection at -20°C. Rhizosphere samples were stored after dilution plating (when performed) or immediately after collection at -20˚C. DNA was extracted using the FastDNA SPIN Kit for soils (Bio101 systems, USA), as described in Hiddink et al. (2005b).

PCR-DGGE. PCR amplification was performed using the U968 (40-bp GC clamp) and L1401 universal eubacterial primers (Heuer and Smalla, 1997) and primers FR1 (58-bp GC-clamp) and FF390 for amplification of fungal SSU rDNA (Vainio and Hantula, 2000). For both primer sets, PCR, DGGE and gel analyses was performed as described by Hiddink et al. (2005b).

Statistical analysis. All data were analyzed with the SAS system for Windows V8 (SAS institute Inc, Cary, NC, USA). Disease ratings for repeated measurements were treated as described by Shah and Madden (2004) for non-parametric data using a box-type-statistic and corrected degrees of freedom. The used macro's were retrieved from the website from Dr E. Brunner of the University of Gottingen (www.ams.med.uni-goettingen.de /de/sof/ld/makros.html). The other plant and soil parameters were analyzed with Proc Mixed (Schabenberger and Pierce, 2004) for repeated measures. Fraction diseased roots were square root-arcsine transformed to obtain normality. Parameters for the disease curves from the field experiment were estimated with the NLIN procedure (Schabenberger and Pierce, 2004) and analyzed with the MANOVA procedure.

Correlations (Pearson Product moment (r)) were calculated between disease, plant and soil parameters (Proc Corr) on the raw data. Shannon-Weaver indices obtained from

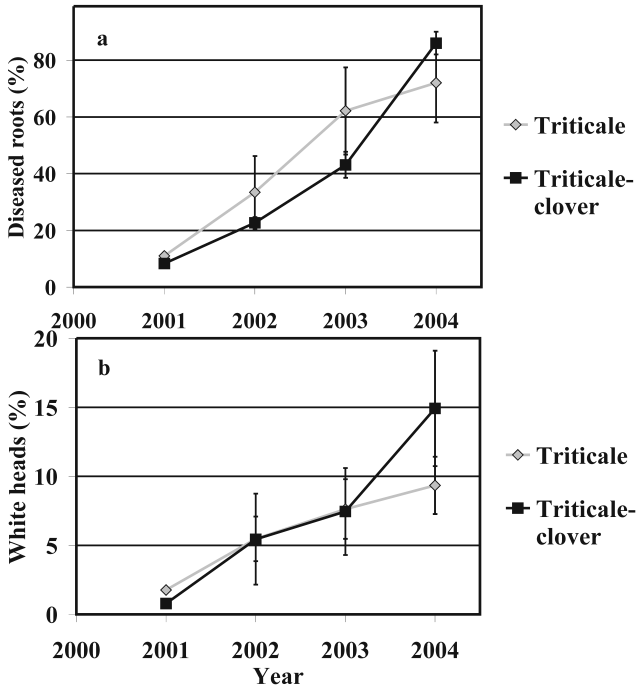

Figure 1. Mean percentage of triticale roots infected with *Gaeumannomyces graminis* var. *tritici* (± s.d. of the mean) in mono-cropped triticale and mix-cropped triticale-clover (a) and the mean percentage (± s.d. of the mean) whiteheads in the field experiment (b).

DGGE analyses were analyzed within planting cycles with one-way ANOVA's. Microbial community profiles obtained from DGGE-gels were analyzed with the Treecon program (Van de Peer and de Wachter, 1994). Similarity between lanes was calculated using the method of Nei and Li (1979). Dendrograms were constructed using UPGMA and bootstrap values were based on 1000 replicates.

Results

Take-all disease development in a field grown triticale-clover mixed crop. The fraction of Ggt-infected triticale roots (Figure 1a) and white heads (Figure 1b) strongly increased over the four experimental years in both mono- and mix-cropped triticale. During 2001-2003, the percentage infected roots in the mixed crop seemed to increase less than in the mono-cropped triticale, but when slopes were compared directly these were not significantly different. However, it resulted in lower percentage diseased roots in the mix-cropped triticale in 2003 (P = 0.07). In 2004, when clover establishment was poor, the percentage diseased roots was higher in the mix-cropped than in the mono-cropped treatment (P = 0.11). Differences in the fraction white heads between the mono- and mix-cropped triticale within years were not significantly different, except in the 2004 year when the fraction white heads was significantly higher in the mixed crop.

Biomass production in the field experiment. The aboveground triticale biomass (shoot weight) was not significantly different between the mono and mixed crop. In 2001, the lack of fertilization resulted in a low biomass yield. In 2002, fertilization resulted in an increase in aboveground biomass compared to 2001, but biomass dropped again in 2003 and 2004 despite fertilization. The yield decrease coincided with an increase in take-all disease. However, triticale grain yield was not significantly different between the mono and mixed crop (data not shown). The clover biomass in both mixed and pure stands was similar in all years except for 2004, when it was significantly (P = 0.05) reduced as a result of a clover pathogen (*Fusarium oxysporum*) that became increasingly important in successive years (data not shown).

Because there was no fertilization in 2001, this year was left out of the correlation analysis between biomass and take-all disease severity. The biomass of mono-cropped triticale had a significant negative correlation with the percentage white heads (r = -0.91, P = 0.0006) and infected roots (r = -0.83, P = 0.006). The same was true for the mix-cropped triticale (r = -0.72, P = 0.03 (white heads) and r = -0.86, P = 0.003 (infected roots)). Also the grain yield showed a significant negative correlation with the percentage white heads (r = -0.56, P = 0.005) and diseased roots (r = -0.66, P = 0.0004, data not shown).

The percentages whiteheads and Ggt infected roots were negatively correlated with the aboveground clover biomass in the triticale-clover crop (r = -0.76, P = 0.02 and r =-0.97, P = < 0.0001, respectively; Figure 2). These correlations point to an effect of clover on Ggt disease severity in triticale.

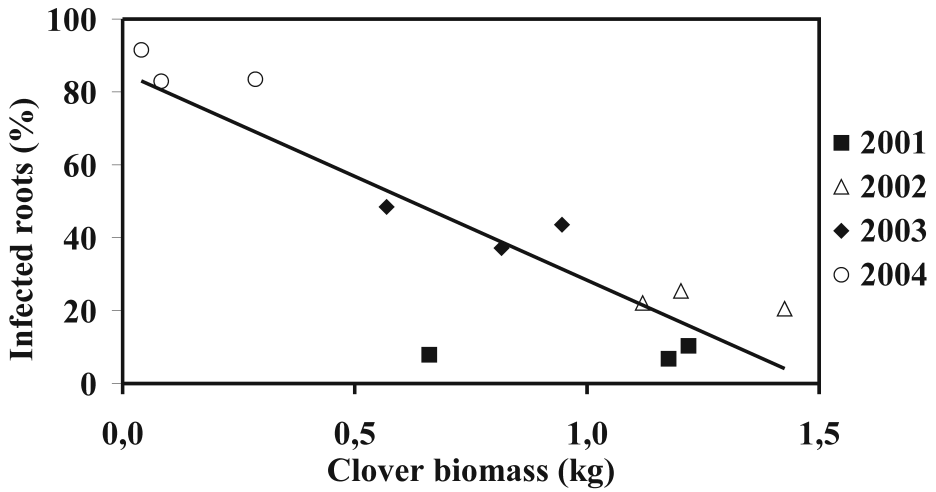

Figure 2. Clover biomass (dry weight) in mixed triticale-clover crops in a field experiment at Wageningen, the Netherlands, plotted against the percentage diseased triticale roots. (Regression line Y = 57.0X+85.3, R^2 = 0.76).

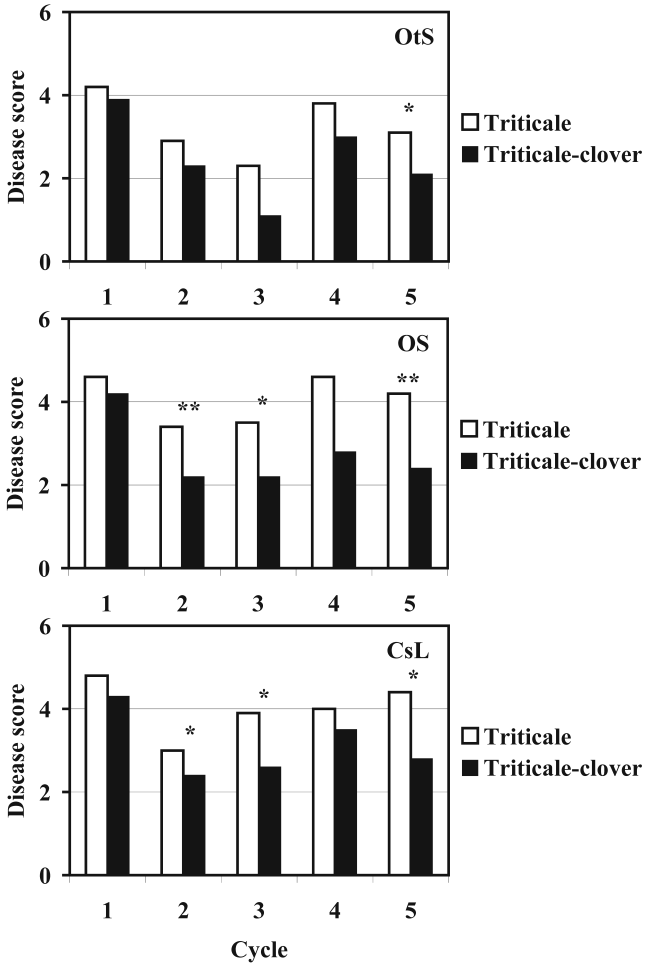

Figure 3. Mean *Gaeumannomyces graminis* var. *tritici* disease rating (0-8 score) on triticale roots in mono- and mix-cropped triticale-clover in the greenhouse experiment (CsL = Conventional silty loam; OS = Organic sandy soil; OtS = Sandy soil with organic treatment). Differences between mono and mix-cropped disease ratings. * = significant at $P = 0.05$; ** = significant at $P = 0.01$).

