Disease suppression and phytosanitary aspects of compost

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2007

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Dit onderzoek is uitgevoerd binnen de C.T. de Wit Onderzoekschool 'Productie Ecologie en Beheer van Natuurlijke Hulpbronnen'

Disease suppression and phytosanitary aspects of compost

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Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. M.J. Kropff,
in het openbaar te verdedigen
op vrijdag 2 februari 2007
des namiddags te 16.00 uur in de Aula

Rijn, E. van. 2007. Disease suppression and phytosanitary aspects of compost. Doctoral thesis, Biological Farming Systems Group, Wageningen University, The Netherlands.

Key-words: compost, disease suppression, phytosanitation, soilborne plant pathogens; *Cylindrocladium spathiphylli*, *Fusarium oxysporum* f. sp. *lini*, *Phytophthora cinnamomi*, *Phytophthora nicotianae*, *Polymyxa betae*, *Pythium ultimum*, *Rhizoctonia solani*, *Verticillium dahliae.*

ISBN 90-8504-584-3

Abstract

In Western Europe, approximately 25% of the 200 million tons of municipal solid waste that is generated each year is of organic origin and therefore compostable. Presently 35% of this organic waste is composted, resulting in 9 million tons of compost, and used mainly in agriculture, horticulture or hobby gardens. Increasing the opportunities to use compost in horticulture and agriculture as a (potting) soil amendment and nutrient source for plants and the soil microbial community would contribute to the recycling of waste and reduce the use of non-renewable peat and artificial fertilizers. This thesis is concerned with disease suppressive properties and phytosanitary aspects of compost.

A major result of this thesis is that a compost cannot be simply assigned the label 'disease suppressive'. Its disease suppressive properties depend on both the plant species and pathogen species involved. Some composts were found to be highly disease suppressive for one or a few pathosystems, but not for others. The same was the case if disease suppression was tested for one pathogen, *Pythium ultimum*, on multiple hosts. Although variation in disease suppression was found as function of compost, host species and pathogen species, stimulation of disease as an effect of compost application was the exception (3% of the cases), and significant disease suppression (54%) or no effect (43%) the rule in a large experiment where 18 composts were tested with 7 pathosystems. Indications were obtained that the microbial change as affected by mixing compost with peat, both in the bulk mix as well as in the rhizosphere of the plants, is a parameter that correlates positively with disease suppression. Prediction of disease based on biotic and abiotic characteristics of compost was quite weak as compared to that based on the peat/compost mixes. The results indicate that there is scope for development of specialty products, designed for certain pathosystems. Different compost batches sampled at different times at the same composting facility showed rather similar levels of disease suppression.

For assessing the phytosanitary risks associated with the application of compost it is necessary to include all risk factors and not only those occurring during composting. It seems safe to use composts produced from Vegetable, Fruit and Garden (VFG) compost although the conditions required for inactivation of some pathogens are still unknown. With respect to the assessment of the phytosanitary risks associated with composting itself, the determination of the degree of inactivation as function of temperature likely overestimates the temperature needed during composting, since other factors such as temporary anoxia, which brings about the formation of a range of toxic compounds, also contributes to inactivation of pathogens, as was shown here for *Polymyxa betae*, a pathogen of sugar beet.

Voorwoord (acknowledgements)

Als de bosjesmannen ’s ochtends een kudu op hun pad aantreffen, zullen zij deze tot in de avond volgen. Andere dieren die hun pad kruisen, zullen ze voorbij laten gaan. Viereneenhalf jaar geleden is mijn promotieonderzoek op mijn pad gekomen. Sindsdien heb ik grote hordes moeten nemen en heeft ziekte mij mijn weg bemoeilijkt, waardoor het nogal eens zware tijden zijn geweest. Maar altijd had ik voor ogen dat dat proefschrift er komen moest. Ik herinner me nog goed hoe ik net met mijn promotieonderzoek was begonnen, rekenend op een uitdagende baan, en hoe ik weken lang vermeend poederschurft van de aardappels krabde. Na weken zou ik met een biotoets vaststellen of de planten van het krabsel ziek werden. Helaas, mijn krabsel-poederschurft had geen effect: was het de toets of het krabsel waar het aan mankeerde? Gelukkig zijn er, met name in de tweede helft van mijn promotieonderzoek, nog vele thema’s op mijn weg gekomen die maar al te interessant waren. Zoals bijvoorbeeld Aad Termorshuizen, mijn co-promotor, het ooit uitdrukte: als jij zit te analyseren, dan beginnen je oortjes ervan te gloeien. Ja, creatief analyseren van ecologische data-sets en het daaruit destilleren van nieuwe concepten, dat vond ik prachtig.

Vele mensen zijn me op mijn pad behulpzaam geweest en ben ik dank verschuldigd. Een aantal mensen wil ik hier in het bijzonder noemen. Allereerst wil ik Aad Termorshuizen bedanken die mij in goede en in slechte tijden altijd heeft gesteund, waarmee ik interessante discussies had en die zijn mening nooit onder stoelen of banken stak. Aad, jouw kijk op zaken heb ik altijd hoog geacht en mede dankzij jou ligt hier nu een voltooid proefschrift. Bedankt voor alles! Ook mijn promotor Ariena van Bruggen ben ik dankbaar voor haar betrokkenheid en de kritische noten die zij plaatste bij mijn onderzoek. Wim Blok wil ik bedanken voor de discussies omtrent de inhoud van de *Pythium*-proeven (hoofdstuk 4) en het beschikbaar stellen van de mooie composteerfoto op de omslag! Leo van Overbeek, Arjen Speksnijder en Rob Pastoor: bedankt voor de bijdrage die jullie hebben geleverd aan het tot stand komen van hoofdstuk 3. Met name aan het moleculaire deel hebben zij een belangrijke bijdrage geleverd.

Veel steun bij mijn onderzoek ontving ik van twee buitenlandse studenten. Muriel Caffort visited our department for several months and performed bio-assays for the compost storage experiments. I had several experiments running at that time and without your enthusiastic help I’m sure I would never had managed it! I am also very grateful to Vasilis Spyropoulos who did his MSc thesis under my supervision. You performed a lot of bio-assays with Pythium and the results contributed significantly to the contents of Chapter 4. You did a great job, Vasilis!

For all the participants in the EU compost project: Claude Alabouvette, Yona Chen, Deborah de Clercq, Dirk Jan van der Gaag, Jan Lagerlöf, A.A. Malandrakis, Nondas Pamplomatas, Birgitta Rämert, Jaak Ryckeboer, Christian Steinberg and Sharon Zmora-Nahum; thank you for your contributions, pleasant company and hospitability during our meetings! It was a pleasure for me to participate with you in the project, sharing the same interest and discussing, eating and drinking together! De composteerders die hun medewerking aan de verschillende projecten verleenden door ons te voorzien van compost, ben ik uiteraard zeer erkentelijk.

Dank ook aan Oscar en Dine die altijd met raad en daad voor me klaar stonden op het

lab en in de kas. Oscar heeft me vooral geholpen bij moleculaire zaken en het maken van de kaft en Dine heeft me praktisch werk voor het *Polymyxa*-onderzoek uit handen genomen. Wampie wil ik hartelijk bedanken voor haar toewijding bij het in orde maken van de lay-out van het proefschrift: vooral de bladspiegel moest in orde zijn, zo was haar devies. Natuurlijk wil ik alle mensen van mijn leerstoelgroep bedanken voor de prettige en gezellige tijd die ik met jullie heb doorgebracht. Allereerst mijn kamergenoot Jeroen, maar ook Lien, Eelco, André, Sasha, Pete, Anne, Niels, Joan, Mbaye, Walter en Egbert. I want to thank my Portuguese, Spanish and Italian colleges and friends Sonia, Carmen, Carlos and Francesca, who made work especially pleasant by giving our group some kind of Mediterranean flavor. In het bijzonder wil ik nog de twee 'oudgediende' promovendi Jan-Kees en Gerbert bedanken, voor jullie betrokkenheid en gezelligheid.

Liesbeth en Luuk, trouwe vrienden: ik vond het fantastisch dat jullie mijn paranimfen wilden zijn opdat ik zonder kleerscheuren door de verdediging van mijn proefschrift zou komen. Heel erg bedankt voor jullie steun, zowel op de dag zelf, als tijdens de voorbereidingen! Jan, Alniek, Hanneke, Bas, Carla, Ines, Michaela en Jeroen, jullie hebben mij als vrienden veel leuke momenten bezorgd tijdens wandelingen, etentjes, gitaar spelen, spelletjes Risk en schaak, wielrennen en wat al niet meer zij. We hebben veel plezier gehad en ik kon bovendien altijd op jullie steun rekenen als ik die nodig had. Bedankt! Susanne, Flor, Maaike en Monique en Geertje, het toneelspelen met jullie was altijd een heerlijke afwisseling op mijn AIO-baan. Ik heb er van genoten en hoop nog veel meer gezellige uurtjes met jullie door te brengen! Ook de mensen van het Johanneskoor wil ik hartelijk bedanken. Ik heb altijd heel fijn met jullie gezongen en vond het erg gezellig.

Mijn lieve ouders, die mij altijd gesteund hebben, door dik en dun, in gezonde en in zieke jaren, ben ik veel dank verschuldigd. Heel erg bedankt! Ook mijn lieve zus en zwager Martina en Cornelis en natuurlijk hun kinderen Rosa en Jonas wil ik bedanken voor de gezellige uurtjes. Elsbeth van de Pijpekamp en mevrouw de Groot, mijn behandelaars, en Dr. A. Langens wil ik bedanken voor hun steun en goede raad welke zeker hebben bijgedragen aan het met goed gevolg voltooien van dit proefschrift. En tenslotte mag beslist niet ongenoemd blijven mijn lieve vriendin Caroline met wie ik interessante inhoudelijke discussies heb gevoerd en met wie ik erg veel leuke dingen heb gedaan die met name de tweede helft van mijn onderzoek tot een interessante en plezierige tijd hebben gemaakt. Nog iemand vergeten? Twee kleintjes maar! Mijn lieve dochtertjes Thisa (6 jaar) en Anna (3 jaar). Wat zou mijn leven er anders uitzien zonder jullie lachende gezichtjes en jullie spel. Fijn dat jullie er zijn!

Februari 2007,

Etienne van Rijn

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Chapter 1

General introduction

In Western Europe, approximately 25% (49 million tons) of the 200 million tons of municipal solid waste that is generated each year is of organic origin and therefore compostable (European Compost Network, 2006). Presently 35% of this organic waste (17 million tons) is composted, resulting in 9 million tons of compost, and used mainly in agriculture, horticulture or hobby gardens (European Compost Network, 2006). The remainder 65% of compostable waste is either used for biogas production (Kumar, 2000) or is incinerated or dumped on landfill sites. Increasing the opportunities to use compost in horticulture and agriculture as a (potting) soil amendment and nutrient source for plants and the soil microbial community would contribute to the recycling of waste and reduce the use of non-renewable artificial fertilizers. Stimulation of the use of compost in horticulture will benefit the composting industry but will also benefit the potting soil industry as the policy in several European countries is to decrease the use of peat in potting soil.

The willingness of farmers and horticulturalists to use compost is directly related to various quality aspects of the compost. Firstly, composts are commonly used in agriculture as an organic amendment to maintain or increase soil organic matter content which is essential for maintaining soil quality by improving the biological, physical and chemical soil conditions (Termorshuizen et al., 2004). Secondly, growers can benefit from an increased use of compost by the fact that many composts have disease suppressive properties and, therefore, use of disease suppressive compost will reduce crop losses caused by soilborne diseases (Hoitink and Fahy, 1986). Thirdly, sanitation plays an important role in the use of compost. Risks of pathogen spread through compost have to be minimized and therefore proper sanitation measures have to be taken during the composting process.

In Section I of this thesis, disease suppressive properties of composts are investigated aiming to get a better insight in which biotic factors (microbial composition of the compost and compost/peat mixes, plant species and pathogen) and abiotic factors (physico-chemical characteristics of the compost and compost/peat mixes) are decisive in disease suppression. Ultimately this may lead to a more effective use of compost as a biological control measure to manage soilborne pathogens. In Section II of this thesis, phytosanitary aspects of composts are studied. Acknowledging that risks of pathogen spread through compost utilization should be minimized, we investigated the possibilities of phytosanitary risk assessment and determined the conditions required to eradicate the soilborne pathogen *Polymyxa betae*.

Section I. Compost-induced suppression of soilborne plant pathogens

Soilborne diseases are an important threat to many agricultural and horticultural crops. For decades, soil fumigants have been used widely to control soilborne diseases, but they have been implicated in causing various environmental problems. Notably methyl bromide

depletes stratospheric ozone and there is also concern about the health consequences of inhalation of fumigants by people living in proximity to treated fields. In addition it has become clear that pesticides with a wide spectrum of activity have unwanted side-effects such as the inactivation of soilborne antagonists of pathogens. As a consequence, their use has been restricted worldwide and therefore alternative methods for the control of soilborne diseases are needed. A commonly used practice to reduce soilborne pathogens is crop rotation. Many diseases build up in the soil when the same crop is grown in the same field year after year. Rotation with non-susceptible crops can help to break this cycle by reducing pathogen levels. However, for economic reasons this is often difficult as farmers are usually specialized in the cultivation of only one or a few crops. This situation frequently occurs for example in greenhouses. Other approaches to manage soilborne pathogens are therefore needed as well. These include physical methods such as solarization (Katan, 1996), biofumigation (Kirkegaard et al., 2000), biological soil disinfestation (Blok et al., 2000) and application of biocontrol agents (Hoitink and Boehm, 1999; Ryckeboer, 2001) or organic amendments (Bailey and Lazarovits, 2003) including composts (Paulitz and Belanger, 2001).

Several studies reveal that composts can suppress plant diseases (e.g. Hoitink and Fahy, 1986; Craft and Nelson, 1996; Diab et al., 2003; Scheuerell et al., 2005). Organic amendments such as composts can introduce compost-specific antagonists and provide the food base needed for the general microflora including specific antagonists. In several cases, these amendments contribute to the general suppressive activity of amended peat, as reported for *Pythium* spp. and *Phytophthora* spp. (Mandelbaum and Hadar, 1990; Hardy and Sivasithamparam, 1991), while in other cases the introduction of compost-specific biocontrol agents play a role in disease suppression (e.g. for *Rhizoctonia solani* in Grebus et al., 1994). Alternatively composts can be suppressive because they induce resistance against plant pathogens or because they produce fungitoxic compounds such as organic acids or ammonia (Hoitink and Boehm, 1999; De Clerq et al., 2004). As the mechanisms of disease suppression vary among different pathosystems, disease suppression of a compost against different plant diseases may vary as well. As crops are exposed to infection by multiple pathogens, it is desirable to select a compost that is suppressive for multiple plant diseases. Research on disease suppressive properties of composts, however, is usually focused on a single or a few pathogens only. Moreover, disease suppression varies according to the type of compost, depending on its biological and physico-chemical characteristics. It would be interesting if reliable predictions could be made on how these compost characteristics affect disease suppressive properties against multiple pathogens.

For the cultivation of many ornamentals peat is used as growing substrate. Peat is cheap and highly suitable as a growing substrate because of its high stability and porosity, and usual absence of weed seeds and plant pathogens. However, peat is a non-renewable resource and peat bogs are getting increasingly appreciated as nature areas that need to be protected. For example, in the UK the area of peat bogs has been reduced since around the start of the nineteenth century by around 94% from an original 95.000 ha to 6.000 ha (UK Biodiversity Action Plan, 2006). The vast majority of the extracted peat in the European Union is used by horticulturalists (11.9 million m^3 yr^{-1}) and gardeners (7.5 million m^3 yr^{-1}; Oosten, 2006). Extraction of peat implies the liberation of fixed carbon at faster rates than the capacity of

bogs to regenerate, thus leading to significant contributions to global warming. The use of compost as an amendment in potting soil will decrease the need of peat and thereby also the release of carbon dioxide.

Peat contains only recalcitrant carbon compounds that are not available to soil microbiota and, as a result, it is generally conducive to soilborne diseases. Amendment of compost to peat or soil results in an increase of compounds that are decomposable by soil microbiota, which has been illustrated by shifts in the microbial community structures (Boehm et al., 1993; Kowalchuk et al., 2002; Yao et al., 2006), and an increase in soil respiration (Diab et al., 2003), which may lead to disease suppression (Kowalchuk et al., 2002; Diab et al., 2003; Scheuerell et al., 2005). Changes in the microbial community of the compost-amended substrate have been demonstrated to be related both to characteristics of the main substrate (peat/soil) and to the type of organic amendment used (Pérez-Piqueres et al., 2006). In some cases, however, a stimulation of the autochthonous microorganisms in the substrate (peat or soil) has been reported while colonization of the substrate by introduced compost-borne organisms was not observed (Saison et al., 2006; Crecchio et al., 2004).

In several cases suppression of soilborne plant pathogens have been shown to be based on microbial processes in the rhizosphere (Raaijmakers and Weller, 1998; Weller et al., 2002). The diversity of rhizosphere microbial communities is affected by the plant species involved and is related to differences in root exudation and rhizodeposition (Jaeger et al., 1999). The influence of compost amendments on the microbial composition of the rhizosphere has been shown to be reduced in the direct neighborhood of the root and to be dependent on the quantity of compost amendment (Inbar et al., 2005). An effect of compost on the rhizosphere microbial community will become apparent only if it exerts a greater influence than the plant on the rhizosphere community. Compost-induced suppression of soilborne pathogens that is generally rhizosphere-mediated may therefore be predicted by the extent to which compost affects the rhizosphere community.

Compost research is often hampered by the fact that repetition of experiments with the same batch of compost is difficult as storage influences organic matter quality and the composition of the microflora. Another important aspect in compost research is that if different parameters of the compost have to be measured, storage is often necessary since different measurements cannot be carried out at the same day or week. The effect of storage on compost quality is also relevant for the distribution of commercial composts as these are stored in bags until use. More knowledge is therefore desired about the effects of storage on microbial communities and microbial activity of composts in relation to the persistence of disease suppressive characteristics.

Section II. Phytosanitary aspects of composting

Composting is an efficient process for treatment of biowastes in order to obtain stable organic matter that contributes to soil microbial activity. Proper composting guarantees the eradication of the majority of plant pathogens residing in biowastes due to heat generated during the thermophilic phase of the composting process (Bollen et al., 1989; Bollen, 1993;

Bollen and Volker, 1996; Ryckeboer, 2001). However, survival at peak compost temperatures >62°C for 21 days has been reported for few soilborne plant pathogens like *Plasmodiophora brassica*, *Fusarium oxysporum* f.sp. *lycoperisici* and *Macrophomina phaseolina* (Noble and Roberts, 2004). Phytosanitary risk assessment associated with compost utilization is therefore desired and should not only focus on the survival of plant pathogens during the composting process but should also pay attention to the composition of the original waste and several aspects related to compost utilization.

Temperature is considered the major factor determining the inactivation of plant pathogens during composting under aerobic conditions and thermophilic anaerobic digestion, while toxic agents are considered to be responsible for pathogen inactivation during decomposition under mesophilic anaerobic conditions (Bollen and Volker, 1996; Ryckeboer, 2003). Composting conditions to eradicate pathogens have been defined on the basis of standard experimental conditions of 30 minutes of incubation in aerated water (Bollen, 1993). For phytosanitary reasons, composting conditions are defined to reach a temperature of >60°C for at least 3 days (Noble and Roberts, 2004). The conditions prevailing during the thermophilic phase of composting are different from the aerated water suspensions used in standard test conditions. During the thermophilic phase of composting, which may last several days, temporary and local anaerobic conditions widely occur (Fernandes and Sartaj, 1997; Veeken et al., 2002; Beck-Friis et al., 2003), leading to decomposition products that may be toxic to multiple organisms. Thus, in a compost heap, combined effects of temperature and anaerobic circumstances may become responsible for pathogen eradication. One of the test organisms to determine the phytosanitary status of a composting process that has been used extensively in the Netherlands is *Polymyxa betae*, the vector of beet necrotic yellow vein virus (BNYVV) in sugar beet. Remarkably, *P. betae* is one of the few pathogens of which the inactivation temperature has not been determined.

Overall objectives

This thesis is concerned with quality aspects of compost, varying from disease suppressive properties to phytosanitary aspects. The study aimed:

- To determine whether disease suppressive characteristics of composts are pathosystem-specific and whether these characteristics are correlated with physico-chemical, microbiological and faunal characteristics of the composts and compost-amended peat substrates.
- To determine the effects of amendment of compost to peat-based substrate on the bacterial, actinomycete and fungal communities in relation to disease suppression.
- To determine the disease suppressive characteristics of a variety of composts for *Pythium ultimum* as function of host plant species, and the relationship of these suppressive characteristics to host-specific rhizosphere bacterial, actinomycete and/or fungal communities.
- To determine the effects of three months of storage under different storing conditions (dry, frozen and cool) on disease suppressive characteristics against flax wilt caused by

Fusarium oxysporum f. sp. *lini,* and relate these effects to any possible changes in microbial composition or microbial activity.
- To assess phytohygienic risks associated with compost utilization in agriculture.
- To determine the composting conditions required for eradication of *Polymyxa betae*.

Outline of the thesis

Section I (Chapters 2-5) deals with disease suppression by compost. Chapter 2 presents the results of investigations aimed at determining whether disease suppressive characteristics of composts are pathosystem-specific and whether these characteristics are correlated with physico-chemical, microbiological and faunal characteristics of the composts and compost-amended peat substrates. Chapter 3 further investigates a subset of the composts of chapter 2. The specific goal was to determine the effects of amending compost to peat-based substrate on the bacterial, actinomycete and fungal communities in relation to disease suppression. Chapter 4 aims at determining disease suppressive characteristics of composts for *Pythium ultimum* as function of host plant species and whether these suppressive characteristics can be related to host-specific rhizosphere bacterial, actinomycete and/or fungal communities. Chapter 5 presents the results of experiments aimed at determining the effects of three months of storage under different storing conditions (dry, frozen and cool) on the bacterial communities and microbial activity of composts in relation to disease suppressive characteristics against flax wilt caused by *Fusarium oxysporum* f. sp. *lini*.

Section II (Chapters 6 and 7) deals with phytosanitary aspects of composting. Chapter 6 describes a phytosanitary risk assessment of compost, including factors like the composition of the biowaste, the degree of sanitation achieved during composting and utilization aspects of the compost. Chapter 7 presents the results of investigations on the composting conditions required for eradication of *Polymyxa betae,* the vector of beet necrotic yellow vein virus (BNYVV) in sugar beet. Survival of resting spores under aerobic and anaerobic conditions under several temperature regimes in a water suspension and in leachate from a compost heap has been determined.

A general discussion of the results of this thesis is presented in Chapter 8.

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Section I

Compost-induced suppression of soilborne plant pathogens

Chapter 2

Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response

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Soil Biology and Biochemistry 38(2006): 2461-2477

Abstract

Compost is often reported as a substrate that is able to suppress soilborne plant pathogens, but suppression varies according to the type of compost and pathosystem. Reports often deal with a single pathogen while in reality crops are attacked by multiple plant pathogens. The goal of the present study was to evaluate the disease suppression ability of a wide range of composts for a range of plant pathogens. This study was conducted by a consortium of researchers from several European countries. Composts originated from different countries and source materials including green and yard waste, straw, bark, biowaste and municipal sewage. Suppressiveness of compost-amended (20% vol./vol.) peat-based potting soil was determined against *Verticillium dahliae* on eggplant, *Rhizoctonia solani* on cauliflower, *Phytophthora nicotianae* on tomato, *Phytophthora cinnamomi* on lupin, and *Cylindrocladium spathiphylli* on *Spathiphyllum* sp. and of compost–amended loamy soil (20% vol./vol.) against *Rhizoctonia solani* on *Pinus sylvestris* and *Fusarium oxysporum* f. sp. *lini* on flax. From the 120 bioassays involving 18 composts and 7 pathosystems, significant disease suppression was found in 54% of the cases while only 3% of the cases showed significant disease enhancement. Pathogens were affected differently by the composts. In general, prediction of disease suppression was better when parameters derived from the compost mixes were used rather than those derived from the pure composts. Regression analyses of disease suppression of the individual pathogens with parameters of compost-amended peat-based mixes revealed the following groupings: (1) competition-sensitive: *F. oxysporum* and *R. solani* / cauliflower; (2) rhizosphere-affected: *V. dahliae*; (3) pH-related: *P. nicotianae*; and (4) specific/unknown: *R. solani* / pine, *P. cinnamomi*, and *C. spathiphylli*. It was concluded that application of compost has in general a positive or no effect on disease suppression, and only rarely a disease stimulating effect.

Introduction

Soilborne diseases are an important threat to many agricultural and horticultural crops. The use of soil fumigants and fungicides is being restricted in Europe and other areas of the world and, therefore, alternative methods for the control of soilborne diseases are needed. Widening the use of crop rotation is a major tool for managing soilborne diseases, but for economic reasons farmers are usually forced to specialize and focus on the cultivation of a single or only a few crops. Such a specialization has been developed most extensively in capital-intensive systems such as greenhouses, where continuous cultivation of crops is common practice. In such systems, other approaches to manage soilborne pathogens are needed as well. These include physical methods such as solarization (Katan, 1996), biofumigation (Kirkegaard et al., 2000), biological soil disinfestation (Blok et al., 2000) and application of biocontrol agents (Hoitink and Boehm, 1999; Ryckeboer, 2001) or organic amendments (Bailey and Lazarovits, 2003) including composts (Paulitz and Belanger, 2001). Increasing the opportunities to use compost in horticulture and agriculture as a (potting) soil amendment and nutrient source for plants and for the soil microbial community would contribute to the recycling of waste and reduce the use of non-renewable artificial fertilizers. Stimulation of the use of compost in horticulture will benefit the potting-soil industry as the policy in several European countries is to decrease the use of peat in potting mixes since peat bogs are increasingly appreciated as nature areas that need to be conserved. Organic amendments are applied to induce the production of fungitoxic compounds such as organic acids or ammonia. Alternatively they may provide substrates for organisms that enhance general antagonism through competition, or for specific agents that invoke antibiosis or induced resistance against plant pathogens (Hoitink and Boehm, 1999; De Clerq et al., 2004).