Take-all disease development in greenhouse-grown triticale-clover. To investigate the observed tendency of lower take-all disease severity in triticale mixed with clover in the field, a greenhouse experiment was performed to elucidate potential mechanisms involved in/contributing to this reduction. Mixed cropping triticale-clover resulted in significantly lower disease severity ($P < 0.0001$) than did triticale mono cropping (Figure 3). Also soil type and cycle affected the disease level significantly. Triticale grown in soil from our experimental field (OtS-soil) had, on average, significantly ($P < 0.001$) lower disease levels than that grown in the other two soils (Os and CsL-soil). Disease severity was reduced

during the first three cycles as was hypothesized, more in the mixed than in the mono crop. In both mono and mixed crop, however, the level of disease increased after the third growth cycle. The interactions crop × soil and crop × cycle were significant, but were considerably lower than those of the main effects and were therefore considered to be less important. The percentages of infected roots showed similar results except that the crop × soil-interaction was not significant (data not shown).

Biomass production in the greenhouse experiment. Triticale shoot biomass (dry weight) was in general higher in the mix-cropped than in the mono-cropped triticale (data not shown). This was, however, cycle dependent, with more biomass in the first two cycles in the mono-cropped triticale for the OtS-soil and in the first cycle for the OS-soil. Total biomass (triticale or triticale + white clover biomass) was highest in the second cycle and decreased during the next cycles significantly in both mono and mix crops.

Triticale fresh shoot weight decreased with increasing disease severity scores (triticale mono-cropped: r = -0.61; $P < 0.0001$; and triticale mix-cropped: r = -0.49, $P = 0.0001$). There was a weak correlation between clover fresh weight and the disease rating on triticale mixed with clover (r = -0.21, $P = 0.1$), but there was no relation between clover fresh weight and percentage infected triticale roots

Nitrate and ammonium concentrations in the field experiment. Nitrate and ammonium concentrations were not affected by the cropping treatments. Both nitrate and ammonium concentrations increased during the years in mono- and mix-cropped soils ($P = 0.0002$ (NO_3); $P < 0.0001$ (NH_4)) because of fertilization with organic manure and incorporation and decomposition of the crop residues. Nitrate concentration was positively correlated with the percentage infected roots in the mix-cropped triticale (r = 0.55, $P= 0.06$) but not in the mono-cropped triticale. Ammonium concentration was positively correlated with the percentage infected roots in both mono- (r = 0.64, $P = 0.02$) and mix-cropped triticale (r = 0.87, $P = 0.0002$)

Nitrate concentrations in the greenhouse experiment. Soil nitrate concentrations increased (due to fertilization) during the first four cycles for all treatments, but in general they were quite low. There was a significant crop × soil-type × cycle-interaction ($P = 0.002$)). However, the main crop-effect was much more important than the interaction (F-value: 51.4 (crop) vs 3.27 (interaction)). Mix-cropping triticale significantly increased the soil nitrate concentration over mono-cropping triticale.

Nitrate concentration was not correlated with the percentage infected roots when treatments were analyzed together. However, for the mix-cropped triticale, the percentage infected roots increased when nitrate concentration increased (Pearson product moment (r) = 0.27, $P = 0.02$).

Ammonium concentrations in the greenhouse experiment. Ammonium concentration showed a soil-type x crop x cycle interaction ($P < 0.0001$), indicating that during the successive cycles ammonium concentration changed differently for each soil and crop combination. However, as was the case for soil nitrate concentrations, also the soil ammonium concentration showed a much higher F-value for the crop-effect than for the

interaction (F-value: 181.0 vs 4.91 resp.). On average, soil ammonium concentrations were higher for mix-cropped treatments than for the mono-cropped triticale treatments ($P < 0.0001$). Irrespective of soil and cycle, ammonium concentrations were negatively correlated with the percentage infected triticale roots, both for the mono-cropped triticale crops (r = -0.29, $P = 0.012$) and for the mixed crop (r = -0.23, $P = 0.06$).

Effect of pH on disease severity. In the field, there was no crop effect on soil pH. Soil pH slightly increased over the years in all treatments (from 5.2 in 2002 to 5.6 in 2004, data not shown) and was positively correlated with the percentage infected roots (r = 0.79, $P = 0.01$) in the mono-cropped triticale, but not in the mix-cropped triticale. In the greenhouse, no pH effect was observed.

Pseudomonas fluorescens in the field experiment. Rhizosphere samples were collected and dilution plated in 2004. Densities of *P. fluorescens* ranged from 6.33 CFU g^{-1} root (f.w.) in the mono-cropped clover to 6.81 CFU g^{-1} root (f.w.) in the mix cropped triticale rhizosphere and were not significantly different among treatments. Densities of 2,4 DAPG-producing (*phlD*$^+$) pseudomonads ranged from log 4.5 CFU g^{-1} root in the mix-cropped triticale rhizosphere to log 5.1 CFU g^{-1} root in the mono-cropped clover rhizosphere. Differences between treatments were not significant.

Pseudomonas fluorescens in the greenhouse experiment. At the start of the greenhouse experiment and prior to infestation with Ggt, rhizosphere densities of fluorescent pseudomonads ranged from log 5.5 CFU g^{-1} root (f.w.) in the OtS-soil to log 5.6 CFU g^{-1} root (f.w.) in the OS-soil and log 6.2 CFU g^{-1} root (f.w.) in the CsL-soil. Densities of *phlD*$^+$-fluorescent pseudomonads were log 5.4, 4.2, and <4.0 (detection limit) CFU g^{-1} root (f.w.) in the rhizosphere of triticale grown in CsL-soil, OtS-soil, and OS-soil, respectively. Although there was a trend towards fewer *phlD*$^+$ *P. fluorescens* isolates in soil that was subjected longer to organic management, the differences were not significant.

Densities of *P. fluorescens* in the triticale rhizospheres in cycle 5 in the OtS-soil were not affected by the treatments (mono-cropped: log 4.6 CFU g^{-1} root, mix-cropped: log 4.9 CFU g^{-1} root). Similarly, fluorescent pseudomonads were not affected by treatments in the clover rhizospheres (mono-cropped: log 4.4 CFU g^{-1} root, mix-cropped: log 5.1 CFU g^{-1} root).

Microbial respiration in the field experiment. Microbial respiration was measured in 2002-2004, with the highest respiration in 2004 (data not shown). Differences were not significant between the crop treatments.

The correlation between disease severity and microbial respiration was positive (r = 0.57, $P = 0.01$), which likely was due to the high respiration levels in 2004, likely associated with the large number of decaying triticale roots due to Ggt infection. When 2004 was left out of the analysis, the correlation was significant and negative (r = -0.65, $P = 0.02$). When treatments were analyzed per year correlations between respiration and disease severity

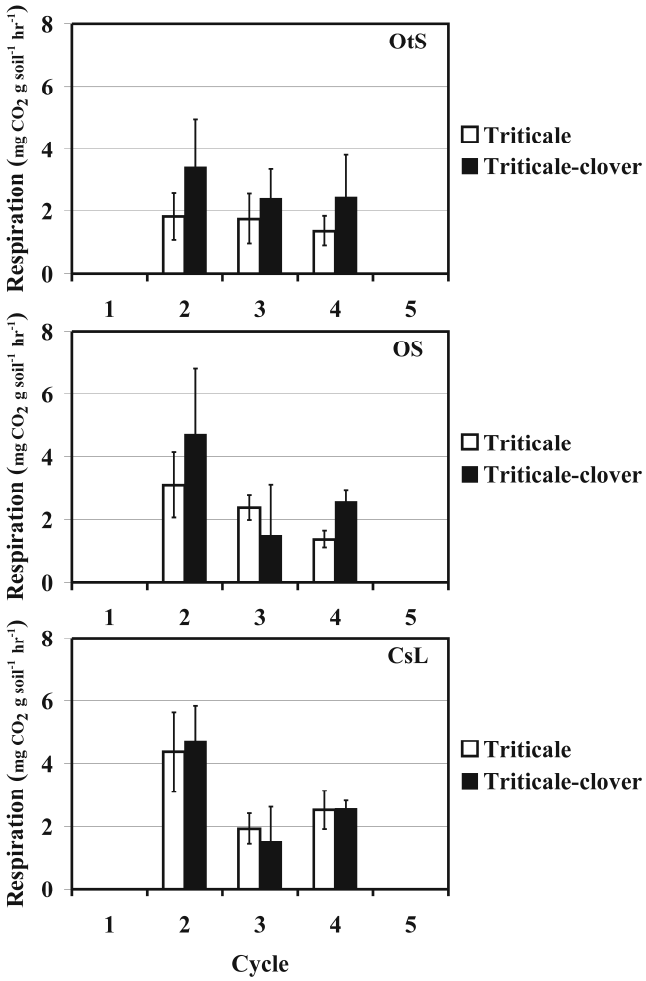

Figure 4. Mean basal respiration of soils (± s.d. of the mean) grown to mono-cropped triticale and mix-cropped triticale-clover in the greenhouse experiment (CsL = conventional silty loam; OS = organic sandy soil; OtS = sandy soil with organic treatment).

were not significant. However microbial respiration increased with higher clover biomass ($r = 0.75$, $P = 0.09$) in the mix-cropped soil when 2004 was excluded from the analysis.