Several studies reveal that composts can suppress plant diseases (e.g. Hoitink and Fahy, 1986; Craft and Nelson, 1996; Diab et al., 2003; Scheuerell et al., 2005). In the majority of these studies, suppressiveness of one type of compost has, however, been tested against a single or a few pathogens only, while in reality a host plant may face infection by multiple pathogens. Furthermore, compost is known as a product that varies considerably in chemical, physical and biotic composition, and, consequently, also in ability to suppress soilborne diseases. So, one may select for a compost which is highly suppressive to one disease while it has no effect against other important root diseases. In this paper we report on investigations aiming to identify physico-chemical and/or biological characteristics that could facilitate the prediction of disease suppression against multiple pathogens. Total microbial activity, biomass, total numbers of culturable actinomycetes, other microorganisms, and genetic and metabolic structure of microbial communities have often been associated with enhanced disease suppression of various diseases (Chen et al., 1988; Tuitert et al., 1998; Diab et al., 2003; Noble and Coventry, 2005; Pérez-Piqueres et al., 2006). The micro- and mesofauna in the compost may also contribute to disease suppression (Friberg et al., 2005). The physico-chemical compounds of composts provide the space and food source for the microbial and faunal populations. The level of suppressiveness of composts may, therefore, be influenced by the decomposition level of the composts (Boehm et al., 1993).

Table 1. Some of the characteristics of the composts studied.

Code	Origin		Composted material[b]	DOC[c] (mg l^{-1})	OM (%)	C/N	EC[c] (mS cm^{-1})	total biomass (mg g^{-1})	actino-mycetes (CFU g^{-1})	bacterivo-rous nema-todes (g^{-1})	fungivorous nematodes (g^{-1})	basal respiration[d]
	Coun-try[a]	Com-pany										
a	F	I	Horse manure (20%) and green wastes (80%) (wheat straws, corn straws, conifer bark)	392	47.0	22.0	1.60	0.80	8.8×10^5	94.0	0.4	40.1
b	F	II	Tree bark, slurries, green wastes	167	37.1	14.7	4.33	0.77	1.0×10^6	11.7	0.0	33.0
c	F	III	Urban biowastes	826	45.6	17.4	2.83	1.41	4.9×10^5	6.6	0.0	83.7
d	F	IV	Organic residue of wine grapes, green wastes	921	66.3	15.2	1.55	0.18	1.0×10^6	36.8	2.1	37.7
e	F	V	Woody wastes, poultry manure	457	37.2	12.4	3.30	0.61	1.7×10^5	53.5	0.0	39.7
f	F	VI	Woodcut, plants, horse manure	1001	39.0	10.8	2.82	0.53	2.6×10^5	0.5	0.1	14.7
g[e]	Gr	VII	Spent mushroom compost (wheat straw (56%), chicken manure (39%), gypsum (5%))	208	39.5	9.5	2.14	1.28	3.1×10^5	46.0	0.0	9.8
h[e]	Gr	VII	Spent mushroom compost: wheat straw (56%), chicken manure (39%), gypsum (5%)	262	46.7	10.0	2.24	1.09	7.4×10^5	30.8	5.8	9.3
i	Gr	VIII	Leonardite[f] (90%), urea (6%), phosphate (2%), catalyst[g] (2%)	57	32.6	16.4	2.58	0.14	2.0×10^3	0.0	0.0	1.8
k	Gr	IX	Wood chips, horse manure	695	34.0	13.3	1.07	0.16	4.0×10^5	87.2	0.5	7.1
l	Il	X	Municipal sewage sludge and yard waste	176	20.5	8.2	2.72	0.85	5.4×10^6	0.3	0.0	4.3
m	NL	XI	Wood chips (88%), manure (2.5%), clay (10%)	291	22.7	14.6	0.71	2.23	1.3×10^6	18.0	58.0	23.1
n	NL	XI	Wood chips (82%), manure (8%), plant residues (5%), clay (5%)	257	23.0	14.2	0.74	2.37	1.8×10^6	65.0	0.8	27.1
o	NL	XI	Wood chips (72%), manure (14%), plant residues (9%), clay (5%)	402	30.7	14.1	1.04	2.49	2.2×10^6	126.0	4.0	40.4
p	NL	XII	Yard waste (woody materials, grass clippings)	188	21.3	15.1	0.87	0.94	4.9×10^5	0.0	0.0	22.6
q	NL	XIII	Yard waste (without grass)	384	28.6	21.5	0.72	0.78	5.0×10^6	28.0	38.0	26.9
r	NL	XIII	Yard waste (without grass)	306	21.8	15.8	0.77	0.90	5.8×10^6	1.1	0.0	44.3
s	NL	XIV	Yard waste (mainly grass)	314	27.7	17.1	0.96	1.01	6.2×10^5	43.8	0.4	25.9

[a] Origin of the composts: F = France, Gr = Greece, Il = Israel, NL = the Netherlands.
[b] Percentages of ingredients are volume-based.
[c] Determined in 1:10 water extract.
[d] Basal respiration (10^{-6} g CO_2 g^{-1} d.w. substrate h^{-1}).
[e] Composts g and h were taken from the same compost heap; compost h was sampled and tested for disease suppressiveness 1 yr after compost g.
[f] Leonardite is a a soft brown coal-like deposit usually found in conjunction with deposit of lignite.
[g] A mixture of microorganisms used by the compost manufacturer (precise composition not known).

The objective of the present study was to test a wide array of composts against various types of pathogens and to select for compost characteristics which may be used to predict suppressiveness against one or more diseases. More specifically the goals were to: (i) determine the suppressiveness of an array of composts against several soilborne pathogens; (ii) determine physico-chemical, microbiological and faunal characteristics of the composts; and (iii) correlate suppressiveness of the composts with the compost and potting mix characteristics. Pathogens included in the present study were the root inhabitants (*sensu* Garrett (Garrett, 1970); i.e., not active outside the rhizosphere) *Fusarium oxysporum* f. sp. *lini* and *Verticillium dahliae* and the soil inhabitants *Cylindrocladium spathiphylli*, *Rhizoctonia solani*, *Phytophthora cinnamomi*, and *P. nicotianae*, of which the latter two species are known to have a low ability to compete (You and Sivasithamparam, 1995; Aryantha et al., 2000). Five pathosystems were tested with potting soil as base substrate and two with loamy soil as base substrate.

Material and methods

Collection and selection of composts

Composts, made from different kinds of waste material combinations (e.g. green waste, domestic biowaste, manure), were collected *at random* from commercial composting plants in four different countries (France, Greece, Israel and the Netherlands) and in three different periods (runs 1, 2 and 3). Composts were stored in loosely closed polyethylene bags at 4°C until use. Two l of each compost were oven-dried at 65°C for chemical analysis. For each run, 9-17 composts were collected. A preselection of composts was carried out to avoid the use of immature composts (which may be phytotoxic or compete with the plants for nitrogen and oxygen) and to exclude composts with a too low level of organic matter (OM). Selection criteria were OM >20% (wt/wt) and concentration of dissolved organic carbon (DOC) in 1:10 water extract <400 mg l^{-1}. Based on DOC and OM data, 6 composts were selected for each run (Table 1). These composts were further studied for disease suppressiveness. The French composts appeared to exhibit in general rather high DOC values of up to 1000 mg l^{-1} in 1:10 water extract and therefore some of these were included in run 3 (Table 1). Non-dried samples of each of these composts were used for biological characterizations including bioassays.

Preparation of potting mixes

About 2-3 weeks before start of the bioassays, of each compost, a 1-l-sample was sent to a commercial laboratory (Groen Agro Control, Delft, the Netherlands) for determination of pH and concentration of mineral elements in the 1:1.5 volume extract. Eight days before starting the bioassays, the composts were incubated at room temperature and potting mixes were prepared one day later. Non-amended mixes consisted of peat (H2-3 on the von Post decomposition scale; Boden, 1994), lime (8 g l^{-1}, Dolokal PG) and PG-mix (0.8 g l^{-1}, 15-10-

20) or a loamy soil with mineral fertilizer added (PG-mix, 0.8 g l^{-1}, 15-10-20). The same batch of peat or loamy soil was used in all tests. The peat (A0, Kekkila, Finland) was stored in compressed bales to which no lime or mineral fertilizers had been added. The loamy soil was sampled in the first 15 cm of a grass meadow, after the surface of the soil was scraped off. The texture of the soil (clay 35%, silt 45.5%, sand 19.5%; wt/wt) was determined by a laboratory of soil analysis (Laboratoire Départemental de Côte d'Or, France). The soil was steam-sterilized for 45 min in a container before use. Amended mixes consisted of peat or loamy soil (80%) and compost (20%, volume basis) and mineral fertilizers. Lime (4 g l^{-1}) was added to some of the compost-amended peat-based mixes to increase the pH. Based on the mineral elements present in the compost extract (1:1.5), mineral fertilizers were added to the compost/peat or compost/soil mixes to obtain similar nutrient levels in the non-amended and amended potting mixes (Kreij and Gaag, 2003). Moist mixes of compost and peat or soil were incubated for one week at room temperature before start of the bioassays. The water content of the peat-based mixes was between 60 and 75% (wt/wt) and of the non-amended mix between 75 and 80%.

Bioassays

Disease suppressiveness of the potting mixes was determined in seven different bioassays: *Phytophthora cinnamomi* Rands / lupin, *Cylindrocladium spathiphylli* Schoult., El-Gholl & Alfieri (teleom. *Calonectria spathiphylli* El-Gholl *et al.*) / spathiphyllum, *Rhizoctonia solani* Kühn AG2-1 / cauliflower, *Phytophthora nicotianae* Breda de Haan / tomato (these three bioassays performed in greenhouses in the Netherlands), *Verticillium dahliae* Kleb. / eggplant (performed in a greenhouse in Greece), *Rhizoctonia solani* AG2-2 IV / pine, and *Fusarium oxysporum* Schlecht. : Fr. em. Snyder & Hansen f. sp. *lini* (Bolley) Snyder & Hansen / flax (these two bioassays performed in greenhouses in France). All bioassays were carried out in peat and compost/peat mixes except the latter two (*R. solani* / pine and *F. oxysporum*), which were carried out in soil and compost/soil mixes. For all bioassays, a randomized complete block design was used. The exact design of the bioassays was based on preliminary experiments, in which the inoculum density was determined that was necessary to arrive at a disease level of about 50-75% of the maximum disease possible in the non-amended controls.

Inoculum of *P. cinnamomi* (isolate P7, kindly provided by Dr. W. J. Blok, Wageningen University) was produced in a mixture of sterilized peat and ground oat as described by Blok *et al*. (2002). The inoculum was mixed with the potting mix at a dosage of 0.1% (vol./vol.). Pots with a diameter of 10.5 cm and a volume of 0.4 l were filled with potting mix. Eight lupin seeds (*Lupinus angustifolius* L. cv. Borweta) were sown in each pot and the number of healthy seedlings was determined after 21 days. Five non-infested and 5 infested pots were sown for each potting mix.

Inoculum of *C. spathiphylli* (isolate AN2000) was produced in the same way as described for *P. cinnamomi* (see above). The inoculum was mixed with the potting mix at a dosage of 0.1% (vol./vol.). Seven-weeks-old spathiphyllum plantlets (*Spathiphyllum wallisii* Hort. cv. Ceres, Braam, De Kwakel, the Netherlands) were planted in pots with a diameter of

9 cm and a volume of 0.2 l. Each replicate consisted of a tray (19 × 31 cm^2) containing seven pots placed on an irrigation mat. For each potting mix five replicates were employed with infested soil and three replicates with non-infested soil. Starting 2 weeks after planting, the number of healthy, diseased and completely wilted or dead plants were recorded every 3-4 days. The final observation was performed 7 weeks after planting.

Phytophthora nicotianae (isolate IK51, kindly provided by G. Zervakis, NAGREF, Greece) was produced in the same way as described for *P. cinnamomi* (see above) and blended with the potting mix at a dosage of 1% (vol./vol.). Seven tomato seeds (*Lycopersicon esculentum* Mill. cv. Jamaica, seeds kindly provided by RijkZwaan, the Netherlands) were sown in a pot (9 cm diameter, 200 ml). The number of healthy seedlings was determined about 10 days after sowing. For each type of potting mix 5 infested and 5 non-infested pots were used.

Inoculum of *R. solani* (AG2-1 isolate 21R21, kindly provided by Dr. J. Postma, Plant Research International, Wageningen, the Netherlands), was grown on Potato Dextrose Agar (PDA; Oxoid, UK). Nine cauliflower seeds (*Brassica oleracea* L. var. *botrytis* cv. Fremont F1 (Royal Sluis, the Netherlands)) were sown in each pot (9 cm diameter). One week after sowing the number of seedlings was recorded and a 3 × 3 mm^2 disk of PDA with actively growing mycelium of *R. solani* was placed in each pot adjacent to a seedling and just below the soil surface near the edge of the pot. Cauliflower seedlings were observed daily and as soon as all seedlings in one of the pots were damped-off, the number of non-damped-off seedlings in each pot was determined. Each treatment of the bioassay had 5 replicates.

Both *Phytophthora* species assays and the *Rhizoctonia*-cauliflower assay were carried out in a greenhouse at 20°C and the *Cylindrocladium* assay in a greenhouse at 24°C. Pots were placed on a dish or tray and irrigated regularly from below using tap water. Spathiphyllum plants were fertilized with a nutrient solution (EC of 2 mS cm^{-1}) once a week starting 4 weeks after potting.

Inoculum of *R. solani* AG2-2IV isolate G6 was grown on barley grains that had been autoclaved twice and supplied with antibacterial antibiotics at a pH of 4.5. The grains were incubated at 25°C for 3 weeks before they were dried, homogenized in a blender, sieved (through pore sizes 3.15 mm in diameter) and blended with soil or soil-compost mixes at 0.5% vol./vol. and thereafter used as soil inoculum. The treatments were performed in plastic trays, each treatment containing 10 pots (7 cm diameter each). Each pot was filled with steam-sterilized loamy soil. Ten to 14 pine seeds of the susceptible *Pinus nigra* var. *austriaca* were sown. Seeds were covered with a thin layer of microgranules of calcined clay to prevent evaporation. After 10 days of growing, pine seedlings were thinned to 10 plants per pot and the 10 pots of a plastic tray were each inoculated with 30 g of the soil inoculum to make up one replicate. Each replicate consisted of 100 seedlings. The control treatment comprised inoculation with non-infested soil or soil/compost mix. The experiment was carried out in a greenhouse at 25°C and each treatment had 3 replicates. The number of healthy pine seedlings was scored daily starting 3 days up to 10 days after infestation.

Inoculum of *V. dahliae* isolate Eg1 was grown in liquid SSN medium (Sinha and Wood, 1968) for 4 weeks under continuous shaking at 120 rpm and 22°C in the dark. After incubation, clusters of microsclerotia, the survival structures of *V. dahliae*, were homogenized in an omni-

mixer (Ivan Sorvall Inc., Newtown, Connecticut) and filtered through a mesh screen of 70 µm diameter. The microsclerotia that did not pass through the mesh screen (the larger microsclerotia) were used in the bioassay. After air-drying, the inoculum was kept at 4°C until needed. Eggplant seeds (*Solanum melongona* L. cv. Black Beauty) were sown in trays containing a mixture of peat and compost mix. Seedlings at the second true-leaf stage were transplanted in individual pots (10 × 10 cm^2). To infest the substrates the inoculum was suspended in water, the density of microsclerotia was determined and an amount of suspension was added to the substrate to reach 25 microsclerotia g^{-1} d.w. substrate. Disease development was assessed once a week estimating disease severity by counting the number of wilted leaves as a percentage of the total number of plant leaves. The experiment was ended when plants in one treatment showed complete wilting (i.e., 6-8 weeks after start of the experiment).

Inoculum of *F. oxysporum* f. sp. *lini* isolate 3 was produced in liquid malt extract medium (10 g l^{-1}, pH 5.5). Five-days-old cultures were filtered through a sterile sintered glass funnel. The density of conidia in the filtrate was determined by direct microscopic counts and adjusted to infest the treatments at a density of 1×10^4 propagules g^{-1} soil/compost mix. The bioassay was set up in polystyrene trays. Each tray consisted of 12 rows containing 8 sowing mini-pots (3 cm diameter). Eight-hundred g of soil or soil/compost mix were distributed in two rows of 8 mini-pots (i.e. 50 g per mini-pot). Four ml of inoculum suspension at a density needed to reach 1×10^4 propagules g^{-1} soil or soil-compost mixes were placed on the top of the 50 g contained in each mini-pot. Non-infested rows of soil and of soil-compost mixes served as control treatment and were irrigated with 4 ml sterile water. Two to 3 flax seeds (*Linum usitatissimum* L. cv. Opaline) were sown in each mini-pot and covered with a thin layer of calcined grains. After emergence (5 days after sowing), the number of seedlings was reduced to one per mini-pot, making a total of 16 plants per treatment. Each treatment was replicated three times. For each replicate, the number of healthy plants was recorded twice a week starting 21 days up to 62 days after sowing.

Calculation of disease suppressiveness of composts

Suppressiveness of the different potting mixes was determined in three experimental runs with each containing six composts. The *P. nicotianae* – tomato pathosystem was only carried out in runs 2 and 3. For each experimental run the disease levels were related to that of the non-amended control in order to be able to compare suppression levels among the different runs. The number of healthy plants in the non-infested controls was not influenced significantly by the composts and the percentage disease suppression induced by the compost in the *Phytophthora* assays was calculated as $100 - 100 \times (CM - IM) / (CM - IC)$, where CM is the number of healthy plants in the non-infested compost-amended treatment, IM is the number of healthy plants in the infested compost-amended treatment, and IC is the number of healthy plants in the infested non-amended potting mix.

In the *Cylindrocladium, F. oxysporum* f. sp. *lini*, *R. solani* AG2-2 (pine), and *V. dahliae* assays the area under the disease progress curve (AUDPC; Campbell and Madden, 1990) was calculated for the number of completely wilted or dead plants and the percentage disease suppression was calculated as $100 - 100 \times (AUDPC_{\text{compost-amended potting mix}} / AUDPC_{\text{non-amended potting mix}})$.

For the *R. solani* AG2-1 (cauliflower) assay, disease suppression (%) induced by the compost was calculated as 100 – 100 × (% damping-off in compost-amended mix) / (% damping-off in non-amended mix).

Characterization of the nutrients concentrations of the potting mixes

One week after preparation of the potting mixes, the mineral element contents, EC (as described in detail below) and pH of water extracts (1/1.5, vol./vol.) of the peat-based mixes were determined by a commercial laboratory (Groen Agro Control, Delft, the Netherlands).

Characterization of biotic variables

For plating of bacteria (incl. actinomycetes), 10 g (d.w. basis) compost and compost mixes was suspended in 90 ml water, sonicated (47 kHz) for 1 min, shaken (180 rpm, 30 min) and serial 10-fold dilutions were made from 10^{-1} to 10^{-8}. 50 µl of the suspension were pipetted onto a Petri dish from the dilutions 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} (copiotrophic bacteria, i.e., growing at high carbon levels) and 10^{-2}, 10^{-3}, and 10^{-4} (oligotrophic bacteria; i.e., growing at low carbon levels, and actinomycetes). Per dilution three plates were used. A standard high-nutrient medium was used to determine the number of copiotrophic bacteria: 0.5 g $MgSO_4 \times 7H_2O$, 0.5 g KNO_3, 1.3 g $K_2HPO_4 \times 3H_2O$, 0.06 g $Ca(NO_3)_2 \times 4H_2O$, 2.5 g $C_6H_{12}O_6$ (glucose), 0.2 g enzymatic casein hydrolysate, 15 g technical agar (Oxoid nr. 3), 1 l demineralized water, and 100 mg l^{-1} sterile cycloheximide after sterilization. For oligotrophic bacteria and actinomycetes the same medium was used at 100-fold dilution of all ingredients except the agar (Agar Noble, Difco Labs, Detroit). Plates were incubated in darkness at 25°C for 7 days (copiotrophic bacteria) or 4 weeks (oligotrophic bacteria and actinomycetes).

Total microbial biomass of compost and compost mix samples was determined using the fumigation extraction method (Joergensen, 1995). Ten g (d.w.) of compost was placed in a glass vial, put in a desiccator, lined with wet tissue paper together with a vial with soda lime, a beaker containing 50 mg ethanol-free $CHCl_3$ (chloroform) and a few boiling chips. The desiccator was evacuated until chloroform boiled for 2 min, incubated in the dark at 25°C for 24 h, followed by removal of the chloroform by 6-fold evacuations. The fumigated as well as the unfumigated control samples were transferred to 250 ml bottles for extraction with 100 ml 0.5 M K_2SO_4 for 30 min in an oscillating shaker at 180 rpm and then filtered through a filter paper (Whatman No. 42) for 1 h. Subsequently, the samples were frozen at -20°C until determination of total organic carbon (TOC) content by ultraviolet persulphate oxidation as described in Joergensen (1995), using an automated TOC/DOC analyzer (Skalar, Breda, The Netherlands).

General microbial activity was characterized by determining basal respiration, i.e. the CO_2-production without addition of extra nutrients. Basal respiration was determined with an automated system in which a continuous air flow of 65 ml min^{-1} was led over 30.0 g f.w. of compost or potting mix in glass tubes (length 24 cm, diameter 3.5 cm) during incubation at 20ºC for 24 h. The CO_2-concentration in this air stream was measured by means of a computer-controlled switching device and an infrared CO_2-analyzer (ADC 7000, Analytical

Development Corporation, Hoddesdon, UK) which allowed hourly measurements. For calculation of the basal respiration the readings of the first 10 h of incubation were omitted. Basal respiration was expressed as μg CO_2 g^{-1} d.w. h^{-1} and determined in duplicate for all compost and potting mix samples. In addition, oxygen uptake rate (OUR) was measured based on the Oxitop® system that determines pressure change that coincides with microbial use of oxygen in closed bottles (Veeken et al., 2005). The Oxitop® system consists of a glass bottle (2 l) with a CO_2 trap (soda lime) in the head space. About 10 g (f.w.) of compost material was sampled, precisely weighed and put in the bottle. 180 ml of demineralized water, 10 ml of pH 7 phosphate buffer (43.08 g l^{-1} KH_2PO_4, 88.86 g l^{-1} $Na_2HPO_4 \times 2H_2O$, 1 l H_2O), 10 ml of macronutrients (4.31 g l^{-1} NH_4Cl, 5.39 g l^{-1} $CaCl_2 \times 2H_2O$, 4.31 g l^{-1} $MgSO_4 \times 7H_2O$, 54 mg l^{-1} $FeCl_3 \times 6H_2O$, 1 l H_20) and 0.2 ml of micronutrients (2 g $^{-1}$ $FeCl_3 \times 4H_2O$, 2 g l^{-1} $CoCl_2 \times 6H_2O$, 0.5 g l^{-1} $MnCl_2 \times 4H_2O$, 30 mg l^{-1} $CuCl_2 \times 2H_2O$, 50 mg l^{-1} $ZnCl_2$, 50 mg l^1 H_3BO_3, 90 mg l^{-1} $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 100 mg l^{-1} $Na_2SeO_3 \times 5H_2O$, 50 mg l^{-1} $NiCl_2 \times 6H_2O$, 1 g l^{-1} EDTA, 1 ml l^{-1} 36% HCl, 0.5 g l^{-1} resazurin, 1000 ml H_2O) were added, the flask was shaken well and the pH was measured to be between 6.9-7.1, therefore no adjustment of pH was necessary. After closure of the flasks with the Oxitop® measuring head, they were incubated in a shaking incubator at 30°C for 1 week. Change of pressure was recorded by the measuring head. Oxygen uptake rate (OUR; mg O_2 kg^{-1} dry substrate d^{-1}) was measured as: $OUR = M_{O2} \times (V_t - V_l) \times \Delta p_{O2} / (R \times T_m \times m)$, where M_{O2} = molecular weight of O_2 (= 32000 mg mol^{-1}), V_t = bottle volume (ml), V_l = sample volume (ml), Δp_{O2} = change in O_2 partial pressure (mbar), R = gas constant (= 83.144 *l* mbar mol^{-1} K^{-1}), T_m = temperature (= 303.14 K), m = dry weight of sample (kg).

Nematodes and other microfauna were extracted by a modified Baermann wet funnel method (Sohlenius, 1979). Five samples (10 g f.w. per sample) of each compost were spread in a thin layer on nylon mesh (mesh size 1 mm) in water-filled funnels. A fliselin filter was put over the mesh to avoid larger particles clogging the mesh. The samples were heated with light bulbs for 24 h; afterwards the extracted animals were collected from the glass tubes, which had been attached to the funnels by rubber rings. The animals were killed by heating to 85°C and preserved in 20% formalin + 2% acetic acid water solution. Total numbers of fungivorous and bacterivorous nematodes per sample were counted under a dissecting microscope (magnification 25×). Classification of nematodes to taxonomic groups was performed under a light microscope (magnification 200×). For each sample, 100 individuals were examined.

Enchytraeidae (potworms) were extracted using a wet funnel technique (O'Connor, 1962). Five 50-g (f.w.) samples of each compost and compost mix were analyzed. The enchytraeids were counted under a dissecting microscope without been further divided into lower taxonomic groups. For quantification of microarthropods (mostly Collembola and Acari), five samples of each compost (16-45 g f.w.) were extracted in modified Tullgren dry funnels (MacFayden, 1961). The extracted animals were preserved in 70% ethanol and counted and classified under a dissecting microscope (magn. 25×). For quantification of macroarthropods (mainly insects and Diplopoda), two samples from each compost (500-600 g per sample and 970 g for G5) were extracted during four days in Tullgren funnels. The extracted animals were preserved in 70% ethanol and examined under stereo-microscope.

Only nematodes were included in the statistical analyses since other animal groups were present in very low numbers.

Physico-chemical characterization of composts

Composts were dried at 65°C, sieved, and the <5 mm fraction was ground and used for further analysis. Organic matter was measured by loss of weight on ignition at 400°C for 8 h. Ash content was determined as the percent of remaining matter. Carbon and N content was measured using an EA 118 Elemental analyzer (Fisons Instruments, Milan, Italy).

Aqueous compost extracts were prepared by shaking compost in distilled water at a 1:10 (wt/wt) ratio for two hours at room temperature. The suspension was centrifuged (12000 rpm, 30 min) and the supernatant was filtered through Schleicher & Schuell 395 paper filter (Tamar Ltd., Jerusalem, Israel) and subsequently through 0.45-µm membrane filters (Supor, Gelman Laboratory, Ann Arbor, Michigan). Electrical conductivity (EC) of the extract was measured using a Radiometer CDM83 (Copenhagen, Denmark) conductivity meter and pH-measurements were done with a Metrom electrode (Herisau, Switzerland). Nitrate concentration was measured using a Radiometer ISE-K-NO3 electrode (Copenhagen, Denmark). Dissolved organic carbon (DOC) concentration was determined using a Formacs^{HT} Total Carbon Analyzer (Skalar, the Netherlands). Part of the extract (200-500 ml) was freeze-dried. Dry dissolved organic matter (DOM) was stored in a desiccator until use. Carbon and N content in the dried DOM was measured as in the bulk. All measurements were conducted in duplicate. 10 mg DOM were hydrolyzed with 1 N H_2SO_4, in a boiling water bath for 4 h. Total carbohydrates were determined on the hydrolisate by the phenol sulfuric acid method (Dubois et al., 1956). The carbohydrate content was determined in three replicate samples of DOM. All the analyses listed above were conducted in the laboratories of the Hebrew University, Rehovot campus, Israel.

Solid state ^{13}C-NMR spectra of the bulk composts were obtained using the Cross polarization magic angle spinning (CPMAS) method with a Bruker Spectro spin Cemagnetics-200 NMR spectrometer. The spectrometer was operated at ^{1}H frequency of 200 MHz and ^{13}C frequency of 50 MHz, with 1 mS contact time, 0.3 S recycle delay time, 75.187 kHz sweep width and 30 Hz line broadening. Samples were spun at 6.8 kHz at the magic angle (54.7° to the magnetic field). These NMR analyses were conducted in the Department of Soil Science of the Technical University of Munich (Freizing, Germany). The NMR spectra were integrated according to four major functional groups (aliphatic-, polysaccharide-, aromatic- and carboxylic-C) and into eight subgroups (alkyl, N-alkyl, O-alkyl, anomeric-, aromatic-, phenolic-, carboxyl- and aldehyde C) (according to Malcolm, 1989). The four subgroups were expressed as % of total carbon and as % of bulk compost.

Statistical analysis

In each experimental run, suppressiveness of compost-amended potting mixes were compared with the non-amended mixes using LSD ($P < 0.05$) after a significant analysis of variance (F-test, $P > 0.05$) (Snedecor and Cochran, 1989).

Multiple regression was carried out for each pathosystem separately. Regressions were carried out using the pure compost data or the compost/peat mix or compost/soil mix data. Regressions were carried out with the statistical software package SAS (version 8.02, Cary, North Carolina) with the 'stepwise' selection. Only variables with a significant input in the model ($P < 0.05$) and a $R_{partial}^2 > 0.15$ were allowed to enter the regression model. Individual data with a relatively large effect on the model parameters having a leverage $H > 2p/n$, where p is the number of parameters in the model and n the number of data points, were omitted from the analysis (Fry, 1994).

Patterns of disease suppressiveness of the 18 different composts, and of biotic and abiotic composition of the composts and the compost-amended mixes were explored using Principal Components Analysis (PCA). The statistical relationships between disease suppression, pure compost parameters and compost/peat mix parameters were assessed by Redundancy Analysis (RDA) (Braak and Šmilauer, 2002), the constrained equivalent of PCA in which the ordination axes based on the disease suppression ('response variables') are constrained to be linear combinations of the compost parameters ('predictor variables') that maximize the total regression sum of squares between the response and predictor variables. To explore the relationships between disease suppression on the one hand and compost parameters or compost/peat mix parameters on the other hand, a manual selection procedure was used with 5000 permutations to allow the Bonferroni correction for multiple simultaneous tests (Braak and Šmilauer, 2002). The selection procedure was stopped when no variables had a further significant ($P < 0.05$) input. PCA and RDA were carried out using CANOCO software version 4.5 (Braak and Šmilauer, 2002). Centering and standardizing of 'Samples' (= composts) was set at 'none', for 'Species', centering was set at 'yes' when PCA or RDA was based on disease suppression and both centering and standardizing were set at 'yes' when PCA or RDA was based on potting soil characteristics.

Results

Disease suppressiveness of composts

In total, 120 bioassays were performed (6 pathosystems with 18 composts and 1 pathosystem (*P. nicotianae* / tomato) with 12 composts). Significant disease suppression was found in 65 (54%) cases and significant disease aggravation in 4 (3.3%) cases (Fisher's Protected LSD, $P < 0.05$; Table 2). The highest and most consistent disease suppression was found for *P. nicotianae* (compost/peat) and for *F. oxysporum* (compost/soil) with medians of 71 and 64% respectively. Significant disease suppression of *P. cinnamomi* and *R. solani* / pine was relatively infrequent, with median suppression of 6.5 and 4.7% respectively. At least three composts induced a disease suppression >50% and one compost >70% for each pathosystem (Table 2). Disease suppressiveness of composts among pathosystems was not correlated (Table 3), except for significant positive correlations among the pathosystems *R. solani* / cauliflower, *F. oxysporum*, and *P. nicotianae* in all combinations; and negative

Table 2. Disease suppressiveness (%) of 18 composts in 7 pathosystems[a].

Compost	*Verticillium dahliae* / eggplant	*Rhizoctonia solani* / cauliflower	*Phytophthora nicotianae* / tomato	*Phytophthora cinnamomi* / lupin	*Cylindrocladium spathiphylli* / spathiphyllum	*Rhizoctonia solani* / pine	*Fusarium oxysporum* / flax	average	median
a	**87.6**	**50.6**	n.d.[b]	9.7	-20.2	-7.1	**71.9**	32.1	30.2
b	**34.8**	32.1	**37.9**	**48.8**	-48.8	8.4	**65.2**	25.4	34.8
c	**46.6**	2.4	28.6	38.1	**32.4**	**92.6**	**56.1**	42.4	38.1
d	**59.1**	-10.1	**85.7**	47.6	**100.0**	27.2	**65.9**	53.6	59.1
e	**37.7**	35.3	**92.1**	-28.6	1.6	1.8	**63.8**	29.1	35.3
f	**56.7**	8.5	**92.1**	**57.1**	**47.4**	4.9	**32.8**	42.8	47.4
g	**43.9**	**38.0**	n.d.	**61.3**	-34.1	1.1	**63.1**	28.9	41.0
h	**63.1**	-4.2	**41.3**	**71.4**	**23.8**	4.5	**47.9**	35.4	41.3
i	**49.9**	12.4	n.d.	-3.2	-10.3	15.4	**58.2**	20.4	13.9
k	**34.5**	-87.3	6.3	23.8	**24.1**	**83.5**	2.1	12.4	23.8
l	-1.9	**67.8**	**45.5**	**58.9**	22.5	**57.0**	**68.1**	45.4	57.0
m	**85.4**	**77.2**	n.d.	3.2	**58.9**	-1.4	**63.3**	47.8	61.1
n	**65.2**	**50.3**	n.d.	3.2	**43.4**	-1.7	**66.7**	37.8	46.9
o	**49.5**	**42.8**	n.d.	3.2	21.8	-0.9	**45.7**	27.0	32.3
p	-21.1	**57.8**	**84.8**	-24.3	-27.9	0.9	**64.2**	19.2	0.9
q	-25.2	**66.4**	**75.8**	-20.8	**63.5**	29.9	**65.8**	36.5	63.5
r	-15.7	49.7	**78.8**	-3.5	-28.0	21.0	**70.5**	24.7	21.0
s	1.9	**68.0**	**66.7**	-17.3	-18.5	-11.3	**67.2**	22.4	1.9
average	36.2	31.0	61.3	18.3	14.0	18.1	57.7		
median	45.3	40.4	71.3	6.5	22.1	4.7	64.0		

[a] Figures in bold indicate significant ($P < 0.05$) disease suppression, i.e., less disease development in the compost-amended compared to the non-amended control. Figures underlined indicate significant disease aggravation as compared to the non-amended control.

[b] n.d. = not determined.

Table 3. Pearson's *r* correlations of disease suppression between the different pathosystems as induced y 18 different compost samples[a].

	V. dahliae / eggplant	*R. solani* / cauliflower	*P. nicotianae* / tomato	*P. cinnamomi* / lupin	*C. spathiphylli* / spathipyllum	*R. solani* / pine
R. solani / cauliflower	-0.26					
P. nicotianae	-0.17	0.50*				
P. cinnamomi	0.35	-0.39	-0.39			
C. spathiphylli	0.26	-0.20	0.10	0.14		
R. solani / pine	-0.19	-0.55*	-0.61**	0.27	0.28	
F. oxysporum	-0.16	0.81***	0.47*	-0.24	-0.20	-0.43

[a] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

correlations between the pathosystems *R. solani* / pine versus *R. solani* / cauliflower and *P. nicotianae*.

Composts differed in their ability to suppress disease for different pathosystems (Table 2, Fig. 1). The first two axes of the Principal Component Analysis (PCA) explained 33 and 20% of the variation of disease suppression in the composts respectively. Composts (represented by dots in Fig. 1) which are furthest away from the origin, e.g. composts c, d and k had the highest variation in suppressiveness while composts o and g are close to the origin and showed relatively little variation in suppressiveness among the pathosystems. For example, for compost k 87% disease stimulation was found in the *R. solani* / cauliflower

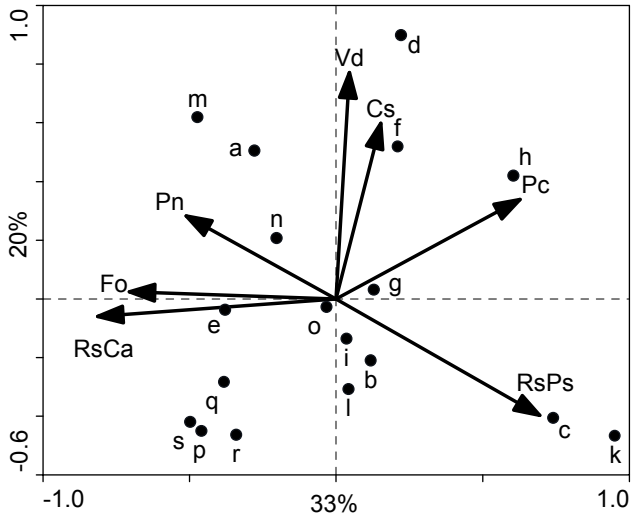

Figure 1. Principal Component Analysis (PCA) based on disease suppressiveness of composts (dots) with indication of bioassays (solid arrows). Cs = *Cylindrocladium spathiphylli* / spathiphyllum, Fo = *Fusarium oxysporum* f. sp. *lini* / flax, Pc = *Phytophthora cinnamomi* / lupin, Pn = *Phytophthora nicotianae* / tomato, RsCa = *Rhizoctonia solani* / cauliflower, RsPs = *R. solani* / pine, Vd = *Verticillium dahliae* / eggplant. For explanation of compost codes, see Table 1.

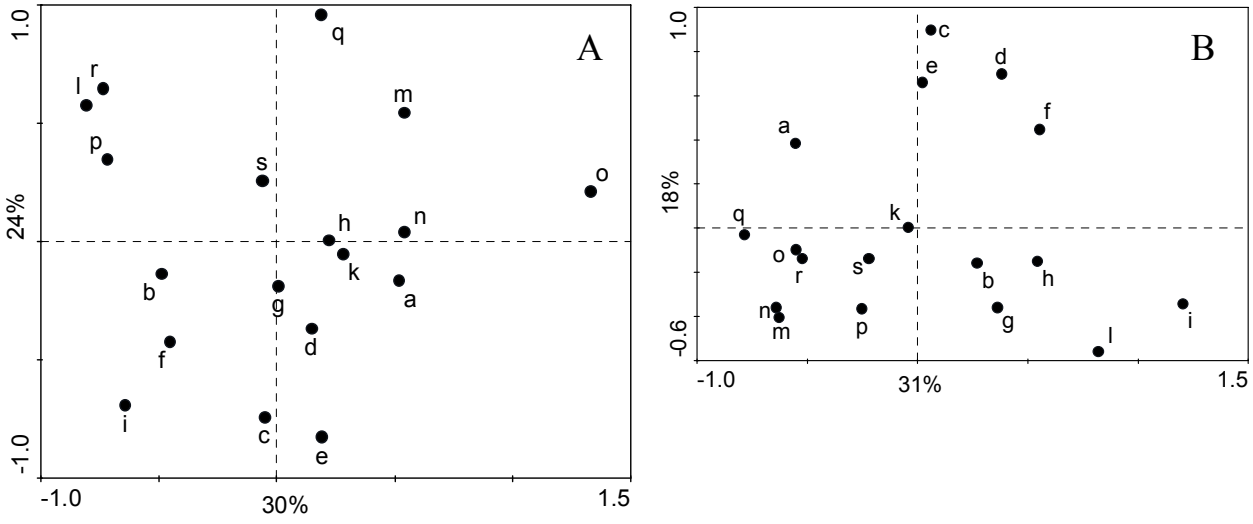

Figure 2. Principal Component Analysis (PCA) based on variation in biotic (A) and abiotic (B) characteristics of pure composts. For explanation of compost codes, see Table 1.

pathosystem, while it induced 84% disease suppression in the *R. solani* / pine pathosystem (Table 2).

Composts made from yard waste (i.e., composts p, q, r, and s; cf. Table 1) behaved more or less similar (Fig. 1). Composts made from wood chips (i.e., composts m, n, and o) and originating from the same composting facility showed comparable disease suppressive effects, although compost o showed no disease suppression in the *C. spathiphylli* assay (Table 2). The two composts that were sampled from exactly the same compost heap but with about a year in between, g and h, were not statistically similar ($P = 0.26$). These composts differed in disease suppressive effects in the *R. solani* / cauliflower pathosystem (significant for the less-aged compost g) and the *C. spathiphylli* assay (significant for the older compost h) (Table 2).

Compost characteristics

The variation among composts based on their biotic or abiotic parameters is illustrated by PCA (Fig. 2). The amount of variation explained by the first two axes is 54 and 49% for the analysis based on biotic and abiotic compost parameters respectively. Groupings of composts differ, indicating that the sets of biotic and abiotic parameters measured do not describe the variation between the composts in a similar way.

Biotic parameters that best describe (determined by RDA) the variation in abiotic characteristics of the composts are respiration (24% of the total variation), density of nematodes (13%), and density of oligotrophic bacteria (4%) (Fig. 3A). Cumulatively, these parameters explain 41% of the variation. Abiotic parameters that best describe the variation in biotic characteristics of the composts are concentration of nitrate (18%), ash content (15%) and concentrations of soluble sugars (8%), Fe (9%), and P (8%) (Fig. 3B). Cumulatively these parameters explain 58% of the variation.

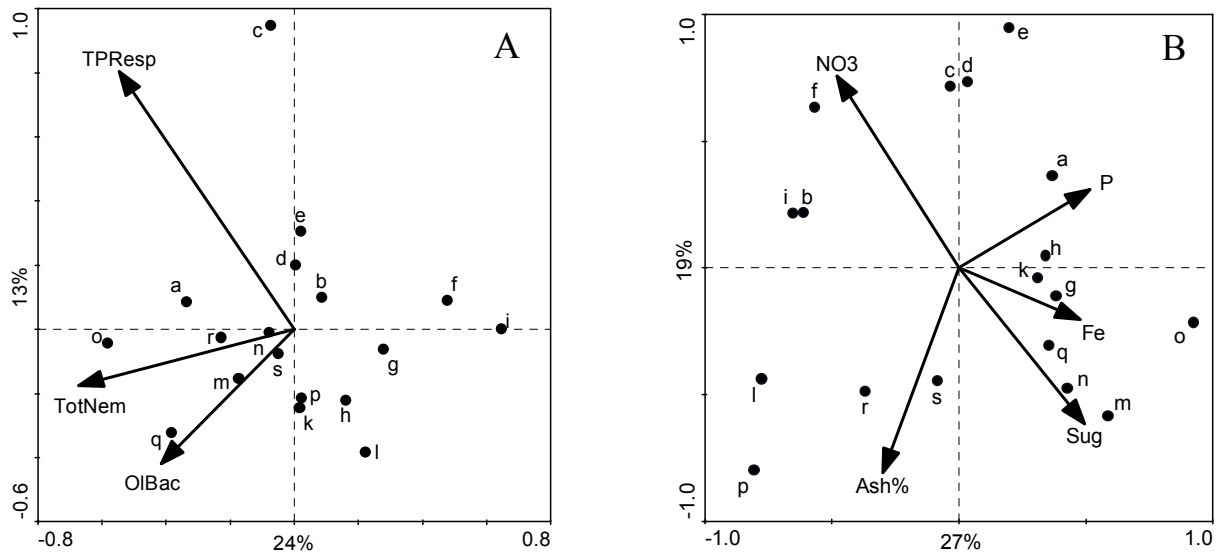

Figure 3. Redundancy Analysis (RDA) based on (A) abiotic characteristics of pure composts explained by biotic characteristics and (B) biotic characteristics of pure composts explained by abiotic characteristics. OlBac = density of oligotriphic bacteria, TotNem = density of all nematodes, TPResp = basal respiration, Ash% = ash content, Fe = concentration of iron, NO3 = concentration of nitrate, P = phosphorus content, Sug = soluble sugar content. For explanation of compost codes, see Table 1.

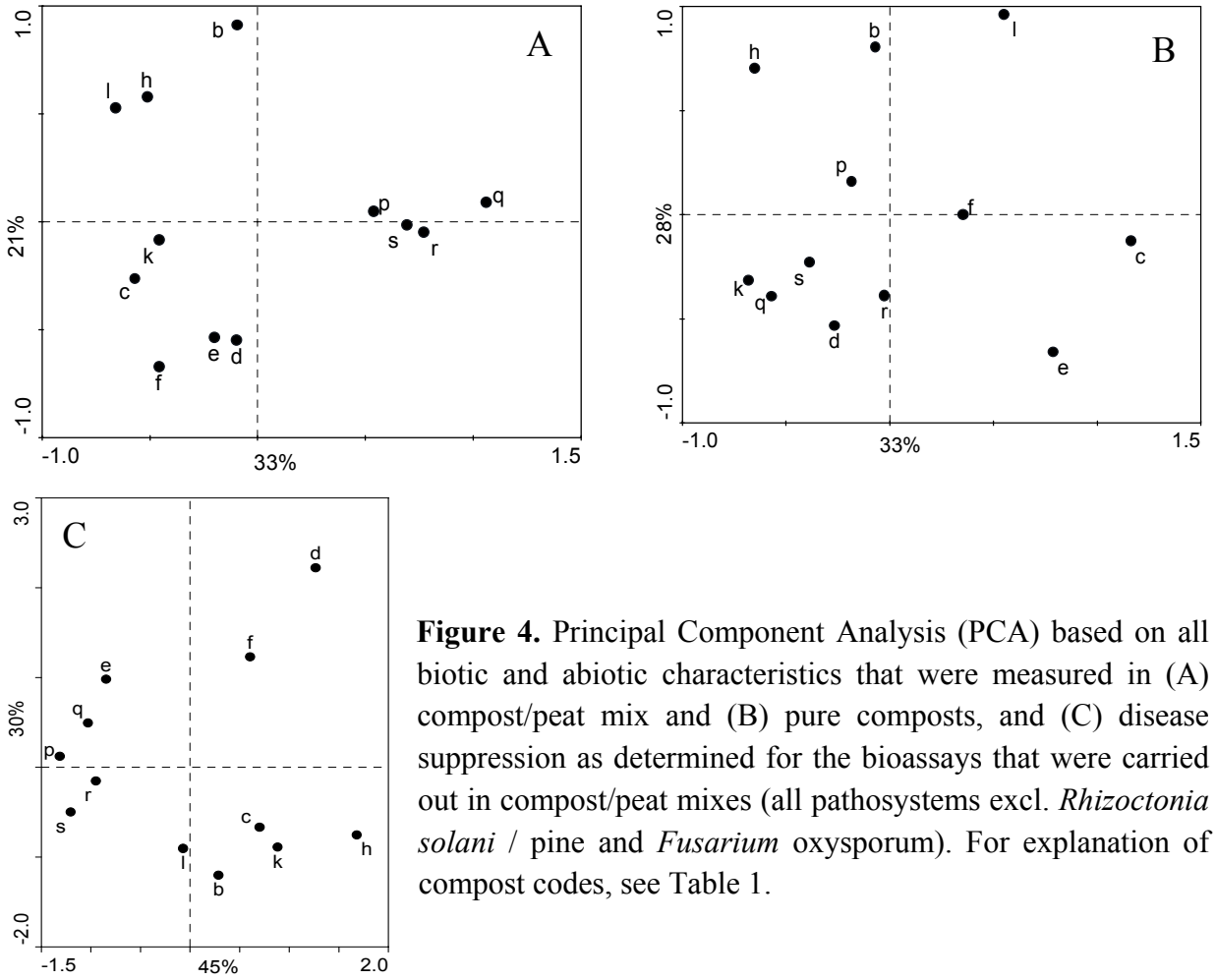

Figure 4. Principal Component Analysis (PCA) based on all biotic and abiotic characteristics that were measured in (A) compost/peat mix and (B) pure composts, and (C) disease suppression as determined for the bioassays that were carried out in compost/peat mixes (all pathosystems excl. *Rhizoctonia solani* / pine and *Fusarium* oxysporum). For explanation of compost codes, see Table 1.

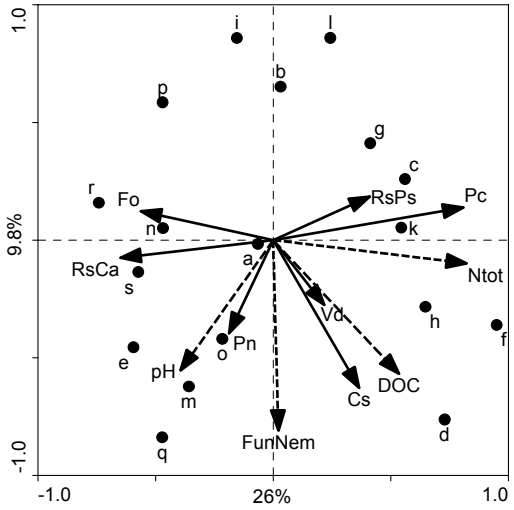

Figure 5. Redundancy Analysis (RDA) based on disease suppressiveness of composts (dots) with indication of bioassays (solid arrows) and explanatory model using pure compost variables (dashed arrows). Pathosystems: Cs = *Cylindrocladium spathiphylli* / spathiphyllum, Fo = *Fusarium oxysporum* f. sp. *lini* / flax, Pc = *Phytophthora cinnamomi* / lupin, Pn = *Phytophthora nicotianae* / tomato, RsCa = *Rhizoctonia solani* / cauliflower, RsPs = *R. solani* / pine, Vd = *Verticillium dahliae* / eggplant. Pure compost variables: DOC = concentration of dissolved organic carbon, FunNem = density of fungivorous nematodes, Ntot = concentration of total N, pH = pH. For explanation of compost codes, see Table 1.

Compost/peat mix characteristics

Fewer parameters were measured for the compost/peat mixes than for the pure composts and most parameters in the mixes were measured for 12 compost/peat mixes only. These 12 mixes showed a different distribution over the first two principal components of a PCA (Fig. 4A) when compared with the same parameters measured in the pure composts (Fig. 4B). For example, before amendment, composts k and q were quite different (Fig. 4A), but after amendment they were identified as similar (Fig. 4B). Distribution of characteristics measured in the compost/peat mixes (Fig. 4A) reflect much better distribution of composts based on disease suppressiveness (Fig. 4C) than characteristics measured in the pure composts (Fig. 4B): in figs. 4A and 4C, composts p, q, r, and s; composts c and k; composts b and l; and composts d and f cluster similarly. Composts e and h were in this respect exceptional. For compost/peat mixes only the oligotrophic bacteria, total biomass (negative) and electrical conductivity (EC) showed significant correlation with the pure compost data; and for compost/soil only the oligotrophic bacteria (results not shown) when individual variables were inspected.