Microbial respiration in the greenhouse experiment. Soil microbial respiration was measured in cycles two, three and four (Figure 4). Mixed cropping of triticale-clover led overall to a higher microbial activity than growing triticale alone ($P = 0.013$). In both the second ($P = 0.008$) and fourth ($P = 0.007$) cycle, mix-cropped soils released more CO_2 than the mono-cropped soils, while in the third cycle there was no statistical difference. The respiration levels were not significantly different among the soil types used.

The percentage Ggt-infected triticale roots was negatively correlated with microbial respiration (r = -0.30, *P* = 0.006). When analyzed separately per treatment this was true for the OS mono-cropped soil, OS mix-cropped soil and CsL mono-cropped soil (r = -0.56, *P* = 0.05, r = -0.45, *P* = 0.11 and r = -0.54, *P* = 0.05, respectively).

When soils were analyzed together, microbial respiration increased significantly with higher fresh clover biomass (r = 0.35, *P* = 0.05). As increased microbial respiration can reduce growth of Ggt (Gerlagh, 1968), stimulation of microbial activity by the mixed crop might be partly held responsible for the disease reduction.

Bulk soil microbial community diversity in the field experiment. Microbial diversity in the field was determined for DNA of bacterial and fungal communities directly extracted from the bulk soil in 2001-2003. Shannon-Weaver indices of the DGGE's ranged from 1.09 to 1.58 for the bacterial communities and from 0.93 to 1.33 for the fungal communities. Mixed cropping did not affect the Shannon-Weaver indices. Microbial community profiles showed very little response to the different crops grown on the soils and showed no significant branching (bootstrap values below 70%).

Rhizosphere microbial communities in the greenhouse experiment. Shannon-Weaver indices based on bacterial (16S-rDNA) or on fungal (18S-rDNA) PCR-DGGE patterns were not significantly different between the mono- and mix-cropped rhizospheres in any of the cycles from all three soils. There was no significant grouping of the 16S microbial community patterns in the rhizosphere. Fungal rhizosphere community patterns showed a clear plant effect (Figure 5) over cycles one and two. The mono-cropped triticale rhizospheres grouped together (bootstrap value 81%) as did the mono-cropped clover rhizospheres (bootstrap value 97%). The mix-cropped clover-triticale and triticale rhizospheres of cycle one clustered significantly together (bootstrap value 83%, Figure 5, 18S). In cycles 3-5, patterns were not significantly different between mono and mixed crops.

Discussion

In this study we investigated the effects of mono-cropping triticale and mixed cropping triticale-clover on *Gaeumannomyces graminis* var. *tritici* disease severity on triticale roots. Overall, clover significantly reduced take-all severity. In the greenhouse experiment, this was clear for all three soils tested, although the difference was not significant in every single growth cycle. In the field experiment, an increasing negative effect of clover on take-all disease increase was clear for the years 2001-2003, but not for 2004, which likely is due to the failure of the clover crop to establish in that growing season. Lower disease severity in mixed crops compared to mono crops has been shown before. Mixed cropping wheat and trefoil (*Medicago lupulina*) reduced Ggt incidence significantly on wheat roots in greenhouse experiments from 76.5% in the mono-cropped treatment to 51.4% in the mixed cropping treatment (Lennartsson, 1988). Villich-Meller (1992) showed that mixed cropping barley-wheat and barley-oats reduced eyespot (*Pseudocercosporella herpotrichoides)*, sharp eyespot (*Rhizoctonia cerealis*) and root disease caused by *Fusarium* spp. Burdon and Chilvers (1976) showed lower damping-off caused by *Pythium irregulare* in a garden cress (*Lepidium sativum*) - Wimmera ryegrass (*Lolium rigidum*) mixture which was attributed to

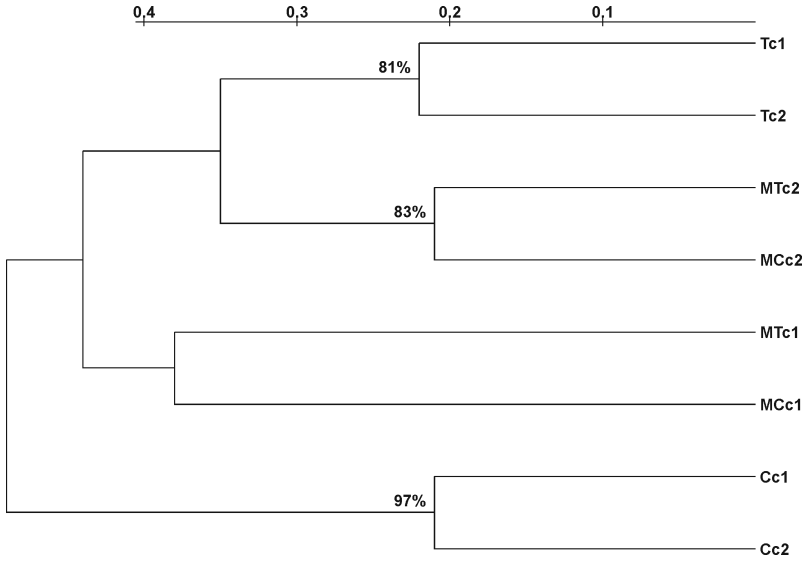

Figure 5. Dendrograms of similarity data of DGGE-gels created from rhizosphere DNA extracts from the fungal (18S) microbial community in triticale (T), clover (C), mix-cropped triticale (MT) and mix-cropped clover (MC) in cycles one and two (c1 and c2) from the OtS-soil.

host dilution effects in controlled experiments where different densities of susceptible hosts were used. Autrique and Pots (1986) showed lower bacterial wilt of potato in a potato-maize and a potato-haricot beans mixture where the mixed crop functioned as a physical barrier lowering root-to-root transmission of the pathogen.

Poor establishment of the clover crop (next to the inoculum build-up) in the field experiment in the fourth year caused the disease to increase dramatically in the mixture, so that infection levels rose as high as in the mono-cropped triticale. From infected clover roots, *Fusarium oxysporum* was isolated, which is a known clover pathogen (Zahid et al., 2001). Due to continuous cropping of clover, populations of pathogenic *Fusarium* spp. (Zahid et al., 2001) can increase and reduce the ability of a clover crop to persist during the growing season. The dependence of the reduction in Ggt disease severity on clover establishment and biomass point to host plant dilution as possible mechanism for disease reduction in mix-cropped systems. This mechanism has been proposed earlier for the reduction in disease severity, both for air-borne (Mundt, 2002) and soilborne pathogens (Burdon and Chilvers, 1976; Villich-Meller, 1992). Besides host plant dilution, as was described for other systems (Autrique and Pots, 1986), clover could also have constituted a physical barrier for Ggt to reach triticale roots, as clover and triticale were sown at the same densities in mono and mixed crops. However, pathogen inoculum was mixed homogeneously through the soil in the greenhouse and the pathogen needed not necessarily pass clover roots to reach susceptible triticale roots. After four years of soil tillage this might also be true for the field experiment.

Clover roots like any other roots are able to influence soil microbial communities via root exudation (reviewed by Rovira, 1965). Increased root density in the mixed crop

leads to higher carbon and nutrient concentrations and this can result in higher respiration levels (Hoitink and Boehm, 1999). Consequently soilborne pathogens, including *Gaeumannomyces graminis*, which has a relatively poor competitive saprotrophic ability (Gerlagh, 1968, Cook, 2003), might face increasing difficulties to spread from root to root. Atmospheric nitrogen can be fixed by clover and become available after decomposition of roots and root nodules (Laidlaw et al., 1996). But also a living clover crop can exude amino acids, nitrate and, primarily, ammonium (Paynel and Cliquet, 2003) and provide accompanying crops with nitrogen. Nitrogen can significantly influence the growth of Ggt (Smiley, 1974; Smiley and Cook, 1973; Sarniquet, 1992). High concentrations of ammonium and use of ammonium fertilizers can decrease Ggt disease severity (Smiley, 1978; Sarniquet, 1992) due to acidification of the rhizosphere by ammonium uptake by the plant. Although the levels of ammonium were higher in the mix-cropped soils, they did not significantly correlate with disease severity in the greenhouse experiment. However, measured ammonium concentrations are representative only for residual ammonium, not for ammonium fluxes in soil or rhizosphere and we do therefore not know what the true effects of ammonium on the disease might be during plant growth.

A direct effect of root exudates on the disease can be another mechanism involved (Dakora, 2003). Phytoalexins in legume root exudates have been described frequently (reviewed by Dakora and Phillips, 1996) and the presence of rhizobia in the soil can suppress soilborne pathogens (Dakora, 2003). For example, biochanin A and formononetin, isoflavonoids excreted by white clover, can reduce germination of arbuscular mycorrhizal spores and subsequent hyphal growth (Tsai and Phillips, 1991) and this may also be the case for soilborne pathogens.

Establishment of antagonists on co-occurring plant species might result in cumulative densities high enough for disease suppression and influence pathogen growth and establishment in mixed cropping systems. Indeed, two plant species can influence each other's rhizosphere microbial communities (Westover et al., 1997) and this was also indicated in DGGE's from our greenhouse experiment. Especially the fungal communities in the OtS-soil showed remarkable similarity between the rhizospheres of triticale and white clover from the mix-cropped treatments. Susceptible roots might become camouflaged by rhizosphere organisms (Gilbert, 1994) and this might be a mechanism of disease reduction in mixed crops. Also incorporation of crop residues of the mixed crop can influence Ggt-antagonistic pseudomonads (Lennartsson, 1988). However, in our field experiment, annual incorporation of clover and triticale crop residues did not result in significantly less disease nor in higher numbers of 2,4-DAPG-producing pseudomonads. Presence of an actively growing clover crop therefore seems to be more important for disease reduction. This is supported by the observations that the disease reduction effect is gone when the clover does not grow well during the growing season (2004 in the field experiment). Successive cycles of mono-cropping triticale did not result in take-all decline but remained at about the same level in the greenhouse experiment and increased steadily in the field experiment. This is in contrast with observations on wheat and barley where monoculturing can induce take-all decline and reduced disease severity after four cycles in greenhouse experiments (Gerlagh, 1968, reviewed by Weller et al., 2002 and Cook, 2003) and after 2-6 years in the field (Shipton, 1975). The time required for the onset of take-all decline in triticale has not been described as far as we know and the time span of our

experiments may have been too short. Also, triticale might not be supportive for 2,4-DAPG-producing pseudomonads, key microorganisms involved in the natural suppressiveness of take-all decline soils in the USA and the Netherlands (reviewed by Weller et al., 2002). Plant-specific support of *P. fluorescens* has been described for wheat where some cultivars enhanced 2,4-DAPG-producing pseudomonads and others did not (Mazzola et al, 2004). The organic management to the OtS soil and OS soil might also influence initiation or enhancing Ggt-suppressiveness as was shown in previous work (Hiddink et al., 2005b) because of low numbers of 2,4-DAPG producing pseudomonads. However, our CsL-soil (from monoculture conventional wheat fields) harbored densities of 2,4-DAPG producing pseudomonads just around the threshold density to suppress take-all (2.5×10^5 CFU g^{-1} root, Raaijmakers and Weller, 1998) which did not result in a significant disease reduction compared to the other soils with lower densities. Because no disease decline occurred in the mono-cropped triticale we assume that 2,4-DAPG-production does not play a mayor role in suppression of *Ggt* in triticale-white clover mixtures.

In conclusion we suggest that clover reduces Ggt disease severity in mix-cropped triticale-clover, which could be the resultant of several mechanisms: microbial competition, increased ammonium release and uptake, with subsequent rhizosphere acidification, root camouflage, direct toxic effects of root exudates of co-occurring crops and probably spatial separation of susceptible roots. These processes would be affected by clover stand, which was quite variable in our experiments. However, use of the right, complementary, crop mixtures can be a tool for sustainable management of soilborne pathogens. Crop mixtures can result in an environment that is less conducive for infection disease development, while supporting simultaneously antagonistic species. An important research challenge for ecological disease management would be to maintain an ecological equilibrium in crop mixtures so that soilborne diseases can be reduced.

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Chapter 6

General discussion

A major problem in agricultural production is the damage incited by soilborne pathogens. According to Lewis and Papavizas (1991), almost 50% of the crop losses can be attributed to soilborne pathogens. In addition, there are indirect costs, since in the absence of soilborne pathogens, narrow rotations with the most profitable crop are most economical. However, in practice rotations need to be wider due to the presence of soilborne pathogens. Since the observations of Lewis and Papavizas in 1991, the situation has not improved. Host resistance against soilborne pathogens is still hardly available. Recently, the EU Council of Agriculture Ministers voted in favor of a proposal of the Commission to ban a large number of pesticides including all soil fumigants. Furthermore, most fungicides are not effective in soil or only effective for a limited period because they are effectively broken down by the resident microflora, bound to soil particles, taken up by the plant or simply because they become diluted in the soil profile. Furthermore, legislation for fungicides for soil or seed applications is often limiting with respect to crop and application time and frequency, and new products are hardly developed. Soilborne diseases are frequently suppressed in organically managed soils, but also there, they do occur at damaging levels. For all these reasons, additional cultural measures are still crucial in managing soilborne pathogens.

Integration of various crop management practices to reduce soilborne pathogens is needed to achieve sufficient control. Sustainable agricultural production heavily depends on the ability of a soil to suppress disease; such soils are often referred to as “healthy soils”, although disease suppressiveness is only one element of soil health (Doran and Zeiss, 2000; Janvier et al., 2007). Improving soil health is a key element for sustainable and effective suppression of soilborne pathogens. This includes crop and disease management practices like reduced soil tillage, organic amendments, seed or soil inoculation with antagonistic microorganisms, cover cropping and mixed cropping as these influence the soil environment in many ways.

The goal of this thesis was to investigate to what extent cultural practices, and in particular mixed cropping, influenced the development of soilborne pathogens and its relation with bacterial and fungal diversity, microbial biomass and several soil physical parameters like nitrogen and organic matter content. In this chapter we summarize and discuss the results obtained in the previous chapters. We will comment on how these results can be used in practice and how they can be used in further research.

Definition of mixed cropping

In literature the terms intercropping, mix cropping and polyculture are used interchangeably (Chapter 3), which can create confusion. Andrews and Kassam (1976; cited in Vandermeer, 1990) defined intercropping as: ‘Growing two or more crops simultaneously on the same field and where crop intensification is both in time and space’. However, intercropping is also used to describe the fallow or cover crop period in between growing two cash crops on the same field. We here use ‘mixed cropping’ as a general term that describes all forms of

growing two or more crops or cultivars together at the same time on the same field in such a way that direct agronomical, biotic and abiotic interactions can occur above- and belowground (Chapter 3). In Chapter 3 several forms of mixed crops are redefined after Vandermeer (1990) and Geno and Geno (2000) and we proposed to use the term 'mixed' instead of intercropping for all types of mixed cropping systems.

Disease suppressiveness in organically managed soils

A way to reduce the impact of soilborne pathogens is to create an environment that is not or less conducive to their development (Alabouvette, 1990). Several farm management factors are thought to be significantly related with disease suppression and those include crop rotation, mixed cropping, tillage, and organic matter management. Organic matter is regarded as one of the driving forces of soil processes (Giller et al., 1997). These soil processes include nutrient mineralisation, which is carried out by the microbial community, and without this process plant growth would be impaired in the absence of manure or artificial fertilizers. Application of more and diverse organic matter can result in an increase in both microbial diversity and activity, which could result in a more intense competition, including competition with soilborne plant pathogens, thus leading to increased disease suppression (Hoitink and Boehm, 1999). On organically managed farms application of organic materials is more common than on conventionally managed farms. As a result this can stimulate or enhance the microbial diversity and activity (Chapter 2) and it can (partly) explain the general observation of increased disease suppression in organically managed soils (Workneh and van Bruggen, 1994; van Bruggen and Termorshuizen, 2003). Indeed, Take-all disease severity (caused by *Gaeumannomyces graminis* var. *tritici*) on wheat and barley was significantly lower in organically managed soils compared to conventionally managed soils (Chapter 2). Take-all is sensitive to competition (Gerlagh, 1968) and in the organically managed soils we studied its development was likely affected by higher microbial activity in the sandy organic soil (Chapter 2). This higher microbial activity can be the result of incorporation of the substantially larger amount of organic matter applied (20 ton ha^{-1} organic manure + 50 ton ha^{-1} humus soil (a combination of compost and sand, used for soil improvement)) in this soil compared to the other soils (including loamy soils), where at maximum 40 ton ha^{-1} organic manure was applied (Chapter 2). On the other hand, no significant effects were observed in loamy soils where application of organic manure was applied in similar amounts in organically and conventionally managed soils although microbial activity tended to be higher in the organically managed loamy soil.

In addition to general suppression of Take-all, also specific disease suppression is a well-known phenomenon. This is induced during continuous cultivation of wheat or barley in the presence of *G. graminis*, resulting in a rise in the populations of 2,4-diactylphloroglucinol (DAPG)-producing pseudomonads, leading to suppression of the pathogen (Raaijmakers and Weller, 1998, reviewed by Weller et al., 2002). However, we did not observe a sufficiently large population (*i.e.* 10^6 CFU g^{-1} soil, Raaijmakers and Weller, 1998) of these organisms in the organically managed soils to explain the observed disease suppression. Furthermore, when introduced in soil, initial survival of the 2,4-DAPG-producing pseudomonads was lower in the organically than in the conventionally managed soils (Chapter 2). In contrast, the effectiveness of the antagonist to reduce take-all disease severity was higher in the conventionally managed soils, implying that the

establishment and activity of the biocontrol agent was higher in the conventionally managed soils than in the organically managed soils.

We thus conclude that the possibility to invade and establish in these active organically managed soils is lower for both the Take-all pathogen and the antagonist. It would be interesting to study the involvement of additional antagonists that are known to suppress Take-all, including phenazine-producing *Pseudomonas* strains, *Bacillus* spp., *Trichoderma* spp. and *Phialophora graminicola* (reviewed by Weller et al., 2002), since the differences in the microbial community structure between the organic and conventionally managed soils (Chapter 2) suggest an involvement of specific antagonists. Therefore, we cannot be conclusive about the exact mechanism for reduced invasiveness from our research but at least for the sandy soils microbial activity was involved (Chapter 2). Studies on incorporation of fresh and recalcitrant organic matter and studies on the rhizosphere and bulk soil microbial communities might add to an understanding of the mechanisms of enhanced disease suppression in organically managed soils.

Disease suppressiveness in mixed crops

The influence of crop rotation and cover cropping on soilborne diseases have been investigated in detail (Mazzola et al., 2002; Janvier et al., 2007) and much of the underlying mechanisms are understood and can be applied. In contrast, mixed cropping appears to be one of the oldest agricultural practices and is preferred by traditional and low input farmers because of its yield stability (Vandermeer, 1990). However, the effect of mixed cropping on airborne and soilborne disease suppression has received far less attention than crop rotation. Especially observations regarding soilborne pathogens in mixed crops are generally coincidental and the underlying processes have not been considered in much detail (Chapter 3).