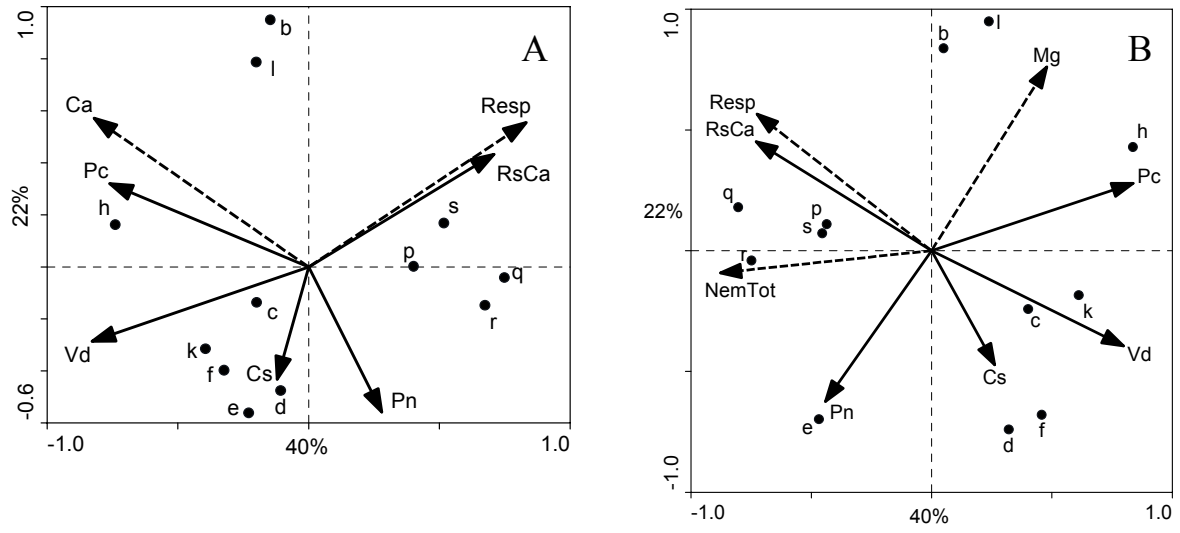

Figure 6. Redundancy Analysis (RDA) based on disease suppressiveness of compost (dots) with indication of all bioassays (dashed arrows) carried out on compost/peat mixes (i.e. all pathosystems excl. *R. solani* / pine and *F. oxysporum*) and two different explanatory models using (A) biotic and (B) abiotic characteristics of the compost/peat mixes (solid arrows). Pathosystems: Cs = *Cylindrocladium spathiphylli* / spathiphyllum, Pc = *Phytophthora cinnamomi* / lupin, Pn = *Phytophthora nicotianae* / tomato, RsCa = *Rhizoctonia solani* / cauliflower, Vd = *Verticillium dahliae* / eggplant. Pure compost variables: Ca = concentration of Ca, Mg = concentration of Mg, NemTot = density of all nematodes, Resp = respiration. For explanation of compost codes, see Table 1.

Relation between disease suppressiveness and compost characteristics

Variation in disease suppressiveness using RDA with the pure compost parameters as explanatory variables revealed that a combination of total N (20% of the variation explained), concentration of dissolved organic carbon (DOC) (10%), density of fungivorous nematodes (13%) and pH (9%) best explained the variation in disease suppressiveness of the 18 composts for 7 pathosystems (Fig. 5). A similar RDA for compost/peat mixes leads to models that explain variation in disease suppressiveness much better. Two different models based on compost/peat mixes were selected: (1) with respiration (38%) and calcium concentration (22%) as explanatory variables (Fig. 6A) and (2) with density of nematodes (31%), respiration (17%) and magnesium concentration (16%) as explanatory variables (Fig. 6B). When a RDA was carried out using all parameters as potential explanatory variables that were measured in the pure composts as well as in the compost/peat mixes, then only compost/peat mix variables were selected as significant indicating that they better predict disease suppression than the pure compost parameters (data not shown).

Multiple regression analysis revealed different models for the various pathosystems (Table 4; Figs. 7 and 8). The models obtained for bioassays carried out on peat mixes explained more of the variation in disease suppression than models obtained for the 2

bioassays carried out on a loamy soil, which in part may be due to the low variation in disease suppression obtained in the *F. oxysporum* f. sp. *lini* pathosystem (Table 2). In many cases one or more composts had to be removed from the analysis due to a too high leverage. No significant regression models were found for *P. nicotianae* based on parameters measured in compost/peat mixes. Respiration was negatively correlated with disease in the *V. dahliae* / eggplant system but positively in the *R. solani* / cauliflower system (Table 4B; Fig. 8).

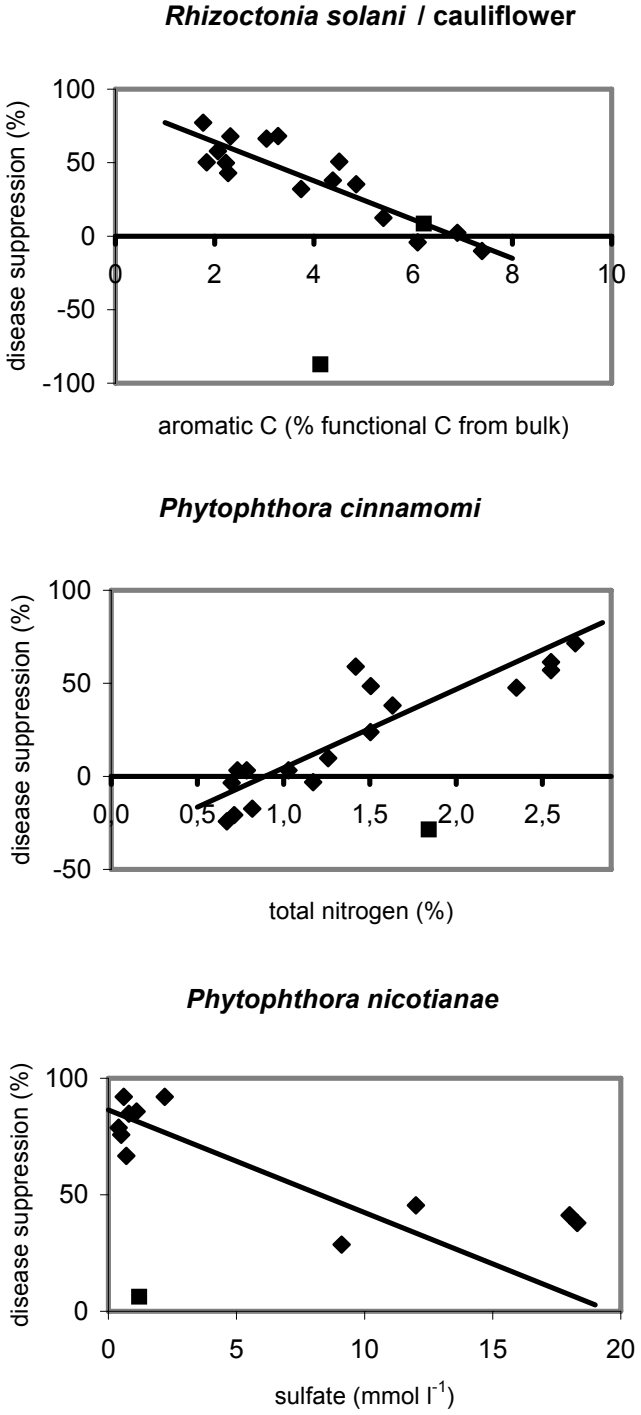

Figure 7. Regression plots for 3 pathosystems based on pure compost parameters. Square symbols refer to composts that were excluded from regression due to their high leverage.

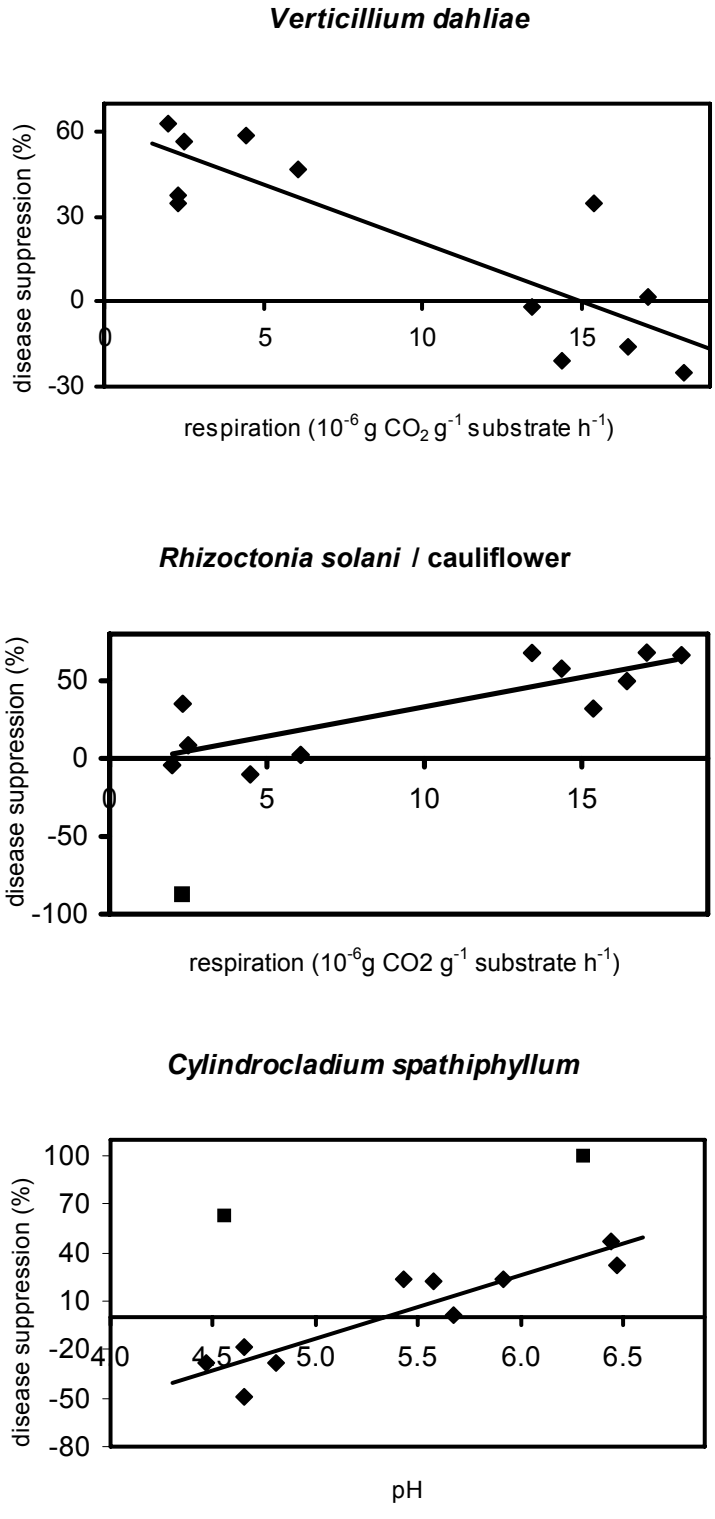

Figure 8. Regression plots for 3 pathosystems based on compost/peat parameters. Square symbols refer to composts that were excluded from regression due to their high leverage.

Table 4A. Best regression models for % disease suppressiveness of compost-amended substrates compared to non-amended controls in 7 different pathosystems based on 59 different biotic and abiotic characteristics measured on the pure composts.

Pathosystem	Model[a]	Composts excluded from regression analysis[b]
Verticillium dahliae / eggplant	47.3***(±5.63) – 2.8x10^{-6}(±4.7x10^{-7})[oligotrophic bacteria***;$R_{partial}^2$ = 0.65] + 4.9x10^{9} (±1.5x10^{-9})[copiotrophic bacteria**$R_{partial}^2$ = 0.15]; R^2=0.80	r
Rhizoctonia solani / cauliflower	90.2***(±7.48) – 13.0(±1.70)[% aromatic carbon from total bulk compost***]; R^2 = 0.80	k
Phytophthora nicotianae / tomato	82.2***(±5.26) – 2.75(±0.58)[sulphate**]; R^2 = 0.71	k
Phytophthora cinnamomi / lupin	-35.2***(±8.23) + 39.7(±5.22)[%total nitrogen***]; R^2 = 0.79[c]	e
Cylindrocladium spathiphylli / spathiphyllum	-120**(±31.7) + 5.24(±1.43)[%soluble sugar C*;$R_{partial}^2$ = 0.27] + 0.11(±0.037)[pdoc*; $R_{partial}^2$ = 0.32]; R^2 = 0.58	d, f, I
Rhizoctonia solani / pine	-18.4(±12.5) + 0.092(±0.036)[pdoc*]; R^2 = 0.35	c, d, f, l
Fusarium oxysporum / flax	no models found	

[a] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; ±: standard errors of the parameters.

[b] Individual data with a relatively large effect on the model parameters having a leverage H > 2p/n, where p is the number of parameters in the model and n the number of data points, were omitted from the analysis (Fry, 1994).

[c] Alternative model: -34.9**(±9.7) + 41.1(±8.87)[%total organic N***]; R^2 = 0.72; composts e and h excluded.

Table 4B. Best regression models for % disease suppressiveness of compost-amended substrates compared to non-amended controls in 6 different pathosystems based on 26 different biotic and abiotic characteristics measured on the composts/substrate mixes.

Pathosystem	Model[a]	Composts excluded from regression analysis[b]
Verticillium dahliae / eggplant	62.1***(±9.34) – 4.15(±0.81)[respiration***]; $R^2 = 0.72$[c]	
Rhizoctonia solani / cauliflower	-257***(±59.3) – 50.4(±11.4)[pH*;$R_{partial}^2$=0.40] + 15.1(±4.22)[Cl**; $R_{partial}^2$ = 0.37]; $R^2 = 0.77$[d]	c
Phytophthora nicotianae / tomato	no models found	
Phytophthora cinnamomi / lupin	23.3(±13.3) – 0.090(±0.015)[total nematodes***;$R_{partial}^2 = 0.72$] + 21.0(±5.77)[EC**; $R_{partial}^2 = 0.17$]; R^2=0.89[e]	
Cylindrocladium spathiphylli / spathiphyllum	no models found	
Rhizoctonia solani / pine	no models found	
Fusarium oxysporum / flax	no models found[f]	

[a] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; ±: standard errors of the parameters.

[b] Individual data with a relatively large effect on the model parameters having a leverage H > 2p/n, where p is the number of parameters in the model and n the number of data points, were omitted from the analysis (Fry, 1994).

[c] Alternative models: (1) -216***(±23.1) + 44.4(±4.49)[pH***; $R^2_{partial} = 0.87$] – 7.31(±2.71)[sulphate*; $R^2_{partial} = 0.06$]; $R^2 = 0.94$; (2) 353***(±57.9) – 46.3(±8.04)[oligotrophic bacteria***]; $R^2 = 0.79$; compost b excluded.

[d] Alternative models: (1) -4.54(±10.3) + 3.78(±0.85)[respiration**]; $R^2 = 0.68$; compost k excluded; (2) -223*(±69.4) + 35.2(±9.44)[transformed oligotrophic bacteria**]; $R^2 = 0.60$; compost k excluded.

[e] Alternative model: 184**(±38.9) – 3.79(±0.85)[Fe**]; $R^2 = 0.77$; composts h, l, k, and r excluded.

[f] Model where composts e, k, and r have a too high leverage: -317*(±104) + 46.4(±13.5)[oligotrophic bacteria**; $R_{partial}^2$=0.33] + 9.48(±3.60)[respiration*; $R_{partial}^2 = 0.29$]; $R^2 = 0.62$.

Discussion

Incidence of disease suppression induced by composts

Disease suppressiveness of composts was studied in compost-amended peat for 5 pathosystems and in compost-amended soil for 2 pathosystems. From the 120 bioassays carried out in this study, 54% showed significant disease suppression and only 3.3% showed significant stimulation. It should be noted that no single compost showed significant disease suppression against all pathogens as well as that the pathogens were not affected similarly by all composts. It should be borne in mind that in our study we infested the substrates with high inoculum densities of the pathogens while in practice a disease starts at low pathogen inoculum density. Usually disease suppressiveness of only a few composts is studied for only one, or at maximum a few, pathosystems and in those cases also, pathogens are generally inoculated at high densities. As incidence of a specific disease usually is unpredictable, disease suppression of compost should preferably be towards various pathogens. In a comparable study involving the suppressiveness of 36 composts against 3 pathogens (*Pythium ultimum* and *P. irregulare* / cucumber and *R. solani* / cabbage), 49% of bioassays showed significant suppression and 14% significant stimulation (Scheuerell et al., 2005). Disease stimulation occurred only for *R. solani.*

In our study, we tested disease suppression of compost against a range of ecologically widely varying plant pathogens, which may be divided into the following groups: (1) the oomycete pathogens *P. cinnamomi* and *P. nicotianae*, classified as soil inhabitants according to Garrett (1970) and known to be competition-sensitive (You and Sivasithamparam, 1995; Aryantha et al., 2000). For the diseases they incite, suppression by compost has been reported frequently, especially for cases where the potting substrate has a low microbial carrying capacity for microbial activity, such as Sphagnum peat mixes (Hoitink and Fahy, 1986); (2) the ascomycete wilt pathogens *F. oxysporum* and *V. dahliae*, which are root inhabitants (*sensu* Garrett, 1970), i.e. they survive in the absence of a host in the form of highly persistent structures (chlamydospores and microsclerotia respectively) and become active only under the influence of root exudates (Ocamb and Kommedahl, 1994); (3) the soil inhabitant basidiomycete *Rhizoctonia solani* of which disease suppression by compost is reportedly to be variable (Tuitert et al., 1998; Hoitink and Boehm, 1999; Ryckeboer, 2001; Pérez-Piqueres et al., 2006) and probably mainly of specific origin (Diab et al., 2003). Although data are scanty about *C. spathiphylli*, it probably should be classified as a soil inhabitant (*sensu* Garrett, 1970) because of its high saprophytic ability (Hunter, 1993). When these ecological groupings based on prior knowledge are compared with the disease suppressiveness of the 18 composts in this study, different groupings appear (Fig. 1): (1) *V. dahliae* and *C. spathiphylli*, (2) *F. oxysporum*, *R. solani* / cauliflower, *P. nicotianae*, and (3) reacting oppositely to group 2, *P. cinnamomi* and *R. solani* / pine. Thus, disease suppression induced by compost may not be related to the traditional ecological groupings of plant pathogens.

A clear result from this study is the existence of a compost × pathogen interaction, which was also reported by Scheuerell et al. (2005) for the pathosystems *Pythium ultimum* and *P. irregulare* with cucumber and for *Rhizoctonia solani* with cabbage. So for optimal

Table 5. Pearson's *r* correlations between selected pure compost characteristics and (1) compost/peat ('Peat') and compost/soil characteristics ('Soil')[a].

Variable	Peat		Soil	
	Pearson's *r*	N	Pearson's *r*	N
bacterivorous nematodes	0.02	12	-	-
fungivorous nematodes	0.00	12	-	-
log(copiotrophic bacteria)	0.57	12	0.79***	12
log(oligotrophic bacteria)	0.84***	12	0.94***	12
total biomass	-0.63*	12	-0.28	12
basal respiration	0.04	12	0.38	12
pH	-0.04	18	-	-
electrical conductivity	0.79***	18	-	-

[a] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

disease control it is best to determine the most likely diseases in a given cropping system. For peat-based cultivations in greenhouses the range of possible soilborne pathogens is indeed rather narrow for many hosts. The compost × pathosystem interaction was also clear for the soil-based assays; *F. oxysporum* and *R. solani* / pine were weakly negatively correlated (Pearson's $r = -0.43$, $P < 0.075$), indicating the importance of the ecology of the pathogen. In addition, the effect of host plant cannot be excluded in this study as different host plants were used for each bioassay.

Prediction of disease suppression

A question of practical importance is whether disease suppression can be predicted for a given batch of compost. The ability to predict disease suppression on the basis of pure compost characteristics is highly desirable, since compost producers can then optimize the composting process based on disease suppression goals, thus enabling growers to choose the appropriate compost for expected disease problems according to the cultivated crop.

Suppression of verticillium wilt showed a positive and negative correlation with the density of copiotrophic and oligotrophic bacteria respectively in the pure composts (Table 4A) and a negative correlation with respiration of the compost/peat mix (Table 4B; Fig. 8A). Copiotrophic rhizosphere-inhabiting bacteria, which also appear in the regression analysis (Table 4) such as the fluorescent pseudomonads are furthermore well-known antagonists of *V. dahliae* (Mercado-Blanco et al., 2004). The negative correlation between respiration in the compost/peat mix and disease suppression is somewhat puzzling. Perhaps the high levels of respiration indicate sufficient nutrients for the microsclerotia to be able to germinate.

No significant regression models were found for suppression of *C. spathiphylli* with characteristics of the compost-amended mixes (Table 4B). Disease suppression has been reported to be pH-dependent (Chase and Poole, 1987), but the pH-levels reported that rendered disease suppression (>6.5; Chase and Poole, 1987), are not realistic for the cultivation of the host plant and were therefore not included in our study. The high level of

disease suppression of compost d was repeatable (results not shown) and most likely due to a specific mechanism or a combination of the pH of the compost-amended potting mix (6.3) in combination with its relatively high organic matter content (66%).

Significant disease suppression for *F. oxysporum* was found for all composts except for compost k. This compost had one of the lowest basal respiration levels in the compost and the compost/soil potting mix. The general suppressiveness of *F. oxysporum* could be explained by high competition-sensitivity of the pathogen (Alabouvette et al., 1986; Serra-Wittling et al., 1996; Alabouvette et al., 2001) in combination with the relatively poor soil used as base substrate. The soil had a low total biomass and respiration as compared to the peat substrate (results not shown) and was probably relatively strongly affected by compost amendment in terms of increase in the density of a competitive microflora. All composts except one were above the threshold for effective competition with the pathogen.

Disease suppression of *R. solani* AG2-1 / cauliflower was negatively affected by pH and positively by basal respiration of the compost/peat mix (Table 4B, first alternative model). A competition-based mechanism may partly explain disease suppression of *R. solani*. On the other hand, the suppression of damping-off caused by *R. solani* AG2-2 / pine could not be attributed to competition for carbon or any other parameter in the compost/soil mix (Table 4B) and a specific suppression has to be proposed. In general, suppression of *R. solani* is a rare phenomenon. For example, in a study involving 36 composts, 17% suppressed *R. solani* against cabbage (Scheuerell et al., 2005). The results of this work indicate that different isolates within one anastomosis group (AG2) can behave differently. AG2 of *R. solani* is known to be quite variable, with for example low-temperature subgroups of AG2-1 attacking bulb species (Schneider et al., 2001), subgroups causing damping-off in many hosts (Reeleder et al., 1996; Villajuan et al., 1996), and subgroups including AG2-2IV infecting mature plants (Engelkes and Windels, 1996). The totally different response of the two isolates of *R. solani* on compost amendment indicate different mechanisms of suppression. On the other hand, the effect of the compost may depend on the substrate (in the present study peat for the bioassay with cauliflower and sand for the bioassay with pine) with which it is mixed. Also an involvement of the host plant in suppression of the pathogen cannot be excluded.

Phytophthora nicotianae and *P. cinnamomi* are often claimed to be highly competition-sensitive (You and Sivasithamparam, 1995; Hoitink and Boehm, 1999), but we could not find regression models that included competition-related parameters. Also Aryantha et al. (2000) for *P. cinnamomi* and Widmer et al. (1998) for *P. nicotianae* did report disease suppressive effects of compost but they could not unravel the mechanism of suppression. Some suggestions for the involvement of pseudomonads (Turnbull et al., 1992) and a hyperparasitic *Acremonium* species (Widmer et al., 1998) have been proposed.

In summary, the following groups of pathogens appear: (1) competitive-sensitive: *F. oxysporum* f. sp. *lini* and *R. solani* / cauliflower; (2) rhizosphere-affected: *V. dahliae*; (3) pH-related (Table 4B): *P. nicotianae*; (4) specific/unknown: *R. solani* / pine, *P. cinnamomi*, and *C. spathiphylli*. Such different mechanisms underlying disease suppression for different pathosystems were also suggested by Scheuerell et al. (2005). Clearly more research has to be done to translate these correlations into practical recommendations.

Pure compost and compost mixture characteristics

RDAs performed to explain disease suppression on the basis of pure compost characteristics gave lower levels of explanation (Fig. 5; 52% with 4 parameters) than when based on compost/peat mixes (Fig. 6; 60-64% with 2 parameters). The parameters of the compost/peat mixes also showed groupings more similar to the disease suppression distribution when analyzed by PCA (Fig. 4). Yet, more regression models could be found using the pure compost parameters. It is possible that the compost/peat mixes may be more indicative for disease suppression when a competition-based suppression is involved (such as in *R. solani* / cauliflower) whereas the pure compost parameters are more indicative when a specific mechanism is present. The multiple regressions of individual pathosystems resulted also in better fits when parameters of the mixes were used than when pure compost characteristics were used; also less composts had to be removed from regression analyses (Table 4). In addition, only a few parameters of the pure composts correlated with parameters of the compost mixes (Table 5). Apparently, composts vary strongly in their reaction when they are mixed with another potting substrate. It may be considered that parameters in a compost/substrate mix which has been incubated for some period of time may better predict disease suppressiveness than parameters of pure compost.

The results show that the set of biotic parameters measured does not, as a group, reflect the abiotic composition of composts and *vice versa*. The best set of biotic parameters explaining variation in abiotic parameters of composts consists of basal respiration, density of nematodes, and oligotrophic bacteria (Fig. 3A). The biotic variation of pure composts is best reflected by the abiotic parameters nitrate, P, Fe, and soluble sugar concentration, and organic matter content (Fig. 3B).

Conclusions

Is it, based on the results of our work, recommendable to apply compost to potting mixes or soil? The answer is yes, although one cannot assume that a certain combination of compost and pathosystem will necessarily behave like another. Partial replacement of peat by compost has in 54% of the cases positive effects on disease suppression, in addition to the positive effects of replacing the non-recyclable peat by a recycled product. If build-up and/or spread of inoculum of the pathogen is also reduced by compost amendment, disease suppression could be even more significant, since for a successful disease development pathogen biomass has to surpass a certain threshold (Otten et al., 2004). For the compost producer it is important to consider that a compost of given composition or made at one factory can be rather consistent in terms of disease suppression.

We conclude that application of composts has a disease suppressive or no effect, and only rarely a disease stimulating effect. Disease suppression can better be predicted based on compost mixes than on pure composts and should be focused on specific pathogens, given the variation in disease suppression between pathosystems.

Acknowledgements

We thank A.P. Schaffers for advise on multivariate analysis. We are grateful to S. Aime, L. Fayolle, R. Hamelink, V. Insunza, M. Savary, D. Volker, and O. de Vos for their help in the greenhouse and laboratories. We thank W.J. Blok, A.H.C. van Bruggen, and J.H.M. Schneider for discussions.

This work was supported by the European Commission (Framework Programme 5; The Compost Management Project) and the Dutch Ministry of Agriculture. The contents of this publication is the sole responsibility of its authors and in no way represent the view of the Commission or its services nor anticipates its future policy in this area.

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Chapter 3

The effect of amending peat-based substrate with 12 composts on the microbial communities in bulk compost/peat mixes in relation to disease suppression against 5 pathosystems

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Soil Biology and Biochemistry, submitted

Abstract

Compost-induced disease suppression of soilborne plant pathogens has been reported repeatedly to be correlated with the microbial composition of the compost/peat or compost/soil mix. Presumably the microbial composition of the mix is a combination of substrate and compost microorganisms and dependent on physico-chemical and microbial characteristics of both the substrate (soil/peat) and the compost. We tested the hypothesis that disease suppression is dependent on the ability of compost to influence the microbial community of the peat-based substrate by investigating the relationship between the bacterial, actinomycete and fungal communities in compost-peat mixes to disease suppression against five pathosystems: *Cylindrocladium spathiphylli*, *Phytophthora cinnamomi*, *P. nicotianae, Rhizoctonia solani* and *Verticillium dahliae.* A specific goal was to investigate the effect of physico-chemical compost and compost-peat mix characteristics on microbial communities in relation to disease suppression. With respect to bacterial, actinomycete and fungal composition, some of the compost/peat mixes showed higher similarities with the microbial composition of the pure peat than with the respective composts and were denoted as 'peat-like'. On average, disease suppression of peat-like mixes with respect to bacteria and/or actinomycetes was significantly lower for *C. spathiphylli*, *P. cinnamomi*, and *V. dahliae* but significantly higher for *P. nicotianae* and *R. solani* than disease suppression of the other mixes. We demonstrated that compost-borne microbial communities may or may not survive after mixing with peat, and that this ability may determine the extent of disease suppression. The ability of compost to change the microbial community of the substrate was significantly related to the pH of the compost-peat mix and the organic nitrogen content and microbial composition of the pure compost.

Introduction

Disease suppression of compost/peat and compost/soil mixes has been reported repeatedly, primarily against soilborne plant pathogenic fungi (Chen et al., 1988; Serra-Wittling et al., 1996; Tuitert et al., 1998; Scheuerell et al., 2005; Termorshuizen et al., 2006). Composts may provide substrates for organisms that enhance general antagonism through competition, or for specific agents that invoke antibiosis or induced resistance against plant pathogens (Hoitink and Boehm, 1999). In multiple cases suppression of soilborne plant pathogens have been shown to be correlated with the microbial composition of the compost/peat or compost/soil mix (Boehm et al., 1993; Kowalchuk et al., 2002; Postma et al., 2005). Shifts in the bacterial and/or fungal community structures of soil and peat as a result of compost amendment have been reported frequently (Boehm et al., 1993; Kowalchuk et al., 2002; Yao et al., 2006) and have been demonstrated to be related both to the soil and to the type of organic amendment used (Pérez-Piqueres et al., 2006).

Compost amendment may stimulate the substrate (peat or soil) microorganisms through the availability of easily decomposable carbon compounds. Thus resident peat or soil microorganisms may outcompete the compost-borne microorganisms as reported by Crecchio et al. (2004) and Saison et al. (2006). Alternatively, the compost mix is colonized by introduced compost-borne organisms as reported by Kowalchuk et al. (2002) and Yao et al. (2006). Presumably the microbial composition of the mix is a combination of substrate and compost microorganisms. Which of both groups will dominate is likely to be dependent on the physico-chemical and microbial characteristics of both the substrate (soil/peat) and the compost and on the ratio of the components in the mix. Total N and pH, for example, have been shown to play an important role in the development of the microbial community structures in soil (Tscherko et al., 2004; Kennedy et al., 2004; Marschner et al., 2004). If suppression of a pathogen is based on antibiosis or induced resistance by specific agents, then disease suppressive properties of a compost may be dependent on the degree of colonization of the compost/peat mix by certain compost-borne microorganisms.

In the majority of studies on disease suppression induced by compost, one type of compost was tested against a single or a few pathogens only, while in reality a host plant may face infection by multiple pathogens, and multiple crops are usually cultivated on a given soil. Therefore, Termorshuizen et al. (2006) studied the effect of 18 composts on their ability to suppress disease in 7 pathosystems and reported a highly significant compost × pathosystem interaction. Physico-chemical and biotic characteristics (culturable microorganisms and mesofauna components) could not explain this interaction. In the current study we elaborate further on 12 of the composts and 5 of the pathosystems studied by Termorshuizen et al. (2006). We tested the hypothesis that disease suppression is related to the ability of compost to change the microbial community of the substrate using DGGE-analysis.

The objective of the present study was to investigate the effect of mixing compost with peat-based substrate on the bacterial, actinomycete and fungal communities in relation to disease suppression against five pathosystems: *Verticillium dahliae* causing wilt in many hosts (test plant eggplant), *Cylindrocladium spathiphylli* causing root rot in *Spathiphyllum*, and *Rhizoctonia solani* (test plant cauliflower), *Phytophthora cinnamomi* (test plant lupin),

and *P. nicotianae* (test plant tomato), all three polyphagous pathogens causing root rot and damping-off. A specific goal was to investigate the effect of physical-chemical compost and compost/peat characteristics on microbial communities in relation to disease suppression.

Materials and methods

Collection and selection of composts

Composts were collected as described in Termorshuizen et al. (2006). In short, composts were made from different kinds of waste materials (e.g. green waste, domestic biowaste, manure) and were collected from commercial composting sites in the Netherlands, France, Greece, and Israel (Table 1). Composts were stored in loosely closed polyethylene bags at 4°C until use. Two liters of each compost were oven-dried at 60°C for chemical analysis. A preselection of composts was carried out to avoid the use of immature composts which may be phytotoxic or compete with the plants for nitrogen and oxygen and to exclude composts with a too low level of organic matter (OM). Selection criteria were OM > 20% (wt/wt), concentration of dissolved organic carbon (DOC) in 1:10 water extract < 400 mg l^{-1}). In the studies of Termorshuizen et al. (2006) 3 series of 6 composts were selected, based on DOC and OM data. In the present study, the composts with compost/peat mixes of series 2 and 3 were investigated.

Preparation of potting mixes

About 2-3 weeks before start of the bioassays a 1-l-sample of each compost was sent to a commercial laboratory (Groen Agro Control, Delft, the Netherlands) for determination of the pH and the mineral element contents in a 1:1.5 volume extract. Eight days before start of the bioassays the composts were incubated at room temperature and potting mixes were prepared one day later. Non-amended mixes consisted of peat (H2-3 on the Von Post decomposition scale), lime (8 g l^{-1}, Dolokal PG) and PG-mix (0.8 g l^{-1}, 15-10-20). The same batch of peat was used in all tests. The peat (A0, Kekkila, Finland) was stored in compressed bales to which neither lime nor mineral fertilizers had been added. Amended mixes consisted of peat (80%) and compost (20%, volume basis) and mineral fertilizers. Lime (4 g l^{-1}) was added to some of the compost-amended peat-based mixes to increase the pH. Based on the mineral elements present in the compost extract (1:1.5) mineral fertilizers were added to the compost/peat mixes to obtain similar nutrient levels in the non-amended and amended potting mixes (Kreij and Gaag, 2003). Moist mixes of compost and peat were incubated for one week at room temperature before start of the bioassays. The moisture content of the peat-based mixes was between 60 and 75% (wt/wt) and of the non-amended mix between 75 and 80%. One week after preparation of the potting mixes, the mineral element contents, EC and pH of water extracts (1:1.5 volume) of the peat-based mixes were determined by the commercial laboratory mentioned above and a random portion of 10 g of each compost (consisting of

Table 1. Characteristics of selected composts.

Code	Origin		Composted material[b]	OM (%)	DOC (ppm)	Total C (% of total bulk)	Total N (% of total bulk)	C/N	$CaCO_3$ (% of total bulk)	Organic N (mg organic N kg^{-1} bulk)	pH	EC (mS cm^{-1})
	Country[a]	Company										
b	F	II	Tree bark, slurries, green wastes	37.1	166.7	22.1	1.5	14.7	5.2	1.4	7.19	4.33
c	F	III	Urban biowastes	45.6	825.6	28.4	1.6	17.4	8.9	1.5	8.06	2.83
d	F	IV	Grape marc, green wastes	66.3	921.1	36.2	2.3	15.2	5.5	2.2	8.11	1.55
e	F	V	Woody wastes, poultry manure	37.2	456.6	21.9	1.8	12.4	5.1	1.6	8.70	3.30
f	F	VI	Woodcut, plants, horse manure	39.0	1000.7	27.5	2.6	10.8	13.5	2.4	8.07	2.82
h	Gr	VII	Spent mushroom compost: wheat straw (56%), chicken manure (39%), gypsum (5%)	46.7	262.1	27.0	2.7	10.0	5.1	2.7	6.75	2.24
k	Gr	IX	Wood chips, horse manure	34.0	695.4	19.9	1.5	13.3	20	1.5	7.74	1.07
l	Il	X	Municipal sewage sludge and yard waste	20.5	175.7	11.6	1.4	8.2	9.3	1.2	6.64	2.72
p	NL	XII	Yard waste (woody materials, grass clippings)	21.3	188.1	10.1	0.7	15.1	0.2	0.7	7.77	0.87
q	NL	XIII	Yard waste (without grass)	28.6	384.0	15.3	0.7	21.5	0.2	0.7	8.09	0.72
r	NL	XIII	Yard waste (without grass)	21.8	306.2	11.1	0.7	15.8	0	0.7	8.39	0.77
s	NL	XIV	Yard waste (mainly grass)	27.7	313.7	13.9	0.8	17.1	0.8	0.8	8.22	0.96

[a] F = France, Gr = Greece, Il = Israel, NL = the Netherlands.
[b] Percentages of ingredients are volume-based.

Table 2. Disease suppressiveness (%) of 12 composts in 5 pathosystems[a].

Compost	*Cylindrocladium spathiphylli* / spathiphyllum	*Phytophthora cinnamomi* / lupin	*Phytophthora nicotianae* / tomato	*Rhizoctonia solani* / cauliflower	*Verticillium dahliae* / eggplant
b	-48.8	**48.8**	**37.9**	32.1	**34.8**
c	**32.4**	38.1	28.6	2.4	**46.6**
d	**100.0**	47.6	**85.7**	-10.1	**59.1**
e	1.6	-28.6	**92.1**	35.3	**37.7**
f	**47.4**	**57.1**	**92.1**	8.5	**56.7**
h	**23.8**	**71.4**	**41.3**	-4.2	**63.1**
k	**24.1**	23.8	6.3	-87.3	**34.5**
l	22.5	**58.9**	**45.5**	**67.8**	-1.9
p	-27.9	-24.3	**84.8**	**57.8**	-21.1
q	**63.5**	-20.8	**75.8**	**66.4**	-25.2
r	-28.0	-3.5	**78.8**	49.7	-15.7
s	-18.5	-17.3	**66.7**	**68.0**	1.9

[a] Figures in bold indicate significant ($P < 0.05$) disease suppression, i.e., less disease development in the compost-amended compared to the non-amended control. Figures underlined indicate significant disease aggravation as compared to the non-amended control.

pooled subsamples of approximately 0.5 g) and compost/peat mix was stored at -20°C for DGGE-analysis.

Bioassays

The disease suppressive properties of the composts were described in Termorshuizen et al. (2006). In the current study, with 12 composts and 5 pathosystems, the highest and most consistent disease suppression was found for *Phytophthora nicotianae* with a median disease suppression of 71% (Table 2). The methodology of disease suppression assays was decribed extensively in Termorshuizen et al. (2006) and is summarized here.

Inoculum of *Phytophthora cinnamomi* was produced in a mix of peat and ground oat as described by Blok et al. (2000). The inoculum was mixed with the potting mix at a dosage of 0.1% (vol./vol.). Pots (diam. 10.5 cm, vol. 0.4 l) were filled with potting mix. Eight lupin seeds (*Lupinus angustifolius* L. cv. Borweta) were sown in each pot and the number of healthy seedlings was determined after 21 days. Five non-infested and 5 infested pots were sown for each potting mix.

Inoculum of *Cylindrocladium spathiphylli* was produced in the same way as described for *P. cinnamomi*. The inoculum was mixed with the potting mix at a dosage of 0.1% (vol./vol.). Young Spathiphyllum plantlets (*Spathiphyllum wallisii* Hort. cv. Ceres, Braam, De Kwakel, the Netherlands) were planted in pots (diam. 9 cm, vol. 0.2 l). One replicate consisted of 7 pots placed on a tray (19 × 31 cm) on an irrigation mat. For each potting mix 5 replicates were employed with infested soil and 3 replicates with non-infested soil. From 2 weeks after potting, plants were observed every 3-4 days and the number of healthy, diseased and completely wilted or dead plants noted. The final observation was carried out 7 weeks

after potting.

Inoculum of *Phytophthora nicotianae* was produced the same way as described for *P. cinnamomi* (see above) and mixed with the potting mix at a dosage of 1% (vol./vol.). Seven tomato seeds (*Lycopersicon esculentum* Mill. cv. Jamaica) were sown in a pot (diam. 9 cm, vol. 0.2 l). The number of healthy seedlings was determined about 10 days after sowing. Five non-infested and 5 infested pots were sown for each potting mix.

Inoculum of *Rhizoctonia solani* AG2-1 was grown on Potato Dextrose Agar (PDA, Oxoid, UK). Nine cauliflower seeds (*Brassica oleracea* L. var. *botrytis* cv. Fremont F1 (Royal Sluis, the Netherlands)) were sown in a pot (diam. 9 cm). One week after sowing the number of seedlings in each pot was noted and a 3 × 3 mm piece of PDA containing actively growing inoculum of *R. solani* was placed adjacent to one seedling and just below the soil surface near the edge of the pot. Cauliflower seedlings were observed daily and as soon as all seedlings in one of the pots were damped-off, the number of healthy seedlings were determined in each pot.

Each potting mix had 5 replicate pots. Both *Phytophthora* assays and the *Rhizoctonia*-cauliflower assay were carried out in a glasshouse at 20°C and the *Cylindrocladium* assay in a glasshouse at 24°C. Pots were placed on a dish or tray and irrigated regularly from below using tap water. Spathiphyllum plants obtained a nutrient solution (EC 2) once a week from 4 weeks after potting.

Inoculum of *V. dahliae* was grown in liquid SSN medium for 4 weeks under continuous shaking at 120 rpm and 22°C in the dark. After incubation, clusters of microsclerotia were homogenized in an omni-mixer (Ivan Sorvall Inc., Newtown Connecticut, USA) and filtered through a mesh screen of 70 µm diam. The inoculum that did not pass the mesh screen (the larger microsclerotia) was used in the bioassay. After air-drying, the microsclerotia were kept at 4°C until needed. Eggplant seeds (*Solanum melongona* L. cv. Black Beauty) were planted in trays containing a mix of peat and compost mix. Seedlings at the second true-leaf stage were transplanted in individual pots (10 × 10 cm). To infest the substrates the inoculum was suspended in water, the density of microsclerotia was determined and an amount of suspension was added to the substrate to reach 25 microsclerotia g^{-1} d.w. substrate soil.

In each series the disease levels were related to that of the non-amended control in order to compare suppression levels between the different series. No significant effect of the composts was found on the number of healthy plants in the non-infested controls and the percentage disease suppression induced by the compost in the *Phytophthora* assays was calculated as: 100 – 100 × (CM – IM) / (CM – IC), where: CM is the number of healthy plants in the non-infested mix, IM is the number of healthy plants in the infested mix and IC is the number of healthy plants in the infested non-amended potting mix. In the *C. spathiphyllum*, and *V. dahliae* assays the area under the disease progress curve (AUDPC; Campbell & Madden, 1990) was calculated for the number of completely wilted or dead plants and the percentage disease suppression was calculated as: $100 - 100 \times (AUDPC_{\text{amended potting mix}} / AUDPC_{\text{non-amended potting mix}})$. For the *R. solani* AG2-1 (cauliflower) assay, the percentage disease suppression induced by the compost was calculated as: 100 – 100 × (% damping-off in amended mix) / (% damping-off in non-amended mix).

Physico-chemical characterization of composts and compost/peat mixes

Composts were dried at 65°C, sieved, and the < 5 mm fraction was ground and used for further analysis. Organic matter was measured by loss of weight on ignition at 400°C for 8 h. Ash content was calculated as the percent of remaining matter. Carbon and N content was measured using an EA 118 Elemental analyzer (Fisons Instruments, Milan, Italy).

Aqueous compost extracts were prepared by shaking compost in distilled water at a 1:10 (wt/wt) ratio for two hours at room temperature. The suspension was centrifuged (12000 rpm, 30 min.) and the supernatant was filtered through Schleicher & Schuell 395 paper filter and subsequently through 0.45-µm membrane filters (Supor, Gelman Laboratory). Electrical conductivity (EC) of the extract was measured using a Radiometer, CDM83 conductivity meter, pH measurements were done with a Metrom electrode. Nitrate concentration was measured using a Radiometer ISE-K-NO_3 electrode. Dissolved organic carbon (DOC) concentration was determined using a Formacs^{HT} Total Carbon Analyzer (Skalar, NL). Part of the extract (200-500 ml) was freeze-dried. Dry dissolved organic matter (DOM) was stored in a desiccator until use. Carbon and N content in the dried DOM was measured as in the bulk. All measurements were conducted in duplicate. 10 mg DOM were hydrolyzed with 1 N H_2SO_4, in a boiling water bath for 4 h. Total carbohydrates were determined on the hydrolizate by the phenol sulfuric acid method (Dubois et al., 1956). The carbohydrate content was determined in three replicate samples of DOM.

DNA extraction and PCR amplifications

DNA extraction was carried out with the MoBio Ultraclean soil DNA extraction kit (Biozym TC, Landgraaf, the Netherlands), according to the manufactures' protocol. PCR was performed in an PTC-100 thermal cycler (Mj Research, Inc., Tilburg, the Netherlands). Primers as listed in Table 3 were used for the specific amplication of 16S rDNA fragments of eubacteria and actinomycetes (Heuer et al., 1997) and 18S rDNA fragments of fungi (Smit et al., 1999; White et al., 1990).

PCR amplification of bacterial 16S rDNA genes was performed according to Heuer et al. (1997). For bacteria the reaction mix (final vol. 50 µl) consisted of 200 µM dNTP, 3.75 mM $MgCl_2$, 1x Stoffel buffer (Applied Biosystems, Foster City, USA), 0.4 µM primer F984GC, 0.4 µM primer R1378, 1.0% formamide, 2 ng μl^{-1} T4 gene 32 protein (Boehringer, Mannheim, Germany) and 0.1 U μl^{-1} Amplitaq Stoffel polymerase. 1.0 µl DNA extract was used as a template. For bacteria a touchdown PCR was performed and included 4 min at 94°C followed by 36 cycles of 1 min at 94°C, 1 min starting at 60°C, and 2 min at 72°C. The annealing temperature was shifted from 60 to 55°C during the first 10 cycles and remained at 55°C during the remaining 26 cycles. Final extension step was at 72°C for 10 min, followed by 10°C for 5 min.

For actinomycetes a nested PCR was performed. The primary PCR reaction mix (final vol. 25 µl) consisted of 200 µM dNTP, 3.75 mM $MgCl_2$, 1x Stoffel buffer, 0.4 µM primer F243, 0.4 µM primer R1378, 1.0% formamide, 4 ng μl^{-1} T4 gene 32 protein and 0.2 U μl^{-1} Amplitaq Stoffel polymerase; the nested PCR mix (final vol. 50 µl) was similar to that of the

bacteria PCR. 1.0 µl DNA extract was used as a template. For actinomycetes the primary touchdown PCR included 4 min at 94°C followed by 32 cycles of 1 min at 94°C, 1 min starting at 63°C, and 2 min at 72°C. The annealing temperature was shifted from 63°C to 58°C during the first 10 cycles and remained at 58°C during the remaining 22 cycles. Final extension step was at 72°C for 10 min, followed by 10°C for 5 min.

A modified protocol was used derived from Anderson et al. 2003. 18S rDNA and ITS regions were PCR-amplified using the primers EF4 (Smit et al., 1999) and ITS4 (White et al.,

Table 3. Primers used in PCR reactions.

System	Primer	Sequence (5'->3')
Actinomycetes	F243	GGA TGA GCC CGC GGC CTA
Bacteria	F984GC	CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G AAC GCG AAG AAC CTT AC
Bacteria	R1378	CGG TGT GTA CAA GGC CCG GGA ACG
Fungi	ITS1F-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCT TGG TCA TTT AGA GGA AGT AA
Fungi	ITS2	GCT GCG TTC TTC ATC GAT GC
Fungi	ITS4	TCC TCC GCT TAT TGA TAT GC
Fungi	EF4	GGA AGG GRT GTA TTT ATT AG

1990). Polymerase chain reactions were carried out using 50 ml reaction volumes containing approximately 50 ng of template DNA, 20 pmol of each primer, 2 mM $MgCl_2$, 250 mM of each of dATP, dCTP, dGTP and dTTP, 10x buffer and 2.5 U of Expand High Fidelity DNA polymerase (Roche). Cycling parameters were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55 C for 30 s, and 72°C for 30 s with a final extension at 72°C for 5 min. These products were diluted 10 times in water and 1µl is then used as template in a nested PCR to generate products for DGGE analysis with the primers ITS1-F (Gardes and Bruns, 1993) with an GC clamp added (Muyzer et al., 1993) and ITS2 (White et al., 1990). PCR was performed as described above, except that T4gene32 protein was omitted from the reactions.

Denaturating gradient gelelectrophoresis (DGGE) was performed with the phorU2 system (Ingeny, Leiden, the Netherlands). PCR products (15 to 20 ml) were applied directly onto 6% (wt/vol.) polyacrylamide gels in 0.53 TAE buffer (20 mM Tris-acetate (pH 7.4), 10 mM sodium acetate, 0.5 mM disodium EDTA) containing a linear denaturing gradient (a 45-65% gradient (eubacteria and actinomycetes) or a 30-80% gradient (fungi) of urea and formamide). The gradients were formed with 6% (wt/vol.) acrylamide stock solutions that contained no denaturant and 100% denaturant (the 100% denaturant solution contained 7 M urea and 40% (vol./vol.) formamide deionized with AG501-X8 mixed-bed resin (Bio-Rad, Veenendaal, the Netherlands)). The gels were electrophoresed for 15 h at 60°C and 100V. After electrophoresis, the gels were stained for 30 min with SYBR Gold I nucleic acid gel stain (Molecular Probes Europe, Leiden, the Netherlands) and were photographed under UV light by using a SYBR Green gel stain photographic filter (Molecular Probes) and a Docugel

V system apparatus (Biozym, Landgraaf, the Netherlands).

Statistical analysis

Banding pattern analysis and comparison of gels was processed by GelcomparII software (version 4.5; Applied Maths, Woluwe, Belgium). Gels were analysed using the instructions from the manual. Each gel contained 4 marker lanes for reference purposes. Distance matrixes, based on the presence/absents of bands, were calculated using Pearson correlation and clustering was performed using the UPGMA algorithm. Patterns of disease suppression of 12 composts for 5 pathosystems were explored using Principal Components Analysis (PCA) (Braak and Šmilauer, 2002). Detrended Correspondence Analysis (DCA), based on DGGE patterns and environmental variables, revealed 1.7 SD (standard deviation) for the length of the gradient, thus indicating that the distribution of the species was not unimodal along the environmental gradients (Braak and Šmilauer, 2002). The statistical relationships between DGGE patterns and disease suppression and between DGGE patterns and compost or compost/peat mix characteristics were therefore assessed by Redundancy Analysis (RDA) (Braak and Šmilauer, 2002). RDA is a constrained ordination technique, based on PCA, in which ordination axes are constrained to be linear combinations of environmental variables. This technique helps to assess the relationship between environmental variables and the multivariate data. RDA in combination with Monte Carlo permutation tests (Braak and Šmilauer, 2002), based on 500 random permutations, was used to determine which of the environmental variables were significantly correlated with DGGE patterns. Environmental variables analyzed with RDA included disease suppression rates of the various pathosystems and physico-chemical characteristics of the composts and compost/peat mixes. PCA and RDA were carried out using CANOCO version 4.5 (Braak and Šmilauer, 2002). Statistical correlations between disease suppression and compost or compost/peat parameters were assessed using SAS software, version 9.1. Data was subjected to analysis of variance and means were separated according to Duncan's multiple range test.