In 30 out of 37 studies, a wide range of soilborne diseases appeared to be reduced in the mixed crop treatment (Chapter 3) compared to their single-cropped equivalents. In the majority of these observations, host dilution was proposed as the most likely disease reducing mechanism. Host dilution can only be a particularly effective mechanism of disease suppression if, within one cropping cycle, secondary infections are involved, *i.e*. novel infections originating from the primary infection. These secondary infections do occur for several soilborne pathogens such as *Pythium* spp., *Rhizoctonia solani* and *Gaeumannomyces graminis*. For monocyclic soilborne pathogens, in general root rot and vascular wilt pathogens (Agrios, 1997), survival structures often result within one growing season only in one infected plant, *e.g. Synchytrium endobioticum* and *Verticillium dahliae.* If disease suppression occurs for the latter group, substitution of the host for a non-host (host dilution in a replacement design) will only result in a proportional reduction of diseased plants if host dilution is the only disease reducing mechanism in mixed crops. Likewise, in a mixed crop with an additive crop no effects are to be expected at all, compared to the single crop, as the host crop is not diluted in an additive design.

We found that for polycyclic pathogens host dilution is not the sole disease reducing mechanism. In the triticale-clover mixed crop, Take-all disease severity on triticale roots was reduced compared to single-cropped triticale, when the triticale-clover was grown in an additive design (no host dilution, Chapter 5). Therefore, also other or additional plant or soil driven processes play a role in the observed disease suppression in

mixed crops (Chapters 3 and 5). Enhanced microbial activity (Chapters 2 and 5), changed rhizosphere microbial communities in mixed crops (Chapter 5), higher populations of microbial antagonists, allelopathic substances and several abiotic factors (Chapter 3) are all known to be related to suppressiveness to soilborne diseases in mix-cropped soils. These processes alone, combined and/or in combination with host dilution could all be reasons for enhanced disease suppression in mixed crops. However, usually the link between microbial indicators and their functions in soil is not clear (Nannipieri, 2003) and this might especially be true for mixed crops. We observed in several experiments a higher microbial activity and a reduction in take-all disease in mix-cropped soils compared to single-cropped soils (Chapter 5). The enhanced soil microbial activity we observed was likely the result of higher quantities of root residues in the mixed cropped soils (Chapter 5). We showed that increased microbial activity correlated with increasing clover biomass in the mixed crop (Chapter 5). However, the soil microbial activity was measured after sampling and removal of the shoots and roots of the mixed crop grown in this soil. The correlation between lower disease in the field and greenhouse and increased microbial activity measured in the laboratory is therefore not conclusive. Measuring microbial activity during growth of the mixed crop itself would have given better insight in the contribution of microbial activity to take-all suppression in the triticale-clover mix crop, preferably in the rhizosphere.

When disease suppressiveness was tested in sampled soils (triticale-clover) with three different pathogens, no enhanced disease suppression was observed in the mix-cropped soil (Chapter 4) compared to the single-cropped soils, while we did observe take-all disease suppression in the standing crop (Chapter 5). Similar observations were made in a barley-Brussels sprouts mixture, where root galling on Brussels sprouts roots was lower in the mix-cropped Brussels sprouts than in the single-cropped Brussels sprouts (unpublished observations, 2001 and 2003), while no enhanced disease suppression was observed in greenhouse disease suppression bioassays with those soils (Chapter 4). Field bulk soil samples were used for all suppressiveness bioassays, and it might have been better to use rhizosphere soils.

The rhizosphere is an area surrounding the root, determined by the mobility of root-excreted substances (Hiltner, 1904). Therefore, roots have only a restricted direct influence on the soil environment. Under normal, *i.e.* non-sterilized, conditions, the rhizosphere is quite small (up to a distance of a few millimetres) because of the rapid consumption of root exudates by the microbial community. Direct effects of a standing mixed crop are expected to occur most significantly in the rhizosphere. In contrast to bulk soil microbial communities, which are soil type dependent (Chapters 3 and 4), rhizosphere-inhabiting microbial communities are plant species dependent (Kowalchuk et al., 2002, Chapter 5). Indeed, in our research with bulk soil collected after mixed cropping we did not observe major influences of mixed cropping on soil microbial activity, soil microbial diversity and general disease suppressiveness (Chapter 4). This limited effect of mixed-cropping on bulk soil microbial communities might be the result of the relatively small volume of rhizosphere soil included in bulk soil samples and replacement of specific rhizosphere communities by general microorganisms as roots decay.

In mixed crops, rhizospheres of the two crops can influence each other (Westover et al., 1997; Song et al., 2007) because of intermingling of the root systems. Clearly, root architecture of both crops determines the extent of root intermingling (De Kroon, 2007).

Depending on the crop species involved, crop mixtures may show complete intermingling of the root systems, but certain crop combinations result in more or less separate root systems (De Kroon, 2007). Thus, root architecture in mixed crops may reflect the competitive (Vandermeer (1981), cited in Vandermeer, 1990) or the facilitative production principle (Vandermeer 1984; cited in Vandermeer, 1990). The competitive production principle is operative when one crop has a an effect on the soil environment in such a way that the other crop responds negatively to it, although both crops are able to grow next to each other and use resources more efficiently than growing alone. The facilitative production principle is operative when one crop changes the (soil) environment in such a way that the other crop is facilitated, i.e it reacts positively on the environmental change by the co-occurring crop (Vandermeer, 1990). In an additive mixed crop, the competitive or neutral production principle is likely more common in crops where root systems do not freely intermingle and competition for space or nutrients forces root systems to penetrate deeper soil layers. For example, barley roots were forced to grow in deeper soil layers by pea roots due to nutrient depletion in the upper layers (Haugaard-Nielsen et al., 2001). As a consequence, root systems of such crops are likely to remain more or less separate, so that the root density of the susceptible host is not affected by the resistant host in the same soil layer and secondary infections may occur. However, if disease were reduced due to larger distances between susceptible roots because of competition, then one crop facilitates the growth of the other with respect to disease, while plant growth can be reduced because of the competition. Which production principle is regarded to be operative will depend on the pathogen-crop combination. However, with respect to disease suppression the facilitative production principle may be more relevant to mixed crops with more intense root intermingling.

Disease suppression in facilitative mixed crops. Intense intermingling of roots results in modified rhizosphere microbial communities as compared to the respective rhizospheres in single crops (Song et al., 2007; Chapter 5). This “combined” rhizosphere, where both crops affect each other, might be more likely to contain antagonistic populations that contribute to pathogen suppression as compared to a rhizosphere of a single crop.

Disease suppression by the use of appropriate cultivars in mixed crops can be enhanced by the presence of a biocontrol agent in the rhizosphere (reviewed by Smith and Goodman, 1999; Whipps, 2001). Combining selected wheat cultivars that support antagonistic microorganisms (Mazzolla and Gu, 2002) can increase the effectiveness of disease suppression. Selected legumes may also contribute to disease suppression in a mixed crop (Chapter 4). Legumes support *Rhizobium* bacteria that can have pathogen suppressive properties besides fixing nitrogen (Dakora, 2003).

In the triticale-clover field and greenhouse experiments we observed that root systems intermingled intensely (Chapter 5). In both greenhouse and field experiments, the microbial community composition was different in the rhizospheres of both mixed crops as compared to that in the rhizosphere of each single crop. Roots might become camouflaged (Gilbert et al., 1997) by the rhizosphere microbial communities of co-occurring plant species, resulting in the hiding of susceptible roots from pathogen infection. The concept is based on the observations that rhizosphere microbial communities of disease resistant plants are more similar to those in the surrounding bulk soil than rhizosphere microbial

communities of more susceptible plants. Lower root exudation or exudation of more complex substrates that are degraded by bulk soil microbial communities instead of typical rhizosphere microbial communities might be the underlying mechanism. It can be questioned if the observed disease suppressive effects are the result of more disease suppressive microbial communities or reduced chemotaxis by the pathogen because of lower or changed exudation profiles. Root camouflage might simply occur if the microbial activity in the bulk soil is so high, that exudates are immediately consumed, as might have happened in the sandy organic soil, when we compared take-all suppression in organic and conventional soils (Chapter 2). The more root systems intermingle and the more different the microbial communities in the combined rhizospheres are from those in the sole cropped rhizospheres, the more likely it is that specific pathogen attracting compounds are degraded and root camouflage acts as a disease reducing mechanism. This concept was developed for single crops and the mechanisms underlying root camouflage in mixed crops could be the subject of further research.

Other mechanisms that can explain facilitation in mixed crops and that has received only little attention thus far are ISR (Induced Systemic Resistance, induced by bacteria) and SAR (Systemically Acquired Resistance, induced by factors such as water stress, salinity and allelopathy) (Hammerschmidt et al., 2001). ISR and SAR can be triggered by environmental factors (Pieterse et al., 1996; Hammerschmidt et al., 2001), for example by rhizobacteria (Pieterse et al., 2001), or by allelopathic substances excreted by co-occurring roots. They can be active against both mono- and polyphagous soilborne pathogens. If ISR or SAR can be significant disease suppressive mechanisms in mixed crops is not clear and should be investigated. Possibly ISR and/or SAR are already active in single crops due to various stress and microbial factors and it remains to be seen if mixed cropping can enhance these effects.

Disease suppression in competitive mixed crops. Disease suppression in mixed crops is induced by factors acting at a scale that is relevant for soilborne pathogens. When rooting zones of crop mixtures with in an additive design do not intermingle because of depletion of nutrients or allelopahtic substances (responsible for the competitive production principle *sensu* Vandermeer (1981; cited in Vandermeer, 1990)), inter-root distances between susceptible hosts may increase thereby reducing the probabillity of secondary infections (Bailey and Gilligan, 1999; Otten et al., 2005, Chapter 3). In contrast, when inter-root distances of the susceptible crop don't increase, the competitive production principle is not operative as a mechanism of disease reduction. In row-mixed cropping, *Pythium* sp. might be limited in its dispersal if one crop does not contribute to its spread, since the maximum distance the pathogen can bridge in the absence of a host has been estimated at about 6 cm (Burdon and Chilvers, 1975). For many other pathogens the distance that can be bridged in the absence of a host is (much) less. Especially pathogens that spread via secondary infections may be reduced (Chapter 5) under this mixed-crop principle. Incidence of disease by monocyclic pathogens (% of susceptible hosts infected) will hardly be affected as they usually do not spread from host to host, unless their mycelium spreads from root to root. However, when the requirements for spore germination and infection change under influence of competition between two crops, disease incidence can be influenced. Consequently, this changes the production principle from competitive into facilitative with

respect to disease suppression.