Results

Effects of mixing peat-substrates with compost on DGGE banding patterns

Numbers of detected DGGE bands in the pure composts, compost/peat mixes and pure peat were for bacteria 21.7 ± 4.0 (stdev), 21.5 ± 3.7 and 22.0 ± 1.4 respectively, for actinomycetes on average 26.3 ± 8.1, 25.4 ± 3.4 and 23.5 ± 3.5 respectively, and for fungi 7.7 ± 3.1, 14.5 ± 2.5 and 15.0 ± 0.0 respectively. The microbial communities as determined by DGGE of the compost/peat mixes were compared by UPGMA with those of the pure composts and the pure peat. Peat mixed with composts e, p, q, r, and s had 16S-rDNA bacterial DGGE banding patterns that had higher similarity with the respective peats than with the respective composts (Fig. 1a). These mixes are here referred to as 'peat-like' with

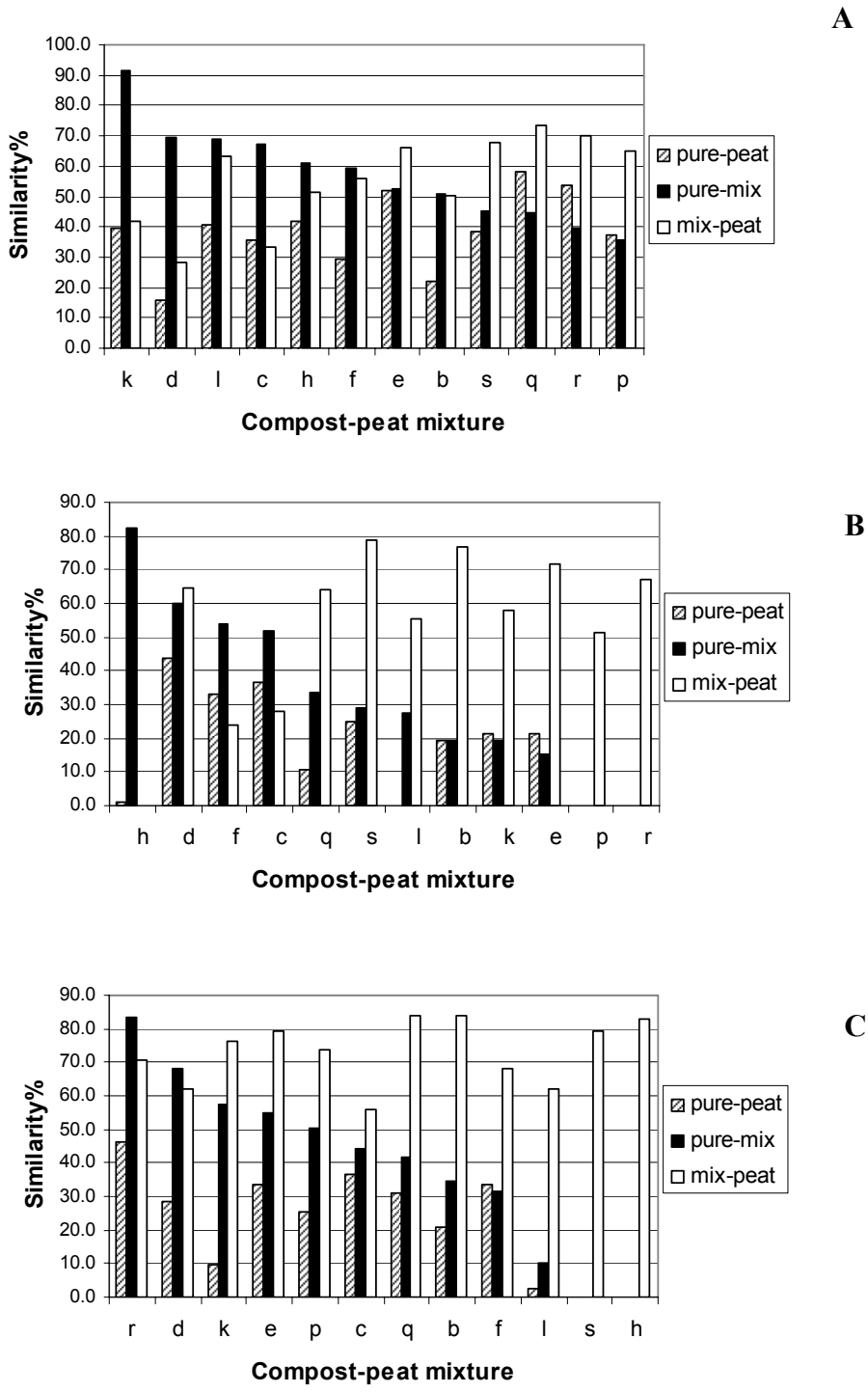

Figure 1. Similarities of DGGE patterns based on presence/absence of bands in the gels of the (A) 16S-rDNA bacterial, (B) 16S-rDNA actinomycete, and (C) 18S-rDNA fungal communities. Left bar: similarity between pure compost and pure peat substrate (indicated as pure-peat); middle bar: similarity between pure compost and the compost/peat mix (indicated as pure-mix); right bar: similarity between compost/peat mix and the pure peat (indicated as mix-peat). For each graph the composts have been ordered in sequence of decreasing similarity of the pure composts with the compost/peat mix.

respect to the bacterial DGGE banding pattern. These peat-like mixes (with respect to bacteria) showed on average significantly higher ($P < 0.01$) similarities with the pure peat than the other mixes (68.4 ± 3.3 (stdev) and 46.3 ± 12.6% similarity respectively) and significantly lower ($P < 0.01$) similarities with the respective composts than the other mixes (43.4 ± 6.4 and 66.9 ± 12.7% similarity respectively). Peat mixed with composts c, d, and k had bacterial DGGE banding patterns that were more alike that of the respective pure composts. Mixes made of other composts took an intermediate position (Fig. 1a). With respect to the actinomycete and fungal DGGE banding patterns, generally most composts were peat-like, except peat mixed with composts c, d, f and h for the actinomycetes (Fig. 1b) and with composts d and r for the fungi (Fig. 1c). Peat-like mixes with respect to actinomycetes showed on average significantly higher ($P < 0.01$) similarities with the pure peat than the other mixes (65.4 ± 10.0 (stdev) and 29.2 ± 26.7% similarity respectively) and significantly lower ($P < 0.01$) similarities with the respective composts than the other mixes (18.0 ± 12.6 and 62.1 ± 14.0% similarity respectively). Peat-like mixes with respect to fungi showed significantly lower ($P < 0.01$) similarities with the respective composts than the other mixes (32.6 ± 21.8 and 75.8 ± 10.9% similarity respectively).

In a PCA, based on the bacterial DGGE banding patterns of pure composts, the group of composts that led to peat-like mixes was, except compost s, separated from the other composts (Fig. 2). These composts resulting in peat-like mixes showed significantly ($P < 0.05$)

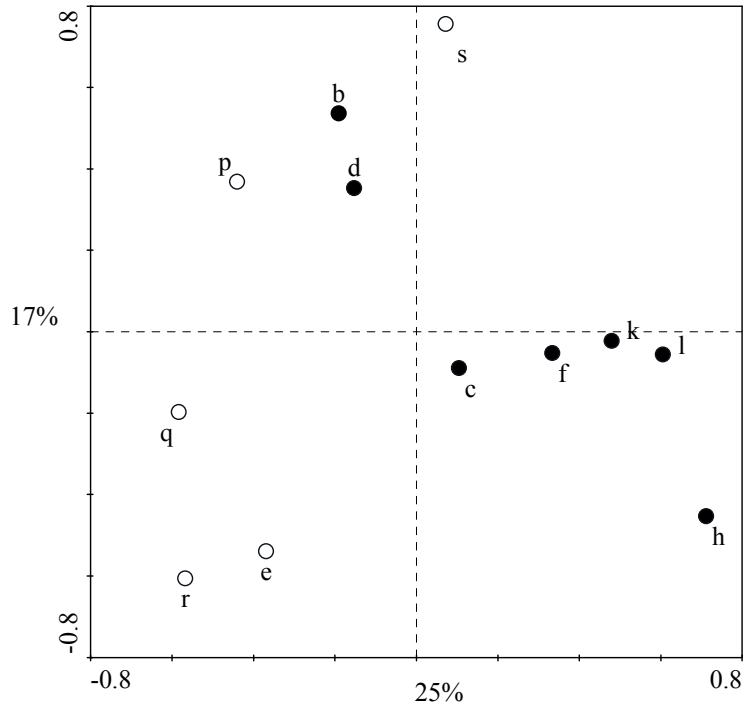

Figure 2. Principal component analysis (PCA) based on bacterial composition of pure composts which resulted in:

O compost/peat mixes that are dominated by the bacterial composition of the peat.

● compost/peat mixes that are not dominated by the bacterial composition of the peat.

For explanation of compost codes, see Table 1.

lower percentages of functional carbon (carboxylic, aromatic, alkyl), organic N, total N and total C, and a significantly higher pH ($P < 0.05$) than the other composts. Composts resulting in peat-like mixes with respect to actinomycetes showed similar differences with the other composts, but in addition they showed significantly lower dissolved organic carbon and organic matter content and no significant differences in pH. Such effects were not present with respect to fungi.

A RDA revealed that pH of the compost/peat mixes contributed significantly to the DGGE banding patterns of the bacteria and fungi ($P < 0.01$ and $P = 0.03$ respectively, but not of actinomycetes ($P = 0.63$) (Table 4). Compost/peat mixes that were peat-like with respect to bacteria (i.e. the mixes e, p, q, r, s) showed significantly lower ($P < 0.05$) pH levels (average pH = 4.83) than the other compost/peat mixes (average pH = 5.83). A positive correlation was found between the pH of the compost/peat mix and the similarity of 16S-rDNA bacterial communities of pure compost and corresponding compost/peat mixes ($r = 0.74$; $P < 0.01$). Organic N content in the pure compost contributed significantly to the DGGE banding patterns of the bacteria and actinomycetes ($P = 0.09$ and $P = 0.01$ respectively; Table 4), but not of fungi ($P = 0.32$) (this parameter was not determined in the compost/peat mixes). Composts that resulted in peat-like actinomycete DGGE banding patterns in the mixes showed significantly lower ($P < 0.05$) organic N levels than the other composts (1.1 and 2.2

Table 4. Significance levels (*P* values) of the association between variation in DGGE profiles and pathosystem and variation in DGGE profiles and selected characteristics of pure compost and compost/peat mixes obtained by including them as environmental variables in RDA and testing for significant relationships using the Monte Carlo permutation test.

	Bacteria *P*[a]	Actinomycetes *P*	Fungi *P*
Characteristics of the pure composts			
Organic matter	0.30	0.30	0.17
Total C	0.09	0.37	0.10
Dissolved organic C	0.02	0.83	0.18
Total N	0.08	0.04	0.22
Organic N	0.09	0.01	0.32
C/N	0.21	0.27	0.02
Characteristics of the compost/peat mixes			
pH	< 0.01	0.63	0.03
NO_3	0.01	0.50	0.16
NH_4	0.23	0.16	0.95
Pathosystem			
Cylindrocladium spathiphylli	0.44	0.68	0.22
Phythophthora cinnamomi	0.04	0.09	0.16
Phythophthora nicotianae	0.09	0.07	0.35
Rhizoctonia solani	0.24	0.26	0.35
Verticillium dahliae	0.06	0.22	0.16

[a] Significance of explanation determined with Monte Carlo permutation test.

mg organic N kg^{-1} bulk respectively). A positive correlation was found between the organic-N content in the pure compost and the similarity of 16S-rDNA actinomycete communities of pure composts and corresponding compost/peat mixes ($r = 0.81$; $P < 0.01$). RDA led to 56 and 25% explanation of the variation of the bacterial and actinomycete bands respectively (Fig. 3).

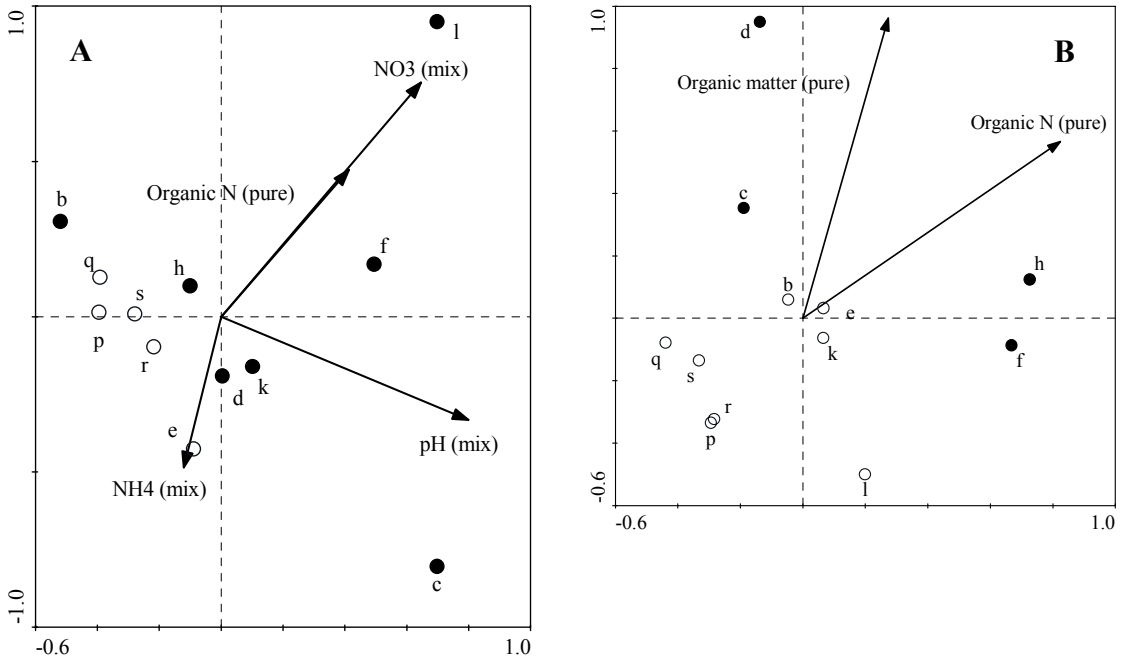

Figure 3. Redundancy analysis (RDA) based on (A) bacterial (total explanation 56%) and (B) actinomycete (total explanation 25%) composition of compost/peat mixes (dots) with indication of explanatory parameters of the pure composts ('pure') and the compost/peat mixes ('mix') indicated as arrows.

- o compost/peat mixes that are dominated by the (A) bacterial or (B) actinomycete composition of the peat.
- ● compost/peat mixes that are not dominated by the (A) bacterial or (B) actinomycete composition of the peat.

For explanation of compost codes, see Table 1.

Disease suppression in relation to the microbial composition as a result of mixing peat with compost

For bacteria and actinomycetes, significant positive and negative correlations were observed between disease suppression of the various pathosystems and the similarity between DGGE banding patterns of the pure composts and of the corresponding compost/peat mixes (Table 5). Between disease suppression of *P. cinnamomi*, *R. solani* and *V. dahliae* and the similarity between DGGE banding patterns of pure peat and the various compost/peat mixes,

Table 5. Correlation between disease suppression for five pathosystems and similarity between the bacterial, actinomycete or fungal DGGE profiles of the pure composts and corresponding compost/peat mixes (A) and of the pure peat substrate and the various compost/peat mixes (B).

Pathosystem	Bacteria		Actinomycetes		Fungi	
	r[a]	*P*[b]	r	*P*	r	*P*
A. Pure compost and compost/peat mix						
C. spathiphylli	0.50	0.097	0.65	0.022	0.08	0.802
P. cinnamomi	0.58	0.046	0.68	0.016	-0.33	0.297
P. nicotianae	-0.64	0.025	-0.13	0.678	0.24	0.445
R. solani	-0.82	0.001	-0.33	0.289	-0.24	0.455
V. dahliae	0.59	0.044	0.71	0.009	-0.11	0.731
B. Pure peat and compost/peat mix						
C. spathiphylli	-0.46	0.137	-0.30	0.340	-0.40	0.199
P. cinnamomi	-0.61	0.036	-0.58	0.048	-0.34	0.273
P. nicotianae	0.44	0.155	0.20	0.538	0.01	0.981
R. solani	0.73	0.007	0.33	0.298	0.12	0.720
V. dahliae	-0.75	0.005	-0.49	0.105	-0.18	0.569

[a] correlation coefficient. [b] significance of linear correlation.

significant correlations of opposite sign were found (Table 5). No correlations were observed for fungi.

In a PCA, based on the disease suppressive properties of the compost/peat mixes, compost/peat mixes that were peat-like in bacterial DGGE banding pattern (mixes e, p, q, r, s) were clearly separated from the other mixes along the 1st axis of the PCA (55% explanation; Fig. 4). These peat-like mixes showed lower disease suppression rates for *C. spathiphylli* (-1.9%; $P = 0.12$), *V. dahliae* (-4.5%; $P < 0.001$), and *P. cinnamomi* (-18.9%; $P < 0.001$) than the other mixes (28.8, 41.9, and 49.3% respectively) (Table 2; Fig. 5). This was however significantly ($P < 0.05$) reversed for *R. solani*: on average 55.0% disease suppression in the peat-like mixes and 1.3% in the other mixes. This opposite behaviour of *R. solani* versus other pathogens was also noted by Termorshuizen et al. (2006). *P. nicotianae* behaved similarly: although all compost/peat mixes except two resulted in high disease suppression (Table 2), the difference between peat-like (with respect to bacteria) and other mixes was significant (79.6 and 48.2 respectively).

The group of peat-like mixes with respect to actinomycetes also showed lower disease suppression than the other mixes (composts c, d, f and h) with respect to *C. spathiphylli*, *V. dahliae*, and *P. cinnamomi* (all $P < 0.02$) and the reversed was again the case for *R. solani* ($P < 0.10$), but not for *P. nicotianae* ($P = 0.48$).

No significant correlations between disease suppression and the fungal DGGE banding patterns could be observed. In a RDA, disease suppression of *P. cinnamomi*, *P. nicotianae* and *V. dahliae* was significantly ($P < 0.10$) associated with variation in bacterial DGGE

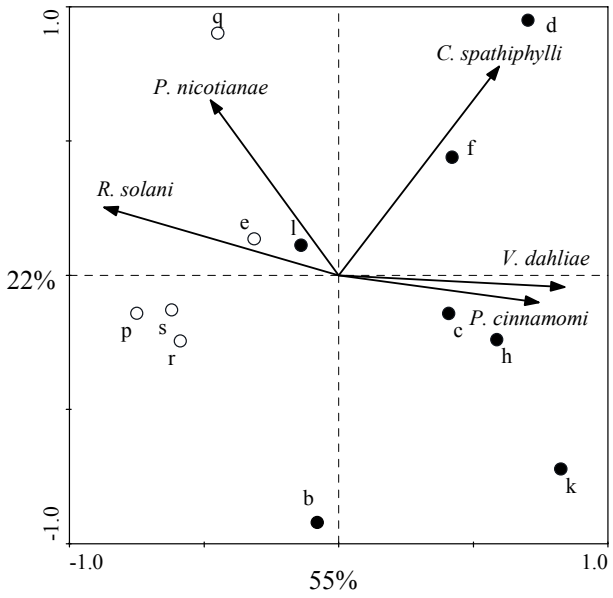

Figure 4. Principal component analysis (PCA) based on disease suppression of compost/peat mixes against 5 pathosystems.

O compost/peat mixes that are dominated by the bacterial composition of the peat.

● compost/peat mixes that are not dominated by the bacterial composition of the peat.

For explanation of compost codes, see Table 1.

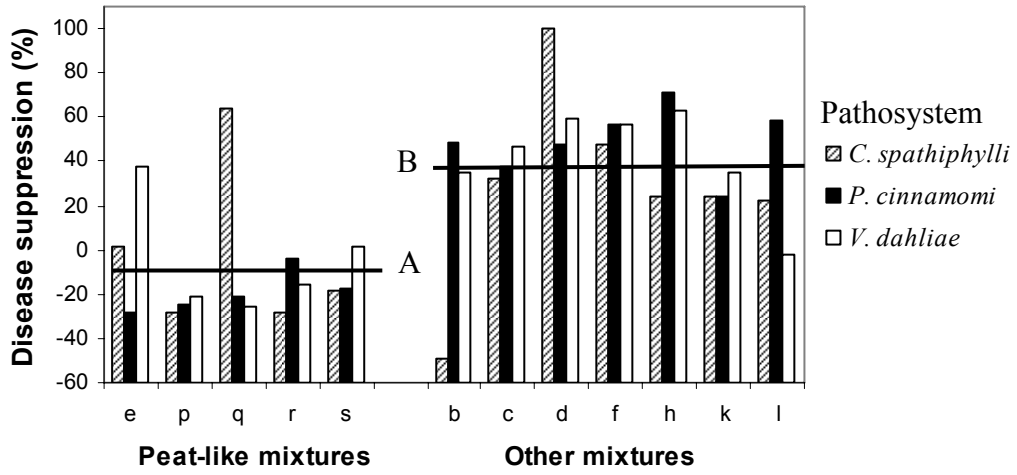

Figure 5. Disease suppression of the compost/peat mixes for the pathosystems *Cylindrocladium spathiphylli, Phytophthora cinnamomi* and *Verticillium dahliae*. The compost/peat mixes grouped according to their bacterial composition: the left group represents compost/peat mixes with a bacterial composition that have highest similarity with the pure peat and the right group represents the other compost/peat mixes. Average disease suppression levels for the two groups (indicated with A and B) are significantly different (t-test, $P < 0.05$). For explanation of compost codes, see Table 1.

Table 6. Selected characteristics of the compost/peat mixes.

Code	pH	EC (mS cm^{-1})	NH_4 (mmol l^{-1})	NO_3 (mmol l^{-1})
b	4.65	2.49	1.0	3.1
c	6.47	1.92	2.4	2.3
d	6.31	0.93	0.1	2.7
e	5.67	1.83	3.4	1.9
f	6.44	1.78	0.0	7.0
h	5.43	2.65	0.4	3.6
k	5.92	1.09	0.2	2.9
l	5.58	2.87	0.8	12.7
p	4.47	0.94	1.7	1.7
q	4.55	0.77	1.2	2.3
r	4.81	0.83	1.7	1.9
s	4.65	0.92	1.6	2.2

banding pattern of the compost/peat mixes (Table 4). The same was the case for disease suppression of *P. cinnamomi* and *P. nicotianae* with respect to variation in actinomycete DGGE banding pattern ($P < 0.10$). None of the tested pathosystems was significantly associated to the variation in fungal composition.

Positive correlations were found between pH of the compost/peat mix (Table 6) and disease suppression of the pathosystems *C. spathiphylli* (r = 0.63; $P = 0.027$), *P. cinnamomi* (r = 0.56; $P = 0.058$) and *V. dahliae* (Table 2; r = 0.76; $P = 0.004$), while it was negatively correlated with disease suppression of *R. solani* (r = 0.62; $P = 0.029$). No correlation was found between pH of the compost/peat mix and disease suppression of *P. nicotianae*. Positive correlations were also found between organic N content in the pure compost (Table 1) and disease suppression of *V. dahliae* (r = 0.93; $P < 0.0001$), *C. spathiphylli* (r = 0.49; $P = 0.109$) and *P. cinnamomi* (r = 0.75; $P = 0.004$), and negative for disease suppression of *R. solani* (r = 0.56, $P = 0.053$ if all compost/peat mixes are included; r = 0.87; $P < 0.0001$ when compost/peat mix k, with an extreme negative value for disease suppression, was excluded). No correlation was found between organic N content and disease suppression of *P. nicotianae*.

Discussion

Disease suppression of all the five investigated pathosystems at least partially was determined by the effect of compost on the microbial composition of the compost/peat mix. If after mixing compost with peat the mixes remained predominantly peat-like, relatively low disease suppression is predicted for *C. spathiphyllum*, *P. cinnamomi*, and *V. dahliae*. For these pathosystems, disease suppression was on average 40.0% for the 7 ‘not-peat-like’ mixes

and -8.4% for the 5 'peat-like' mixes. However, a reverse reaction occurred for *P. nicotianae* and *R. solani*. Changes noticed in the bulk (i.e., non-rhizosphere) samples, as reported in the current study, likely have also effect on the microbial rhizosphere composition, which is bulk soil-derived (Costa et al., 2006). In another study (Chapter 4) we report on the effects of compost-amendment on microbial rhizosphere communities: if the bacterial rhizosphere composition (determined using DGGE) of a plant is affected by compost, disease suppression is affected as well.

This study was part of a larger study involving 18 composts and 7 pathosystems, where a major conclusion was a significant compost × pathosystem interaction with respect to disease suppression (Termorshuizen et al., 2006). Respiration and Ca content or respiration and density of nematodes in the compost/peat mix best correlated with the variation in disease suppression (Termorshuizen et al., 2006). Microbial changes likely go together with variation in these parameters. As the peat was a constant factor in this study, the variation among the composts was the single factor explaining the variation in microbial communities in the compost/peat mixes. If natural soils would be the target of compost amendment also the variation in their microbial communities would need to be taken into account.