Disease suppression not directly related to the facilitative or competitive production principle. Host dilution (as in a replacement design), is a disease reducing mechanism that cannot be regarded as a competitive or facilitative production principle with respect to disease suppression. In this situation susceptible plants are physically separated by replacing the susceptible crop with a non- or less susceptible crop creating such large distances between host plants that root to root spread by pathogens is impeded. For polyphagous pathogens like *Rhizoctonia solani* and *Pythium* spp. that can infect both mono- and dicotyledonous hosts, mixed cropping is expected to be less effective than for pathogens with smaller host ranges. However, not all nutrient sources are equally suitable for the pathogen. Disease progress in a 'less-suitable / suitable host' mixture can still be expected to be reduced significantly compared to a sole crop situation (Otten et al., 2005). Also the capacity of saprotrophic growth on alternative nutrient sources reduces the effectiveness of mixed crops against these generalist pathogens when sufficient nutrient sources are available. For these pathogens, practices to manage organic matter can be more important for disease reduction than mixed cropping.

Conclusions and priorities for further research

Some degree of suppression of soilborne pathogens is observed in all unsterilized soils; this is referred to as 'general disease suppression' (Gerlagh, 1968). This aspecific suppression appears to be higher in organically compared to conventionally managed soils (Chapter 2). The degree of general suppression is greatly determined by soil physical characteristics and organic matter quantity and quality. Soil microbial activity was highest in soil where take-all disease was lowest and is therefore likely contributing to the observed suppression.

Increased disease suppressiveness appears to be quite common in mixed crops and many crop combinations can reduce at least some pathogens (Chapters 3 and 5). However, most observations in the literature on disease suppression induced by mixed cropping are coincidental, resulting from the unplanned presence of a soilborne pathogen in a mixed-crop field experiment. The mechanisms responsible for this observed disease suppression are pluriform and might act simultaneously.

The observed disease suppression in mixed crops (Chapters 3 and 5) is likely additional to general suppressiveness of soils. Mixed cropping can enhance the mechanisms responsible for general suppression like microbial activity, but may also induce specific disease suppression.

The most important observation from this thesis is that the effects of mixed cropping on disease suppression mainly occur in the rhizosphere and that management and measurement of disease suppressiveness should be focused on the rhizosphere (Chapters 3-5). An important consequence of this conclusion is that mixed cropping does not lead to such an inprovement in soil quality that general, aspecific disease suppression is increased. Thus, the effects of mixed cropping systems on soilborne pathogens depend more on the crops growing at that moment than on the mixed crops grown previously. Consequently, disease suppressiveness bioassays with bulk soil (Chapter 4) will be suboptimal to study effects of mixed cropping on disease suppression. Also the sampling and handling of field soil may be such a disturbance that any effects of mixed cropping on microbial communities and

their disease suppressing activities are nullified.

Although the positive effect of mixing crops on disease suppression has been observed quite frequently, more research is needed prior to advising mixed cropping as a management tool for soilborne pathogens. First of all, the number of crop combinations is large and current theories do not predict the effect on a given pathogen and its host. A large number of crop mixtures would need to be tested for disease suppressive effects in order to derive indicators that are consistently associated with these effects, so that the underlying mechanisms can be unravelled. Micro-arrays spotted with a range of known antagonists and genes involved in disease suppression can be used to identify microorganisms in the rhizosphere that may be responsible for the suppressive effects. These data could then be linked to data on the chemical composition in the rhizosphere as affected by root exudation. Root exudation characteristics that result in disease suppressive microbial communities in the rhizosphere can then be used to select crops or cultivars usable for disease suppressive crop mixtures. Mazzola and Gu (2002) used this approach to enhance suppression of apple replant disease by growing wheat cultivars that specifically support certain antibiotic producing pseudomonads. Once exudation characteristics that lead to suppression of particular diseases have been determined, this knowledge might be used in breeding programs to specifically breed for cultivars with disease suppressive capabilities in mixed cropping systems.

Additional to root exudation characteristics, root architecture in crop mixes and the extent of root intermingling could be studied. The possibility for the roots of one crop to affect infection of roots of another crop is largely determined by the extent of root intermingling. When root systems remain separate, rhizosphere interactions do not occur and effects of disease suppression may be limited to host dilution effects.

Another interesting topic that has not been addressed in detail in this thesis is the effect of nutritional status on disease suppression in the standing mixed crop. In our experiments available nitrogen was usually measured after the growing season or at the start of a bioassay. It does therefore not reflect the nutrient availability during crop growth and infection in the mixed crop. Monitoring the availability of nutrients (for example available nitrogen) and linking these data to infection can result in increased insight in pathogen behaviour in mixed crops.

If crop mixtures are found that can enhance disease suppression in practice, a suitable crop rotation still needs to be identified. In single cropping, crop rotation is the most important measure to manage soilborne pathogens. However, if mono- and dicotyledonous crops are mixed, soilborne pathogens affecting crops in either of these groups will be stimulated to some extent. It is an open question whether disease incidence would be less in mixed crops that are not rotated properly than in the same crops grown as single crops in rotation. Finally, implementation of mixed cropping in practice depends not only on the crops involved, but also on proper fertilization schemes and sowing and harvesting techniques.

Despite all the remaining questions and the limited possibilities to extrapolate our results to other crop mixtures and their pathogens, mixed cropping is not just an interesting object of scientific research. Soil- and airborne pathogens in mixed crops are in many cases not as damaging as they would be in the single crops. Furthermore, mixed cropping can have other positive effects, such as reduced erosion, increased nutrient efficiency, water

retention, weed suppression and increased farmer's income (Vandermeer, 1990). These are important benefits of mix-cropping, especially for small scale farmers in low input agricultural systems. Mixed farming, including mixed cropping, may be the most sustainable way of agricultural production in the broadest sense, even in modern high input agriculture provided that appropriate management techniques are developed.

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Summary

One of the constraints limiting agricultural production are pathogens and in particular soilborne pathogens. Even with all modern techniques hardly any sustainable solutions are available. Adequate, chemical, measures are limited and often have unwanted environmental side effects. Stimulating disease suppressive organisms and enhancing soil health by the use of organic production principles and growing mixed crops seem promising tools to manage soilborne pathogens.

In *chapter two*, disease suppressiveness in organically managed soils was compared to conventionally managed soils. Take-all disease severity on roots of barley and wheat, caused by *Gaeumannomyces graminis* var. *tritici*, was significantly lower in organically-managed than in conventionally-managed soils, but a clear soil type (primarily texture) effect was also present. Population levels of fluorescent *Pseudomonas* spp. and in particular 2,4-diacetylphloroglucinol-producing (*phlD*$^+$) pseudomonads, which are key factors in the Take-all decline phenomenon, were quantified. They appeared to occur at lower levels in organically-managed than in conventionally-managed soils. Also, organic management adversely affected the initial establishment of the introduced *phlD*$^+$ *P. fluorescens* strain Pf32-*gfp*, but not its overall survival. In spite of its equal survival period in both types of managed soils, the efficacy of biocontrol of Take-all disease by the introduced strain Pf32-*gfp* was significantly stronger in conventionally- than in organically-managed soils. Collectively, these results suggest that *phlD*$^+$ *P. fluorescens* do not play a critical role in the take-all suppressiveness of the soils included in this study. Furthermore, the role of more general mechanisms involved in Take-all suppressiveness in the organically-managed soils was investigated. The higher microbial activity observed in the organically-managed sandy soil combined with the significantly lower disease severity suggests 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.

In *chapter three*, disease suppressive agricultural systems were reviewed. Some agricultural practices are regarded more sustainable than others and mixed cropping is regarded as a sustainable way of agricultural production. When growing crop mixtures, higher total yields per area can be obtained, available nutrients are taken up more efficiently and soil erosion can be reduced. Also, mixed crops are regarded less disease conducive. This has been shown for airborne pathogens on many occasions and also for soilborne pathogens scientific data is available. In 30 of 37 listed publications mixed cropping showed a disease reducing effect while in only seven publications no or a negative effect was found. Host dilution appeared to be the most important disease-reducing mechanism presented. Although many interactions with other known disease suppressive mechanisms are likely to occur at the same time, they are often not investigated in detail. These knowledge gaps should be filled before the disease suppressive properties of mixed cropping can be adequately managed and observations extrapolated to other crop mixtures and pathogens. Mixed cropping deserves more attention as a sustainable way of agricultural

production in today's agriculture, because of the promising results in disease suppression and other ecological benefits.

In *chapter four*, the effect of mixed cropping on disease suppressiveness of soils was tested for two cropping systems, Brussels sprouts – barley, and triticale – white clover. Disease suppressiveness of field soils was evaluated in bioassays for the soilborne pathogens: *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *lini*, and *Gaeumannomyces graminis* var. *tritici* on various host plants, depending on the pathogen. For both cropping systems, field soils collected from mixed cropping plots did not enhance disease suppressiveness over soils from single cropped plots. In some cases, soil cropped to barley alone was significantly more suppressive to *F. oxysporum* than soils cropped to Brussels sprouts or the mixture of Brussels sprouts and barley. Denaturing gradient gel electrophoresis (DGGE) profiles of amplified 16S- and 18S-rDNA fragments obtained from bulk soil were investigated. In most cases, fungal and bacterial diversity showed no significant differences between mixed and single-cropped soils. In conclusion, in this study, mixed cropping of soils with Brussels sprouts and barley or with triticale and white clover, did not enhance microbial diversity or disease suppressiveness of soils to three different soilborne plant pathogens. We can therefore conclude that soil health is not readily improved when mixed cropping is used.

In *chapter five*, the effect of mixed cropping triticale with white clover on disease incidence and severity of Take-all was studied in both a field experiment and under greenhouse conditions over multiple cropping cycles. In the field experiment, Take-all disease severity on triticale roots increased in both single- and mix-cropped triticale during the first three years. However, disease severity increased to a slightly lesser extent in the mix-cropped triticale. After three cropping cycles the percentage infected triticale roots was lower in the mix-cropped triticale. Due to bad clover establishment, the effect was not significant in the fourth growing season, on the contrary, Take-all disease severity tended to be higher in the mixed cropped triticale. In the greenhouse experiment, Take-all disease severity on triticale roots declined significantly in mix-cropped triticale-white clover during 5 successive cycles compared to the sinlge-cropped triticale. Several known mechanisms that can affect *Gaeumannomyces graminis* var. *tritici* were investigated, including microbial activity, nitrogen concentration and population density of *phlD*$^+$-pseudomonads. In the greenhouse experiment, microbial respiration was significantly higher in the mix-cropped treatment. It was positively correlated with clover biomass and negatively correlated with the fraction of *Gaeumannomyces graminis* var. *tritici* infected roots. Also in the field experiment, a decrease in clover biomass correlated significantly with the increasing Take-all disease incidence over the years. We conclude that clover biomass in mixed triticale-clover crops relates to the reduction in Take-all disease. Disease reducing mechanisms might be the result of changed root architecture, enhanced microbial activity, stimulation of antagonists in the rhizosphere and possibly increased ammonium concentrations in the rhizosphere.

In c*hapter six*, the most important findings in this thesis are summarized and discussed. The results in this thesis provide a better understanding of the way soil and plant health can address problems regarding soilborne pathogens in organically and conventionally managed agriculture. The general conclusions are:

a) Aspecific suppression appears to be higher in organically compared to conventionally

managed soils. The degree of this aspecific suppression is greatly determined by organic matter quantity and quality and the resulting soil physical and biological characteristics.

b) Applying mixed crops under organic management might have an additional soilborne disease suppressive effect because of host dilution and changes in microbial communities in the rhizosphere.

c) Increased disease suppressiveness appears to be quite common in mixed crops and many crop combinations can reduce at least some pathogens. Mixed cropping can enhance the mechanisms responsible for general suppression like microbial activity, but may also induce specific disease suppression.

d) The effects of mixed cropping on disease suppression mainly occur in the rhizosphere. Consequently management and measurement of disease suppressiveness should be focused on the rhizosphere. Therefore, effects of mixed cropping systems on soilborne pathogens depend more on the crops or cultivars growing at that moment than on the mixed crops grown previously.

e) Mixed cropping generally does not lead to such an improvement in soil quality in bulk soil that general, aspecific disease suppression is increased, unless the overall biomass and soil-incorporated crop residues are drastically increased.

Although the positive effect of mixing crops on disease suppression has been observed quite frequently, more research is needed before mixed cropping can be advised as a management tool for soilborne pathogens. Although many questions remain to be solved and the extrapolation of our results to other crop mixtures and their pathogens is limited, we do not regard mixed cropping as just an interesting object of scientific research. Integration of mixed cropping in current agricultural production could increase the sustainability of agricultural production in the broadest sense, even in modern high input agriculture provided that appropriate mixed-crop management techniques are developed.

Samenvatting

Bodemgebonden plantenpathogenen zijn een belangrijke oorzaak van verliezen in de agrarische productie. Weliswaar zijn veel bodemgebonden pathogenen goed te beheersen door middel van vruchtwisseling, maar de kosten van een brede vruchtwisseling zijn hoog en daardoor vaak niet uitvoerbaar. Verder zijn er maar weinig duurzame maatregelen beschikbaar die bodempathogenen voldoende kunnen beheersen. Chemische middelen zijn nauwelijks beschikbaar en hebben bovendien als nadeel dat ze vaak ongewenste neveneffecten hebben op het milieu. Dit proefschrift had tot doel om te onderzoeken of door middel van verhoging van de biodiversiteit van de bodem een betere onderdrukking van bodempathogenen bewerkstelligd kon worden. Dit werd gedaan door (1) het ziektewerende vermogen van biologisch beheerde gronden te vergelijken met gronden die gangbaar beheerd zijn en (2) de toepassing van mengteelten te onderzoeken in vergelijking met teelten met één gewas. Beide opties verhogen de biodiversiteit: biologische landbouw, omdat deze vorm van landbouw sterk afhankelijk is van gebruik van diverse vormen van organische stof als meststof, en mengteelt omdat dan op één areaal minimaal twee in plaats van het gebruikelijke ene gewas geteeld wordt. In beide gevallen leidt dit tot een groter of gevarieerder aandeel organische stof in de bodem. Dit is van belang, omdat organische stof de voedselbron is voor het bodemleven. Een verhoging van de omvang en de diversiteit van het bodemleven kan een negatief effect hebben op bodempathogenen.

In *hoofdstuk 2* werd ziekteonderdrukking in biologisch beheerde gronden vergeleken met die in gangbaar beheerde gronden. Aantasting door de tarwehalmdoder (*Gaeumannomyces graminis* var. *tritici*), een schimmel die wereldwijd veel verliezen geeft in granen, op gerst- en tarwewortels was minder ernstig in biologisch beheerde gronden dan in gangbaar beheerde gronden. De grondsoort, met name de textuur, beïnvloedde dit verschil tussen biologisch en gangbaar sterk. Fluorescerende pseudomonaden, een bepaalde groep van bodembacteriën, spelen een belangrijke rol bij 'Take-all decline', het verschijnsel waarbij door continuteelt van graan de aantasting door de tarwehalmdoder in de loop der jaren af- in plaats van toeneemt zoals bij de meeste andere pathogenen. Deze fluorescerende pseudomonaden bleken in relatief geringe aantallen aanwezig in de biologisch beheerde gronden vergeleken met de gangbaar beheerde gronden. Introductie van een bepaald *Pseudomonas*-isolaat, waarvan de relatie met 'Take-all decline' bekend was (isolaat Pf32-*gfp*) liet zien dat de populatie in eerste instantie sneller terugliep in biologisch beheerde gronden. Hoewel het uiteindelijke populatieniveau in de biologisch en gangbaar beheerde gronden gelijk was, bleek de ziekteonderdrukking door deze geïntroduceerde antagonist significant beter in de gangbaar beheerde gronden dan in de biologisch beheerde gronden. Deze resultaten suggereren dat deze groep van pseudomonaden niet van groot belang is voor de onderdrukking van de tarwehalmdoder in de biologisch beheerde gronden. Daarentegen lijken algemenere vormen van ziekte-onderdrukkende mechanismen belangrijker in de biologisch beheerde gronden. In de biologisch beheerde zandgrond bleek een hogere microbiële activiteit samen te gaan met een geringere aantasting door de tarwehalmdoder. Dit suggereert dat microbiële activiteit een rol speelt bij de ziekteonderdrukking in biologisch beheerde gronden. De samenstelling van de bacterie- en schimmelgemeenschappen in de biologisch beheerde gronden, zoals bepaald met een moleculaire methode (DGGE), bleek te verschillen van die in de gangbaar

beheerde gronden. Dit is een aanwijzing dat er mogelijk ook andere specifieke ziekteonderdrukkende groepen aanwezig zijn in de biologisch beheerde gronden.

In de rest van het proefschrift is mengteelt onderzocht als methode om de biodiversiteit van grond te verhogen. In *hoofdstuk drie* wordt mengteelt gedefinieerd, worden diverse agrarische systemen beschreven en wordt op basis van de literatuur geëvalueerd in welke mate deze systemen zouden kunnen bijdragen tot beheersing van bodemgebonden pathogenen. Door de teelt van menggewassen kunnen hogere opbrengsten per oppervlakte worden gerealiseerd, is het gebruik van beschikbare nutriënten efficiënter en kan bodemerosie sterk gereduceerd worden. Daarnaast is het optreden van ziekten in mengteelten over het algemeen minder heftig. Dit is vaak beschreven voor bovengrondse pathogenen, maar ook in het geval van bodemgebonden ziekten is wetenschappelijk bewijs voorhanden. In 30 van de 37 gevonden publicaties over dit onderwerp bleek dat bodemgebonden ziekten in mengteelten minder schade aanrichten in vergelijking met een enkel gewas. In slechts zeven publicaties werd geen of een negatief effect gevonden. Minder waardplanten per oppervlakte-eenheid werd het meest genoemd als oorzaak voor de geringere aantasting. Hoewel het voor de hand ligt dat ook andere ziekteonderdrukkende mechanismen optreden in mengteeltsystemen, zijn deze vaak niet onderzocht. Deze kennislacunes moeten opgevuld worden voordat de ziektewerende eigenschappen van mengteeltsystemen adequaat toegepast kunnen worden. Vanwege de veelbelovende ziektewerende resultaten en ook de andere ecologische voordelen van mengteelt verdient het gebruik ervan meer aandacht als een duurzame wijze van agrarische productie.