The results suggest that *C. spathiphylli*, *P. cinnamomi* and *V. dahliae* are suppressed by introduced compost-specific bacteria and actinomycetes, but not by fungi. It should be borne in mind that with DGGE only the dominant species are detected and one band is not necessarily linked to a single species (Schmalenberger and Tebbe, 2003), thus banding patterns do not necessarily represent biodiversity. Still, although the incidence of matching bands from different samples may represent different taxons, it would be unlikely that multiple matching bands from different samples represent diffent taxons at all occassions. Because of the relation between banding pattern similarities between samples and disease suppression, we show here that DGGE-analysis of 16S-rDNA bulk samples can have an ecologically meaningful result. Behaviour of disease suppression of *R. solani* and *P. nicotianae* was reversed compared to that of the other pathogens. Perhaps these opportunistic pathogens, which cause seedling damping-off, took more advantage of the more nutrient-rich conditions of the compost/peat mix than the other pathogens which are more specialized in attacking older plants.

It would be interesting if predictions could be made on how compost and compost/peat characteristics affect microbial composition in compost/peat mixes. Compost/peat mixes that were dominated by the microbial composition of the peat with respect to bacteria showed, on average, significantly lower pH levels (4.8) than the other compost/peat mixes (pH 5.8). Microbial community composition is known to be strongly influenced by soil pH (Steenwerth et al., 2003; Marschner et al., 2004; Baeckman et al., 2003; Pankhurst et al., 2001; Lei and Vandergheynst, 2000). At low pH levels (< 5.5) the growth of bacteria is generally reduced (Alexander, 1977; Boulter et al., 2000), while fungi and actinomycetes generally tolerate lower pH levels (Golueke, 1972; Goodfellow and Williams, 1983; Pankhurst et al., 2001). For the pathosystems *C. spathiphylli*, *P. cinnamomi* and *V. dahliae*, disease suppression was positively correlated with pH. Disease suppression of *C. spathiphylli* has been reported to be pH-dependent, but the pH levels that rendered disease suppression (> 6.5; Chase and Poole, 1987) were higher than those observed in our study. Disease suppression of *P. cinnamomi* has

been reported to be maximal within the pH range of 5.5-7.0 (Broadbent and Baker, 1974), which is in line with our results. In order to prepare disease suppressive compost/peat mixes for *C. spathiphylli*, *P. cinnamomi* and *V. dahliae*, it is therefore recommendable that pH of compost/peat mixes is adjusted to at least 5.5-6.0. Gaag et al. (2004) observed a negative correlation (r = 0.73) between pH of the potting mix and suppression of damping-off of cauliflower seedlings caused by *R. solani*. Increasing the pH of the yard waste amended mixes using lime to a level of 5.6 rendered the mix less or non-suppressive. This is in line with the conclusions of Grosch and Kofoet (2003) who demonstrated that the hyphal growth rate of *R. solani* (AG 1 through AG 5) at pH 4 was significantly lower than at pH > 5.

Compost/peat mixes that were dominated by the microbial composition of the peat with respect to actinomycetes showed, on average, significantly lower organic N rates than the other compost/peat mixes (1.1 and 2.2 mg organic N kg^{-1} bulk on average, respectively). In line with this, organic N in the pure compost was significantly positively correlated with the colonization of the compost/peat mix by compost actinomycetes (r = 0.81; $P < 0.01$) and significantly ($P < 0.05$) associated with variation in actinomycete composition in the compost/peat mixes. This is in line with studies of several authors reporting on correlations between N availability and microbial rhizosphere and bulk soil communities (Kennedy et al., 2004; Marschner et al., 2004; Tscherko et al., 2004; Ros et al., 2006). Kennedy et al. (2004) and Ros et al. (2006) demonstrated with T-RFLP-analysis and 16S-rDNA DGGE profiles respectively that addition of mineral N induced a significant change in the soil bacterial community structure. The variation in bacterial composition between soils that were amended with mineral N and soils that were not amended with mineral N was significantly larger than the variation in bacterial composition as a result of amendment of different composts (Ros et al., 2006). Compost-borne microorganisms may colonize the nutrient-poor peat substrate only if sufficient compost-derived N is available. Positive correlations were found between organic N in the pure compost and disease suppression of *C. spathiphylli*, *P. cinnamomi* and *V. dahliae.* This is in line with the results of Broadbent and Baker (1974) who demonstrated that nitrogen contents were higher in soils suppressive than in soils conducive to root rot caused by *P. cinnamomi*. pH in the compost/peat mix and organic N in the pure compost were significantly correlated (r = 0.70; $P = 0.010$) and therefore it is in this study not possible to unravel their effects on disease suppression. However, the RDA indicated that pH contributed significantly to the DGGE banding patterns of bacteria and fungi, but not of actinomycetes, while organic N contributed strongly to the DGGE banding patterns of actinomycetes (Table 4). Thus possibly both factors play a role in the colonization of compost/peat mixes by compost-borne microorganisms.

The bacterial, actinomycete and fungal composition of the compost/peat mixes showed in respectively 42, 67 and 83% of the investigated composts-peat mixes higher similarities to the native populations of the peat than to the native populations of the respective composts. Likely these mixes were dominated by native peat populations. Changes of the microbial composition of the main substrate as a result of compost amendment have been reported in literature in some cases, while in others no significant change in the microbial communities was observed. Saison et al. (2006) demonstrated that the effect of compost amendment on the microbial community (pH 7.8) as assessed by phospholipid fatty

acid profiles could be ascribed to physico-chemical characteristics of the compost rather than to compost-borne microorganisms as the effect was independent of compost sterilization. Yao et al. (2006) demonstrated strong effects of compost amendment (69 t ha^{-1}) on the dominant microbial populations in the compost-soil mix (pH 6.5) as determined by DGGE and these changes could be detected up to 1 year after application. On the other hand, after compost amendment (consisting of municipal solid waste; 24 t ha^{-1}) to soil (pH 8.3), no significant variation in the bacterial communities in the soil was observed by Crecchio et al. (2004).

In conclusion, we demonstrated that compost-borne microbial communities may or may not sustain after mixing with peat. The ability of these communities to survive in the mix may well be related to disease suppression of certain pathogens. The driving factor may well be the pH level and/or the organic N concentration, although further research is needed to verify this. Our results could be important in the prediction of compost-induced disease suppression.

Acknowledgements

This study was undertaken as part of the first author's PhD program and was funded by the EU project QLK5-CT-01442. We thank A.H.C. van Bruggen for critically reading the manuscript and R. Pastoor for technical assistance.

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Chapter 4

Compost-induced suppression of damping-off caused by *Pythium ultimum* is host and compost dependent

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Soil Biology and Biochemistry, submitted

Abstract

Disease suppression of compost-amended potting mixes has been reported repeatedly, especially against soilborne fungal plant pathogens, and it is generally thought that the rhizosphere microbial community plays a crucial role. The composition and activity of this community is the resultant of edaphic and plant factors, and the relative strength of these factors may thus be decisive for whether a compost is suppressive or not. If this is true, disease suppression would be dependent on the effect of compost on the microbial rhizosphere community and this dependency would be affected by the host species studied. We tested this hypothesis by determining disease suppression of nine composts against damping-off caused by *Pythium ultimum* for five host species. Analysis of variance showed a significant ($P < 0.01$) compost × plant species interaction, indicating that disease suppression depends on the combination of type of compost and plant species. Relatively low disease suppression rates (< 20%) were observed for plant species that showed high (> 70%) similarity between the rhizosphere bacterial DGGE banding patterns of a compost-amended mix and the non-amended peat (assigned as 'strong' plant species), while high or low disease suppression rates were observed for plant species that showed low similarities to the banding pattern in non-amended peat ('weak' plants). Composts that had highest bacterial DGGE similarities among all various hosts tested ('strong' composts) showed higher disease suppression than composts that had low similarities ('weak' composts). Our results suggest that disease suppression may be predicted by a combination of 'weak' plants and 'strong' composts. In conclusion, we demonstrated that disease suppression of *P. ultimum* through compost amendment is determined by interacting plant species - compost effects on the rhizosphere bacterial community, but not, or to a lesser extent, on the actinomycete or fungal community. For prediction of disease suppression of Pythium damping-off, the effects of both

the host and the amended substrate on rhizosphere bacterial communities should be investigated.

Introduction

Suppression of soilborne plant pathogens has been shown to be based on microbial processes in the rhizosphere (Weller et al., 2002). For example, Raaijmakers and Weller (1998) demonstrated that antibiotic-producing pseudomonads are key components in suppression of *Gaeumanonmyces graminis* var. *tritici* in monoculture of wheat. Disease suppressiveness induced by compost mixed with peat or soil has been reported repeatedly, primarily against soilborne plant pathogenic fungi (Chen et al., 1988; Serra-Wittling et al., 1996; Tuitert et al., 1998; Scheuerell et al., 2005; Termorshuizen et al., 2006), but also against soilborne plant pathogenic nematodes (Barbosa et al., 2004, Jin et al., 2004). Mechanisms of compost-induced disease suppression include general disease suppression (based on the activity of the general soil microflora through increased microbial activity of multiple organisms), specific disease suppression (based on activity of a specific antagonist), or induced resistance (through plant-mediated changes in the defense system) (Hoitink and Boehm, 1999). Compost amendments can affect the rhizosphere microbial community (Boehm et al., 1993) and their ability to modify this community may be crucial for inducing disease suppression. Plant rhizospheres are selectively enriched for those microorganisms originating from the bulk soil (Costa et al., 2006) that are adapted to utilization of specific plant-released organic compounds (Garbeva et al., 2004). Thus, in studies on alfalfa and rye (Miethling et al., 2000) on clover, bean and alfalfa (Wieland et al., 2001) and on chickpea, canola and Sudan grass (Marschner et al., 2004), plant species was identified as the major determinant of microbial rhizosphere composition, rather than edaphic factors or plant developmental stage. On the other hand, the importance of bulk soil parameters in defining microbial rhizosphere communities has also been stressed (Groffmann et al., 1996; Marschner et al., 2005a). The change of the microbial composition in the rhizosphere compared to the surrounding bulk soil is referred to as the 'rhizosphere effect' (Smalla et al., 2001). For example, for wheat, a small rhizosphere effect was found (numbers of copiotrophic bacteria in the rhizosphere 1.3× those in the bulk soil) in a sandy loam soil amended with fresh corn debris, while a pronounced rhizosphere effect (numbers of copiotrophic bacteria in the rhizosphere 10× those in the bulk soil) occurred in a coarse loamy soil without amendment of fresh plant debris (Semenov et al., 1999).

The influence of compost amendments on the microbial composition of the rhizosphere has been shown to decrease with increasing proximity to the root and to increase with higher levels of compost amendment (Inbar et al., 2005). Since the rhizosphere community is in the first place determined by the quantity and quality of root exudates, an effect of compost on the rhizosphere community is likely to become apparent only if it dominates to some extent the rhizosphere effect. So, compost-induced suppression of soilborne pathogens may be predicted by the extent to which compost affects the rhizosphere community. In that case, a negative relation between the magnitude of the rhizosphere effect

as mediated by the plant and disease suppression would be found. This would corroborate the statement of Gilbert et al. (1994), that a rhizosphere, which is relatively strongly influenced by the bulk soil, may be less attractive to typical rhizosphere organisms. Whether edaphic or plant factors primarily determine the composition of the rhizosphere community may relate to their relative 'strengths' (Garbeva et al., 2004) and compost-mediated disease suppression may thus be plant species and compost type dependent, at least for those pathogens that are primarily suppressed through aspecific competition.

Pythium ultimum is a common and polyphagous soilborne pathogen causing seed decay, pre- and post-emergence damping-off and root rot (Hendrix and Campbell, 1973). Disease suppression in the rhizosphere has been reported for this pathogen (Chen et al., 1988) and a relation with general (aspecific) disease suppression is well-established in literature (Chen et al., 1988; Mandelbaum and Hadar, 1990; Boehm et al., 1993, Veeken et al., 2005). Chemical compounds such as amino-acids, fatty acids and carbohydrates present in root and seed exudates can stimulate the germination of sporangia and oospores of *P. ultimum* (Martin and Loper, 1999). Suppression of *Pythium* disease has been achieved through enrichment of growth substrate with microorganisms (bacteria and fungi) that are able to rapidly metabolize these chemical stimulants, thus decreasing the pathozone (*sensu* Gilligan and Bailey, 1997) of *Pythium* (Chen et al., 1988; Mandelbaum and Hadar, 1990).

The goal of this study was to test the hypothesis that the level of compost-induced disease suppression is dependent (1) on the degree to which compost influences the microbial rhizosphere community, and (2) on the host species. We determined the disease suppressive ability of nine different composts against *P. ultimum* for five host plants and related differences in disease suppression to the microbial rhizosphere composition.

Material and methods

Overview

Three series of bio-assays were carried out to test the effect of plant species and compost type on disease suppression of damping-off caused by *Pythium ultimum*. In experiment 1, two plant species, and in experiments 2 and 3 five plant species were tested (Table 1). Denaturing gradient gel electrophoresis (DGGE) was carried out for PCR-amplified 16S and 18S-rDNA isolated from rhizosphere samples collected in experiments 1 and 2 (Table 1).

Collection and selection of composts

Composts made from either separately collected organic household waste or from green waste were collected from commercial composting sites at different stages of maturity (Table 2). After collection, the dry-sieved fractions < 10 mm were stored for 1 day in loosely closed polyethylene bags at 4°C until use. On the day of collection, 2 l of each compost was oven-dried at 60°C for 24 h for chemical analysis and 0.5 l was stored at -20°C until determination of the Oxygen Uptake Rate 8 weeks later.

Table 1. Overview of bioassays done and of DGGEs carried out. ✓ = done, n.d. = not done.

Compost code	Exp. #	tomato	carrot	pea	sugar beet	cucumber	DGGE
a	1	n.d.	n.d.	✓	n.d.	✓	✓ [a]
b	1	n.d.	n.d.	✓	n.d.	✓	✓ [a]
c	1	n.d.	n.d.	✓	n.d.	✓	✓ [a]
d	2	✓	✓	✓	✓	✓	✓ [b]
e	2	✓	✓	✓	✓	✓	✓ [b]
f	2	✓	✓	✓	✓	✓	✓ [b]
g	3	✓	✓	✓	✓	✓	n.d.
h	3	✓	✓	✓	✓	✓	n.d.
i	3	✓	✓	✓	✓	✓	n.d.

[a] only for cucumber and pea.
[b] for all plants tested.

Chemical analyses

Air-dried compost samples (6 g, ground and subsequently sieved over a 2-mm sieve) were added to 30 ml dematerialized water and shaken for 30 min. Measurements of pH and EC were carried out with a pH/mV meter (InoLab, Weilheim, Germany). The organic matter content of the samples was assessed gravimetrically by dry combustion of the organic material in a furnace at 500-550°C (loss-on-ignition method). Total N and C were measured with a Fisons Type EA 1108 Element Analyzer (Milano, Italy) according to the method of Dumas (Anonymous, 1997).

Bioassays

Disease suppressiveness of the composts against *P. ultimum* was determined in three series of bioassays in each of which three composts were tested. Compost/peat mixes contained 65% (vol./vol.) Finnish Sphagnum peat (Kekkila Finnpeat, A0, Dega, Delft, the Netherlands), 20% compost and 15% perlite (Agra perlite #3, Rhenen, the Netherlands). The substrate without compost served as control and contained 85% (vol./vol.) Finnish Sphagnum peat and 15% perlite. The pH of potting mixes was adjusted to a value of 5.5-6.0 by adding lime (Dolokal). For fertilization Osmocote Start (N-P-K-Mg, 12-11-17-2), a slow-release fertilizer, was added in an amount of 2 g l^{-1} potting mix. After mixing, water was added to the

Table 2. Origin and physico-chemical properties of composts.

Exp.	Compost sample	Company	Source material	Maturity (weeks)	Organic matter content (wt/wt, %)	pH	EC (ms cm^{-1})	Total C (wt/d.w., %)	Total N (wt/d.w., %)	C/N	OUR[a]
1	a	I	Urban biowaste	8	52.0	7.8	3.87	26.9	1.98	13.6	12.7
1	b	II	Green waste	22	26.2	7.9	0.14	15.8	0.96	16.4	8.4
1	c	III	Green waste	12	21.9	7.5	0.13	14.2	0.83	17.0	10.4
2	d	I	Urban biowaste	2	39.2	7.8	3.00	18.7	1.22	15.3	19.7
2	e	IV	Urban biowaste	8	25.2	7.5	1.71	13.2	0.80	16.6	10.3
2	f	V	Green waste	12	12.4	7.3	0.62	6.2	0.32	19.5	6.0
3	g	I	Urban biowaste	8	29.4	7.7	3.14	15.6	1.25	12.4	10.1
3	h	V	Green waste	12	10.4	7.6	0.49	6.5	0.35	18.5	14.9
3	i	II	Green waste	22	24.5	7.9	1.30	13.1	0.82	15.9	7.2

[a] OUR: Oxygen Uptake Rate (mmol O_2 kg^{-1} organic matter (d.w.) h^{-1}).

mix in such an amount that pressing the material by hand lead to just a few water drops, which equals a pF of about 1.7-1.9. The moistened potting mixes were stored for one week at 20°C, in plastic boxes loosely closed with a lid to prevent excessive evaporation.

Pythium ultimum isolate G (kindly provided by J.G. Lamers, Applied Plant Research, Wageningen UR, Lelystad, the Netherlands) was originally isolated from soil from the Noordoostpolder (the Netherlands). For the experiments, the isolate was grown on Potato Dextrose Agar (Merck, Darmstadt, Germany) for 1 week at 20°C, and plugs with mycelium were added to a potting soil-oat meal mix. Potting soil (Lentse potgrond no. 4, sieved over a 4-mm sieve), 2% (d.w./d.w.) oat meal, and tap water (pF 1.7-1.9) were put in 500-ml Erlenmeyer flasks, closed with a cotton wool plug, covered with aluminum foil and autoclaved (121°C, 20 min) twice on consecutive days. Subsequently, the flasks were inoculated with 5 agar plugs containing growing mycelium of *P. ultimum*. The flasks were incubated at 25°C for 2 weeks and hand-shaken every 2-3 d to ensure a thorough colonization of the mix by the fungus. Shortly before sowing, the inoculum was mixed with the potting mixes at low and high inoculum density for each plant species: 0.03% (vol./vol.) and 0.3% for both pea and sugar beet, and 0.1% and 1.0% for carrot, cucumber and tomato. The amounts were derived from the results of a preliminary experiment in which a non-amended control mix had been infested with different amounts of inoculum. With the amounts of inoculum chosen we aimed at a disease incidence of 50-85% in the non-amended control for at least one of the inoculum densities. The inoculum was mixed thoroughly with the potting mix by shaking amounts of approx. 1-3 l for at least 5 min in plastic bags. A noninfested mix served as a control.

Disease suppressiveness of the three compost/peat mixes against *P. ultimum* was determined for *Cucumis sativus* L. cv. Chinese slangen (cucumber), *Lycopersicon esculentum* Mill. cv. Moneymaker (tomato), *Beta vulgaris* L. cv. Anastasia (sugar beet), *Pisum sativum* L. cv. Kelvedon Wonder (pea), and *Daucus carota* L. cv. Amsterdam (carrot). Seeds were obtained from Pieterpikzonen B.V. (Heerenveen, the Netherlands). Seeds were disinfested by washing them in 1% NaOCl for 1 min. followed by rinsing in running tap water for 5 min. The seeds were sown the same day.

For each treatment five 450-ml plastic pots were filled with the potting mixes. Pots received 10 (cucumber and pea) or 15 (tomato, sugar beet and carrot) seeds per pot. The pots were placed on saucers in a greenhouse compartment in five blocks with random placement of the pots within the blocks. The bioassay was performed in a well-controlled greenhouse compartment at 20°C and 60-80% relative humidity. The plants were rated for percent emergence and post-emergence damping-off. After 21 d the final readings were taken.

Disease suppression was calculated only for the infestation levels that led to 50-85% disease incidence in the non-amended controls. Percent disease suppression was calculated as: $100\% \times (\#\text{diseased plants}_{\text{non-amended control}} - \#\text{diseased plants}_{\text{compost-amended mix}}) / \#\text{diseased-plants}_{\text{non-amended control}}$. Diseased plants were defined as the number of sown plants – the number of healthy plants. No correlation was observed between the applied inoculum density for the different plants and disease suppression.

Rhizosphere samples of healthy plants from non-infested treatments in experiments 1 and 2 were taken for DGGE analysis at the end of the experiments (after 21 days). Roots were

shaken to remove loosely adhering soil, then three g (f.w.) of root material with adhering soil was placed into a test tube with 15 ml 120 mM phosphate-buffer (Na_2HPO_4/NaH_2PO_4), sonicated for 1 min, vortexed for 1 min and sonicated again for 1 min. The samples were stored in -20^{o}C till DNA extraction and PCR amplifications took place.

DNA extraction, PCR amplifications and DGGE

DGGE banding patterns were determined for rhizosphere microbial communities of all plant species × compost combinations (including the non-amended controls) of bioassays 1 and 2. DNA extraction was carried out with the MoBio Ultraclean soil DNA extraction kit (Biozym TC, Landgraaf, the Netherlands), according to the manufactures' protocol. PCR was performed in an PTC-100 thermal cycler (Mj Research, Inc., Tilburg, the Netherlands). Primers as listed in Table 3 were used for the specific amplication of 16S rDNA fragments of eubacteria and actinomycetes (Heuer et al., 1997) and 18S rDNA fragments of fungi (Smit et al., 1999; White et al., 1990).

PCR amplification of bacterial 16S rDNA genes was performed according to Heuer et al. (1997). For bacteria the reaction mixture (final volume 50 µl) consisted of 200 µM dNTP, 3.75 mM $MgCl_2$, 1x Stoffel buffer (Applied Biosystems, Foster City, USA), 0.4 µM primer F984GC , 0.4 µM primer R1378, 1.0% formamide, 2 ng μL^{-1} T4 gene 32 protein (Boehringer, Mannheim, Germany) and 0.1 U μl^{-1} Amplitaq Stoffel polymerase. 1.0 µl DNA extract was used as a template. For bacteria a touchdown PCR was performed, which included 4 min at 94°C followed by 36 cycles of 1 min at 94°C, 1 min starting at 60°C, and 2 min at 72°C. The annealing temperature was shifted from 60°C to 55°C during the first 10 cycles and remained at 55°C during the remaining 26 cycles. Final extension step was at 72°C for 10 min, followed by 10°C for 5 min.

For actinomycetes a nested PCR was performed. The primary PCR reaction mixture (final volume 25 µl) consisted of 200 µM dNTP, 3.75 mM $MgCl_2$, 1x stoffel buffer, 0.4 µM primer F243, 0.4 µM primer R1378, 1.0% formamide, 4 ng μl^{-1} T4 gene 32 protein and 0.2 U/µl Amplitaq Stoffel polymerase; the nested PCR mixture (final volume 50 µl) was similar

Table 3. Primers used in PCR reactions.

System	Primer	Sequence (5'->3')
Actinomycetes	F243	GGA TGA GCC CGC GGC CTA
Bacteria	F984GC	CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G AAC GCG AAG AAC CTT AC
Bacteria	R1378	CGG TGT GTA CAA GGC CCG GGA ACG
Fungi	ITS1F-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCT TGG TCA TTT AGA GGA AGT AA
Fungi	ITS2	GCT GCG TTC TTC ATC GAT GC
Fungi	ITS4	TCC TCC GCT TAT TGA TAT GC
Fungi	EF4	GGA AGG GRT GTA TTT ATT AG

to that of the bacteria PCR. 1.0 µl DNA extract was used as a template. For actinomycetes the primary touchdown PCR included 4 min at 94°C followed by 32 cycles of 1 min at 94°C, 1 min starting at 63°C, and 2 min at 72°C. The annealing temperature was shifted from 63°C to 58°C during the first 10 cycles and remained at 58°C during the remaining 22 cycles. Final extension step was at 72°C for 10 min, followed by 10°C for 5 min.

18S rDNA and ITS regions were PCR-amplified using the primers EF4 (Smit et al., 1999) and ITS4 (White et al., 1990). Polymerase chain reactions were carried out using 50 ml reaction volumes containing approximately 50 ng of template DNA, 20 pmol of each primer, 2 mM $MgCl_2$, 250 mM of each of dATP, dCTP, dGTP and dTTP, 10x buffer, 1 µl of T4gene32 protein (Roche Diagnostics, Mannheim, Germany) and 2.5 U of Expand High Fidelity DNA polymerase (Roche). Cycling parameters were 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 5 min. These products were diluted 10 times in water and 1 µl is then used as template in a nested PCR to generate products for DGGE analysis with the primers ITS1-F (Gardes and Bruns, 1993) with an GC clamp added (Muyzer et al., 1993) and ITS2 (White et al., 1990). PCR performed as described above, except that T4gene32 protein was omitted from the reactions.