In *hoofdstuk vier* is het effect van mengteelt van (1) spruitkool met gerst en (2) triticale met witte klaver op het ziekteonderdrukkende vermogen van de grond onderzocht. De mate van ziekteonderdrukking van de veldgronden waarop deze mengteelten werden geteeld werd vergeleken met de ziekteonderdrukking van veldgronden beteeld met de enkelvoudige gewassen. De mate van ziekteonderdrukking werd getoetst door grondmonsters te nemen en deze in potproeven te toetsen met de volgende bodemgebonden pathogenen: *Rhizoctonia solani* (veroorzaakt wortelrot en kiemplantenziekte in tal van gewassen), *Fusarium oxysporum* fsp. *lini* (veroorzaakt verwelking bij vlas; staat model voor tal van verwelking-inducerende pathogenen), en *Gaeumannomyces graminis* var. *tritici* (tarwehalmdoder). Grond waarop een mengteelt werd toegepast bleek geen hogere ziekteonderdrukking te bezitten vergeleken met grond waarop een van beide gewassen alleen geteeld werd. De bacterie- en schimmeldiversiteit, bepaald met een moleculaire methode (DGGE) bleken niet significant te verschillen tussen de gronden. We concluderen hieruit dat de in dit onderzoek toegepaste mengteelten niet leiden tot een verhoging van ziektewering tegen de drie onderzochte bodemgebonden pathogenen.

In *hoofdstuk vijf* is het effect van de teelt van triticale-witte klaver op aantasting door de tarwehalmdoder onderzocht in zowel het veld als in een potproef in de kas, gedurende meerdere teeltcycli. In het veld werd de aantasting op wortels van triticale heviger gedurende de eerste drie jaar in zowel triticale alleen als in de mengteelt triticale met witte klaver. Maar de aantasting nam in de mengteelt minder sterk toe dan in de alleen geteelde triticale. In het vierde groeiseizoen verdween dit verschil echter, wat waarschijnlijk veroorzaakt werd door de slechte stand van de witte klaver in de mengteelt. In het kasexperiment verminderde de aantasting van triticiale door de tarwehalmdoder in het mengsel triticale-witte klaver in vergelijking met de triticale gedurende alle vijf cycli.

Diverse ziekteonderdrukkende mechanismen die de tarwehalmdoder kunnen beïnvloeden werden onderzocht. Daartoe werd het belang van de microbiële activiteit en diversiteit, stikstofgehalte en populatiedichtheid van fluorescerende pseudomonaden onderzocht. In het kasexperiment bleek dat de microbiële activiteit significant hoger was in de met triticale-klaver beteelde grond. De microbiële activiteit was ook positief gecorreleerd met de klaverbiomassa in de mengteelt en negatief gecorreleerd met de mate van aantasting door de tarwehalmdoder. Ook in het veld correleerde een grotere klaverbiomassa met een geringere aantasting door de tarwehalmdoder over de jaren heen. We concluderen hieruit dat de klaverbiomassa in de mengteelt een negatieve invloed heeft op de aantasting door de tarwehalmdoder. Dit wordt mogelijk veroorzaakt door veranderingen in het wortelgroeipatroon, verhoging van de microbiële activiteit, stimulering van antagonisten in de rhizosfeer en verder wellicht ook door hogere ammoniumconcentraties in de rhizosfeer.

In *hoofdstuk zes* worden de belangrijkste bevindingen uit dit proefschrift samengevat en samenhangend besproken. De resultaten in dit proefschrift geven informatie over en een verbeterd inzicht in de methoden waarmee de problemen met bodemgebonden pathogenen in biologisch en gangbaar beheerde gronden bestreden en voorkomen kunnen worden. De belangrijkste conclusies zijn:

a) Algemene onderdrukking is sterker in biologisch beheerde gronden dan in gangbaar beheerde gronden. De mate van deze algemene onderdrukking wordt grotendeels bepaald door de soort en het gehalte aan organische stof en daardoor door de fysieke en biologische eigenschappen van de bodem.
b) In biologisch en gangbaar beheerde gronden zou het telen van een mengteelt vanwege de plantgerelateerde ziektewering een aanvulling kunnen zijn van de al aanwezige bodemgebonden ziektewering.
c) Ziekteonderdrukking is een algemeen voorkomend verschijnsel in mengteelten en veel gewascombinaties kunnen tenminste sommige pathogenen onderdrukken. De teelt van een menggewas kan sommige algemene ziektewerende mechanismen zoals microbiële activiteit versterken, maar ook specifieke ziekteonderdrukkende mechanismen (bijvoorbeeld antagonisten) zouden gestimuleerd kunnen worden.
d) Het effect van een mengteelt op ziekteonderdrukking is voornamelijk aanwezig in de rhizosfeer. Ziektewering door middel van mengteelten moet dus gericht worden op deze rhizosfeer. Hiermee benadrukken we dat de ziekteonderdrukkende effecten van mengteelten vooral een direct gewas- of cultivareffect zijn, op het moment dat het menggewas wordt geteeld. Er lijkt dus niet een langer durend effect te zijn dat meetbaar is in een volgend gewas.
e) Hieruit volgt dat het telen van een menggewas niet tot grote verbetering van de algemene ziekteonderdrukking van de grond leidt, tenzij het menggewas een grotere hoeveelheid ondergeploegde gewasresten geeft dan de enkele gewassen.

Alhoewel er regelmatig een sterkere ziekteonderdrukking is gezien in mengteelten, kan het gebruik van mengteelten nog niet geadviseerd worden als methode om bodemgebonden pathogenen te onderdrukken.Vele vragen zijn nog onbeantwoord en generalisatie van onze resultaten naar andere mengteeltsystemen is nog maar beperkt mogelijk. Desondanks zien we het gebruik van mengteelten niet slechts als een interessant wetenschappelijk onderzoeksobject. Integratie van mengteelten in de huidige agrarische productiesystemen zou een verduurzaming van de agrarische productie kunnen zijn in de breedste zin, zelfs in

moderne kostenintensieve agrarische productiemethoden indien adequate teelt- en oogstmethoden voorhanden zijn.

Curriculum vitae

Gerrit Albertus Hiddink werd geboren op 4 mei 1973 in Doetinchem. In 1990 behaalde hij zijn HAVO-diploma aan het Ulenhof College te Doetinchem. Datzelfde jaar begon hij aan de studie Nederlandse Landbouw met specialisatie tuinbouw aan de HAS in Dronten. Als stage en afstudeerscriptie voor de HAS deed hij literatuur- en veldonderzoek naar de benodigde arbeidstijd bij de oogst en verwerking van bolgewassen. Daarnaast werkte hij aan een afstudeerproject voor ALF (Agrarisch Laboratorium Flevoland) naar het effect van bladbemesting in leliebollen. Na het behalen van het HAS-diploma in 1995 is hij onder andere werkzaam geweest als agrarisch medewerker, assistent waagmeester en hovenier. In 1997 begon hij aan een studie Gewasbescherming en Plantenveredeling aan Wageningen Universiteit, met als specialisatie Ecologische Gewasbescherming (T15b). In 1999 studeerde hij af op de relatie tussen de dichtheid van microsclerotiën van *Verticillium dahliae* in de bodem en aantasting in trompetboom en aubergine, onder leiding van Jan Kees Goud en Aad Termorshuizen. Dit onderzoek werd gedurende negen maanden voortgezet als toegevoegd onderzoeker bij de leerstoelgroep Biologische Bedrijfssystemen. Na deze aanstelling volgden zes maanden als technisch onderzoeker Fytopathologie en Toedieningstechnieken (gewasbeschermingsmiddelen) bij het PBG in Naaldwijk. In November 2000 startte hij als Assistent in Opleiding in het project: ‘Enhanced biodiversity for sustainable crop protection‘ waarin gewerkt werd aan het deelproject: ‘Cropping diversity and Soilborne plant pathogens’ (NWO-project: 014.22.032). Na afloop van het contract nam hij de zorg voor zijn zoon Ian op zich en schreef onderwijl aan zijn proefschrift. Vanaf augustus 2005 tot en met december 2005 was hij werkzaam als wetenschappelijk onderzoeker bij PPO in Lisse waar onderzoek werd gedaan naar Tabaksratelvirus in gladiool, biofumigatie-effecten van koolgewassen op *Pythium* in de bloembollenteelt, effect van mycorrhiza’s op bolproductie van hyacint en *Pseudomonas fluorescens* als biologische bestrijder in de teelt van bloembollen. Vanaf 1 januari 2006 is hij werkzaam bij het groentezadenveredelingsbedrijf Enza Zaden B.V. in Enkhuizen als zaadpatholoog. In 2008 werd het promotietraject afgesloten met het proefschrift dat nu voor u ligt.

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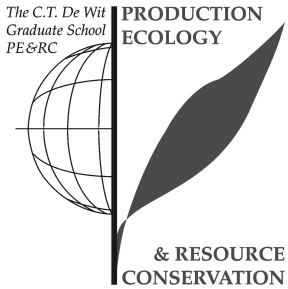
PE&RC PhD Education Certificate



With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of Literature (5.6 ECTS)
- Cropping diversity and soil-borne pathogens (2001)

Laboratory Training and Working Visits (1.4 ECTS)
- PCR en haar toepassingen; Hogeschool Brabant (2000)

Post-Graduate Courses (5.8 ECTS)
- Enhanced biodiversity for improved crop protection; PE&RC (2001)
- Applied soil ecology, linking theory to practice; PE&RC (2003)
- Basic and Advanced statistics; PE&RC (2003)

Competence Strengthening / Skills Courses (2.6 ECTS)
- Scientific writing; Wageningen University Language Centre (2006)
- NWO talentendag workshops (netwerken en subsidie aanvragen); NWO (2004)
- Afstudeervak organiseren; OWI Wageningen University (2003)

Discussion Groups / Local Seminars and Other Scientific Meetings (7 ECTS)
- Agricultural production systems; PE&RC discussion group (2000-2004)
- Weekly Phytopathology meetings (Wageningen University (2000-2004)
- Workshop NWO Agrobiodiversity; NWO (2004)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (2.8 ECTS)
- PE&RC annual meetings (2000-2004)
- KNPV gewasbeschermingsdagen (2000-2004)
- Werkgroep bodempathogenen (2000-2004)

International Symposia, Workshops and Conferences (7.0 ECTS)
- ISME Amsterdam (2001)
- ICPP Christchurch (2003)
- Rhizosphere Munich (2004)

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