Denaturating gradient gelelectrophoresis (DGGE) was performed with the phorU2 system (Ingeny, Leiden, the Netherlands). PCR products (15 to 20 ml) were applied directly onto 6% (wt/vol.) polyacrylamide gels in 0.53 TAE buffer (20mM Tris-acetate (pH 7.4), 10 mM sodium acetate, 0.5 mM disodium EDTA) containing a linear denaturing gradient (a 45-65% gradient (eubacteria and actinomycetes) or a 30-80% gradient (fungi) of urea and formamide). The gradients were formed with 6% (wt/vol.) acrylamide stock solutions that contained no denaturant and 100% denaturant (the 100% denaturant solution contained 7 M urea and 40% (vol./vol.) formamide deionized with AG501-X8 mixed-bed resin (Bio-Rad, Veenendaal, the Netherlands)). The gels were electrophoresed for 15 h at 60°C and 100V. After electrophoresis, the gels were stained for 30 min with SYBR Gold I nucleic acid gel stain (Molecular Probes Europe, Leiden, the Netherlands) and were photographed under UV light by using a SYBR Green gel stain photographic filter (Molecular Probes) and a Docugel V system apparatus (Biozym, Landgraaf, the Netherlands).

Organic matter stability

Organic matter stability was determined using the Oxitop® system (Reuschenbach et al., 2003). This system measures a pressure drop in the headspace, which can be directly related to O_2 consumption when CO_2 is trapped and nitrification is inhibited. The Oxitop system consists of a glass bottle (2 l) with a CO_2 trap (sodalime) in the head space. About 10 g (fresh weight) of compost material was sampled, precisely weighed and put in the bottle. Then 180 ml of demineralized water, 10 ml of pH 7 phosphate buffer (43.08 g l^{-1} KH_2PO_4, 88.86 g l^{-1} $Na_2HPO_4.2H_2O$, 1 l H_2O), 10 ml of a macronutrients solution (4.31 g l^{-1} NH_4Cl, 5.39 g l^{-1} $CaCl_2.2H_2O$, 4.31 g l^{-1} $MgSO_4.7H_2O$, 54 mg l^{-1} $FeCl_3.6H_2O$, 1 l H_20) and 0.2 ml of a micronutrients solution (2 g l^{-1} $FeCl_3.4H_2O$, 2 g l^{-1} $CoCl_2.6H_2O$, 0.5 g l^{-1} $MnCl_2.4H_2O$, 30 mg l^{-1} $CuCl_2.2H_2O$, 50 mg l^{-1} $ZnCl_2$, 50 mg l^{-1} H_3BO_3, 90 mg l^{-1} $(NH_4)_6Mo_7O_{24}.4H_2O$, 100 mg l^{-1}

$Na_2SeO_3.5H_2O$, 50 mg l^{-1} $NiCl_2.6H_2O$, 1 g l^{-1} EDTA, 1 ml l^{-1} 36% HCl, 0.5 g l^{-1} resazurin, 1000 ml H_2O) was added, the flask was shaken well, and the pH was checked to be between 6.9-7.1. After closure of the flasks with the Oxitop® measuring head, they were incubated in a shaking incubator at 30°C for one week. The measuring head recorded change of pressure (once per h). Oxygen uptake rate (OUR; mg O_2 kg^{-1} dry substrate d^{-1}) was measured as: $OUR = M_{O2} \times (V_t - V_l) \times \Delta p_{O2} / (R \times T_m \times m)$, where M_{O2} = molecular weight of O_2 (= 32000 mg mol^{-1}), V_t = bottle volume (ml), V_l = sample volume (ml), Δp_{O2} = change in O_2 partial pressure (mbar), R = gas constant (= 83.144 l mbar mol^{-1} K^{-1}), T_m = temperature (= 303.14 K), m = dry weight of sample (kg).

Statistical analysis

Statistical analysis of disease suppression data was performed on square-root and arcsine transformed data. Banding pattern analysis and comparison of gels was performed using GelcomparII software (version 4.5; Applied Maths, Woluwe, Belgium). Gels were analyzed using the instructions in the manual. Each gel contained 4 marker lanes for reference purposes. Distance matrixes, based on the presence/absents of bands, were calculated using Pearson correlation and clustering was performed using the UPGMA algorithm. Disease suppression data and similarities were subjected to analysis of variance and means were separated according to Duncan's multiple range test ($P < 0.05$). All statistical analyses were carried out with SAS version 9.1 (SAS Institute Inc., Cary, USA).

Results

Disease suppression

Significant disease suppression ($P < 0.05$) was found in 17 out of 30 compost-plant species combinations evaluated (ranging from 18.2 to 99%, median 45.9%) (Table 4). No significant disease stimulation was found. Analysis of variance indicated strongly significant main effects of plant and compost (both $P < 0.0001$) and a significant ($P = 0.0033$) compost × plant interaction (Table 5). For cucumber only 1 (out of 9) composts induced significant disease suppression. For carrot, sugar beet and pea significant disease suppression was obtained for 2 and 3 (out of 5) and 5 (out of 9) composts respectively (Table 4). For tomato, disease suppression occurred with all composts (Table 4). Disease suppression induced by the various composts showed high positive correlations for carrot and pea (r = 0.80); carrot and sugar beet (r = 0.74); and pea and sugar beet (r = 0.92). Average disease suppression was significantly ($P < 0.05$) lower for cucumber (2.2%; experiments 2 and 3) than for tomato (49.6%) and carrot (26.9%). Highest average disease suppression was found for tomato.

None of the composts showed disease suppression for all the tested plants (Table 4). Composts originating from urban biowaste (composts d, e, and g) showed significantly ($P < 0.05$) higher disease suppression (18.7, 33.6, and 34.1% respectively) than the green waste composts (composts f, h, and i; 6.3, 11.8, and 5.4% respectively).

Table 4. Percentage of suppression of damping-off (%) caused by *Pythium ultimum* for 5 plant species, induced by 9 different composts in a peat-substrate/compost mix (80/20, vol./vol.) compared to a non-amended peat substrate.

Exp.	Compost sample	Tomato				Carrot				Pea				Sugar beet				Cucumber				Compost average	
1	a[a]	n.d.[b]				n.d.				77.4	A[c]	a[d]	*[e]	n.d.				99.0	A	a	*		
1	b	n.d.				n.d.				70.6	A	a	*	n.d.				-0.1	BC	b			
1	c	n.d.				n.d.				27.8	BC	a	*	n.d.				0.0	BC	a			
2	d	75.5	A	a	*	9.8	B	b		10.0	CD	b		19.1	AB	b	*	4.7	BC	b		18.7[f]	AB
2	e	63.3	AB	a	*	66.1	A	a	*	19.5	C	b	*	18.2	AB	b	*	16.8	B	b		33.6	A
2	f	43.3	AB	a	*	12.1	B	ab		0.7	D	b		2.9	B	b		0.0	BC	b		6.3	B
3	g	32.0	B	a	*	66.1	A	a	*	59.3	AB	a	*	38.1	A	a	*	1.8	BC	b		34.1	A
3	h	37.9	B	a	*	16.8	AB	a		9.1	CD	a		3.4	B	a		3.3	BC	a		11.8	B
3	i	48.4	AB	a	*	12.1	B	ab		1.1	D	b		6.8	B	b		-6.8	C	c		5.4	B

[a] For details, see Table 2.
[b] n.d. = not determined.
[c] Significant differences ($P < 0.05$) between composts are indicated with upper case letters. Significance is based on analysis of variance, means were separated according to Duncan's multiple range test.
[d] Significant differences ($P < 0.05$) between plants are indicated with lower case letters. Significance is based on analysis of variance, means were separated according to Duncan's multiple range test.
[e] An asterisk indicates significant disease suppression ($P < 0.05$). Significance is based on analysis of variance, means were separated according to Duncan's multiple range test ($P < 0.05$).
[f] Averages per compost were calculated using square root-arcsine transformed data and were back-transformed to average disease suppression rates.

Table 5. Analysis of variance of disease suppression data for 5 plants and 9 composts[a].

Effect	df	Mean Square	F-value	Pr > F
Plant species	4	0.176	22.05	< 0.0001
Compost sample	8	0.096	12.06	< 0.0001
Interaction	23	0.017	2.16	0.0033

[a] Analysis was carried out with square-root, arcsine transformed data.

DGGE patterns of microbial rhizosphere communities

The average similarities of the 16S-rDNA bacterial communities of the rhizosphere samples from the compost-amended treatments compared with the non-amended treatments were relatively high for cucumber (83%; exp. 2), while those for carrot and tomato were significantly lower (47-53%) (Table 6). We interprete these results as that bacterial rhizosphere communities (as determined by DGGE) of cucumber are relatively more influenced by the plant than by the type of compost, while this is less so for carrot and tomato. Pea and sugar beet take an intermediate position, with 24 and 21 percentage points lower average similarities than cucumber. For composts d and e, but not compost f, the bacterial rhizosphere composition based on DGGE patterns clustered for all hosts except cucumber and pea (Fig. 1), indicating that for these compost/host combinations, composts performed a strong influence on the bacterial rhizosphere composition, while compost f was less influential (Fig. 1). Similar clusterings were

Table 6. Percentage of similarity of the DGGE-patterns of the 16S-rDNA bacterial rhizosphere communities from the compost-amended peat mix and the non-amended control.

		Compost											
Experiment	Plant species	a		b		c		Average similarity			stdev		
1	Pea	62		71		83		72	a[a]		11		
1	Cucumber	77		73		90		80	a		9		
1	Average	70	A[b]	72	A	87	A	76			-		
		d		e		f							
2	Tomato	52		62		44		53	b		9		
2	Carrot	35		43		63		47	b		14		
2	Pea	71		66		40		59	ab		16		
2	Sugar beet	54		50		80		62	ab		16		
2	Cucumber	87		90		74		83	a		8		
2	Average	60	A	62	A	60	A	63			14		

[a] Significant differences ($P < 0.05$) between the average similarities per plant species are indicated with lower case letters. Significance is based on analysis of variance; means were separated according to Duncan's multiple range test.

[b] Significant differences ($P < 0.05$) between the average similarities per compost are indicated with upper case letters. Significance is based on analysis of variance; means were separated according to Duncan's multiple range test.

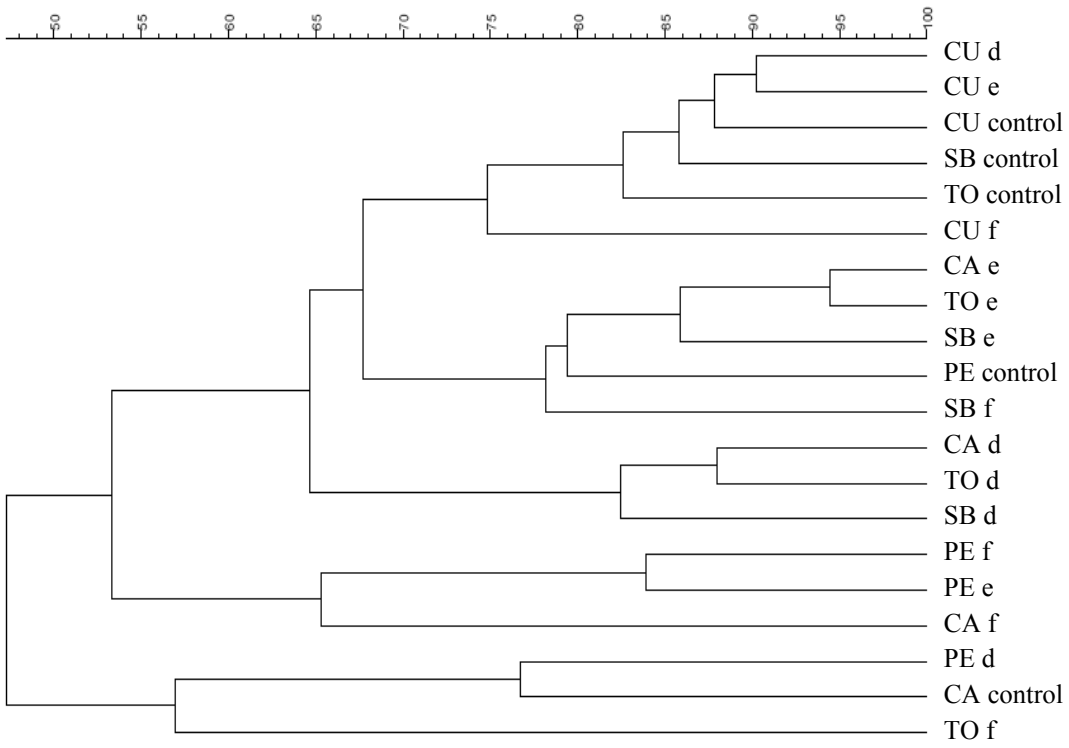

Figure 1. Dendrograms based on presence/absence of bands in the gels of 16S-rDNA bacterial communities in rhizosphere samples of five plant species (CA = carrot, CU = cucumber, PE = pea, SB = sugar beet, TO = tomato), 3 composts (d, e, f) and a non-amended control (control). Samples were collected 21 days after sowing from plants not affected by *Pythium ultimum*. Data concern experiment 2.

obtained for the actinomycete and fungal DGGEs (results not shown). If similarities in DGGE patterns are relatively high among rhizospheres originating from different plant species growing on substrates amended with the same compost, then DGGE patterns are likely to be more determined by the compost than by plant species. For compost f, compost-amended peat mixes showed a lower ($P < 0.05$) average similarity for bacterial DGGEs between all various hosts tested than composts d and e, but a higher (significant only for compost e, $P < 0.05$) average similarity for fungal DGGEs (Table 7).

Correlation of DGGE banding pattern, oxygen uptake rate and organic matter content with disease suppression

If the similarity between the rhizosphere bacterial DGGE banding patterns of a compost-amended mix and the non-amended peat was high (> 70%), low disease suppression rates (< 20%) were observed (Fig. 2). If these similarity values were lower, low as well as high disease suppression rates were observed, depending on host species and compost type (Fig. 2). Compost treatments that had high bacterial DGGE similarities between the hosts tested (i.e., composts e and d) showed higher disease suppression than those that had low

Table 7. Average percentage and standard deviation of similarities of the DGGE-patterns of the 16S-rDNA (bacteria), 16S-rDNA (actinomycetes), and 18S-rDNA (fungi) rhizosphere communities of each compost tested with 5 plant species.

Compost code	Bacteria similarity average		stdev	Actinomycete similarity average		stdev	Fungal similarity average		stdev
d	78[a]	a[b]	7	50	a	43	51	bc	19
e	75	a	10	58	a	18	48	c	19
f	52	b	15	56	a	12	66	ab	12
non-amended control	60	b	25	63	a	17	77	a	15
Average	66	A[c]	12	57	B	6	61	AB	13

[a] The numbers indicate average similarities between all possible plant combinations

[b] Significant differences ($P < 0.05$) between the average similarities per plant or per compost are indicated with lower case letters. Significance is based on analysis of variance, means were separated according to the Duncan's multiple range test.

[c] Significant differences ($P < 0.05$) between the average similarities per group of microorganisms are indicated with upper case. Significance is based on analysis of variance, means were separated according to the Duncan's multiple range test.

similarities (compost f and the non-amended control; Fig. 3). Neither the actinomycete nor the fungal DGGEs showed such relations with disease suppression.

Oxygen Uptake Rate (OUR) of the composts did not show correlations with disease suppression, neither for the disease suppression rates of all plants together, nor for the disease suppression rates per individual plant species. The similarity of bacterial DGGE banding patterns of compost-amended mixes averaged over plant species (Table 7) showed positive trends with OUR, organic matter content, total C, total N, pH and EC and negative trends with total C/total N ratio and maturity of the various pure composts (data not shown). These trends were not significant due to the limited number of observations (3). Negative correlations were

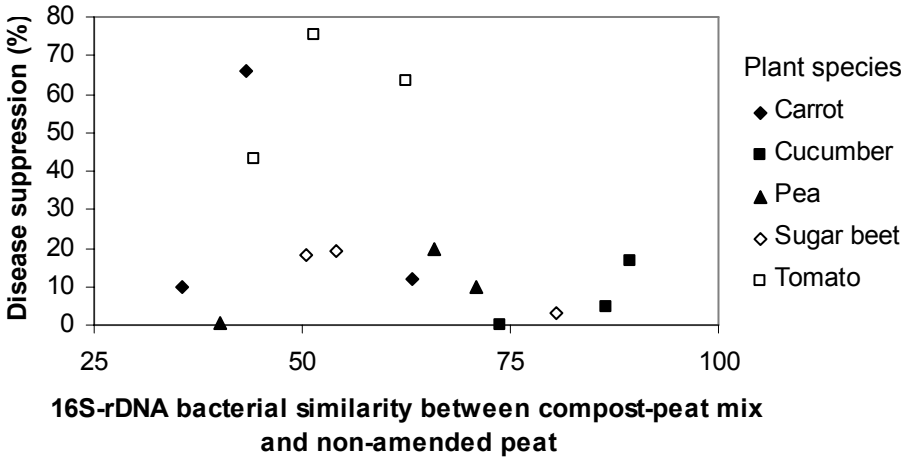

Figure 2. Correlation between similarities of rhizosphere DGGE patterns based on 16S-rDNA bacterial communities of plants grown in compost-peat mixes or non-amended peat and disease suppression. Data concern experiment 2.

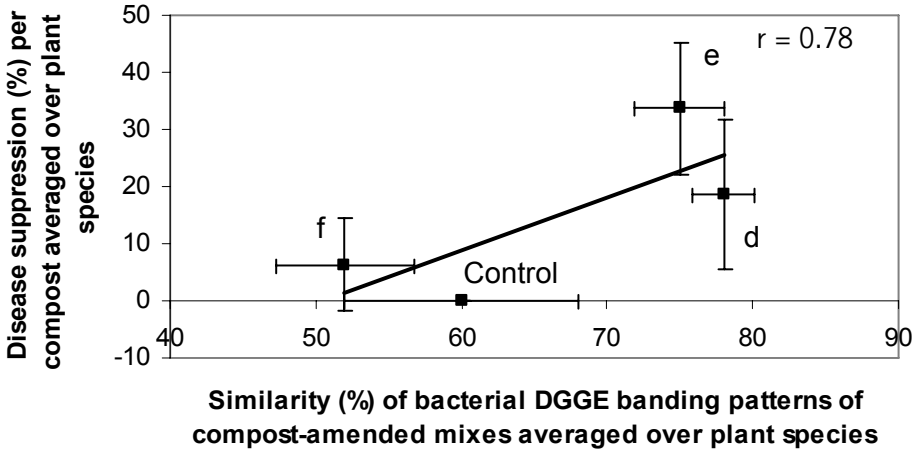

Figure 3. Correlation between disease suppression of compost and their influence on the bacterial community of the rhizosphere of the plant based on the results of experiment 2. Of experiments 1 and 3 no similarities per compost could be calculated. For actinomycetes, no significant differences in similarities were observed. Error bars indicate standard error. Codes refer to composts as explained in Table 2.

found between disease suppression of pea and sugar beet and the total C/total N ratio in the pure compost (r = -0.79, P = 0.05 and r = -0.91, P = 0.01 respectively) and positive correlations were found between disease suppression of sugar beet and EC (r = 0.89, P = 0.02) and total N (r = 0.84, P = 0.03). Composts originating from urban biowaste (maturity 2–8 weeks) showed significantly higher ($P < 0.05$) total N concentrations and significantly lower ($P < 0.05$) total C/total N ratios than composts originating from green waste (maturity 12-22 weeks).

Discussion

This study shows that results of assays quantifying disease suppression of Pythium damping-off on one host do not necessarily predict disease suppression of the same pathogen on another host. For example, compost sample i showed significant disease suppression only for tomato (48%), while it was insignificant for the other hosts (<13%). Thus, we found a significant interaction between plant species and compost. So, for example, compost samples g and i were not significantly different in disease suppression when tomato was used as test plant (disease suppression levels of 32 and 48% respectively), but for carrot, pea and sugar beet disease suppression induced by compost g was significantly higher than that induced by compost i (38-66 and 1.1-12% respectively). Although these results seem to hamper the choice of a compost that is generally applicable as an amendment that can enhance disease suppressiveness of growth substrates, it should be borne in mind that 57% of the bioassays showed significant disease suppression and there was no compost significantly stimulating disease. Thus, there seems to be no risk associated with the usage of compost, while replacing

peat with compost would reduce the exploitation of non-renewable peat reserves and contribute to the recycling of waste (Termorshuizen et al., 2004).

Termorshuizen et al. (2006) showed a significant compost × pathosystem interaction for disease suppressiveness of 18 compost-amended substrates against 7 pathogens, indicating that disease suppressiveness of a compost is not only host-, but also pathogen-dependent. These and our current results suggest that disease suppression induced by compost is a process that is mediated by a 3-way interaction involving compost sample, plant species, and pathogen. If this is true, then disease suppression induced by organic amendments should be optimized for specific conditions of pathogen, plant species, and type of amendment.

The genetic and functional diversity of the rhizosphere community depends on plant species through the quantity and quality of root exudation and rhizodeposition (Ferguson and Menge, 1982; Lemanceau et al., 1995; Brimecombe et al., 2001; Jaeger et al., 1999; Bergsma-Vlami, 2005) as well as on edaphic factors (Garbeva et al., 2004), since most, if not all, rhizosphere organisms originate from the bulk soil (Costa et al., 2006). Several edaphic factors related to soil type are known to have a strong effect on the microbial rhizosphere composition such as pH (Marschner et al., 2005a) and soil texture (Groffmann et al., 1996). Also root age for perennial plants and seasonality have been reported to affect microbial rhizosphere DGGEs (Marschner et al., 2005b). Whether a plant species has a stronger effect on the microbial rhizosphere composition than bulk soil factors depends on the specific characteristics of both factors (Latour, 1996; Berg et al., 2005; 2006). So, for example for maize (Chiarini et al., 1998) and *Carex arenaria* (Ridder-Duine et al., 2005), bulk soil effects were reported to dominate over plant effects and Kennedy et al. (2004) reported that for 7 perennial grassland species, lime and nitrogen amendment had a significantly greater effect on bacterial rhizosphere ribotypes than plant species. Tscherko et al. (2004) concluded that the major determinants of the rhizosphere microflora of *Poa alpina* vary along a successional gradient. In the pioneer stage it was primarily determined by the soil environment and under more favourable environmental conditions the host plant selected for a specific microbial community.

The composition of microbial rhizosphere communities of plants growing in compost-amended substrates is determined by a balance between the 'strengths' of the influence of the compost and that of the plant. Assuming that disease suppression induced by compost is rhizosphere-mediated, a compost added to a peat substrate that has no influence on the rhizosphere community would not induce disease suppression. If, on the other hand, compost amendment contributes to the composition of the microbial rhizosphere community, disease suppression would be possible. In their review Garbeva et al. (2004) hypothesized 'strong' and 'weak' plant species and types of soil with respect to their influence on the microbial rhizosphere communities. According to their model, wheat is for example considered a 'strong' and tomato a 'weak' plant species and sand and clay 'weak' and 'strong' soil types respectively. Applying this terminology to this concept, with respect to bacterial rhizosphere communities our results can be summarized as follows: 'weak' plant species: carrot and tomato; 'strong' plant species: cucumber; 'weak' compost: f; and 'strong' composts: d and e. Highest disease suppression would be predicted by a combination of 'weak' plants and 'strong' composts. This would fit in the 'root camouflage' concept introduced by Gilbert et al.

(1994), who suggested that rhizosphere microbial communities that are more similar to the microbial community of the surrounding bulk soil may be less attractive to pathogens. Likely in a nutrient-poor medium such as peat the rhizosphere effect is lessened after compost amendment and especially after amendment of 'strong' composts. In this study, 'strong' composts were young vegetable, fruit and garden (VGF) composts with high values of OUR, organic matter content, total C, total N, pH and EC and low total C/total N ratio.

Suppression of damping-off caused by *P. ultimum* has been shown to be stimulated by a high microbial activity of the substrate (Chen et al., 1988; Mandelbaum and Hadar, 1990; Boehm et al., 1993; Scheuerell et al., 2005; Veeken et al., 2005) and for Pythium bulb rot it has been shown that different microbial communities can lead to similar suppression of Pythium bulb rot (Kowalchuk et al., 2003), although competition for carbon in root exudates may not be the main mechanism explaining disease suppression (Os and Ginkel, 2001). McKellar and Nelson (2003) obtained indications that suppression of *P. ultimum* is aspecific, depending on microbial consortia able to quickly consume linoleic acid rather than on individual species. Buyer et al. (2002) reported weaker rhizosphere effects on fungal than on bacterial communities in substrate utilization studies. In our study, differences in disease suppression of *P. ultimum* for the different plant species could be well explained by bacterial compositions of the rhizosphere but not by actinomycete and fungal compositions of the rhizosphere.

In this study, we used DGGE banding pattern as a representation of microbial composition. With this technique, only dominant rDNAs are detected and one band position is not necessarily linked to a single species (Muyzer et al., 1993). Furthermore, the quality of DNA extraction depends on edaphic factors. Therefore, DGGE banding patterns do not reflect the total microbial community. Still, we show here that DGGE-analysis can yield ecologically meaningful results.

In conclusion, we demonstrated that disease suppression of *P. ultimum* through compost amendment is determined by interacting plant - compost effects on the rhizosphere bacterial community, but not, or to a lesser extent, on the actinomycete or fungal community. For prediction of disease suppression of Pythium damping-off the rhizosphere effects of both the host and the amended substrate should be investigated. Our results could be important in defining strategies to find means of prediction of disease suppressiveness of compost.

Acknowledgements

This study was undertaken as part of the first author's PhD program and was funded by the EU project QLK5-CT-01442. We thank A.H.C. van Bruggen and O. de Vos for critically reading the manuscript, L. van Overbeek and A. Speksnijder (Plant Research International, Wageningen, the Netherlands) for discussions and R. Pastoor and H. Halm for technical assistance.

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Chapter 5

Storage method affects disease suppression of flax wilt induced by composts

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Soil Biology and Biochemistry, submitted

Abstract

A factor constraining compost research is that repetition of experiments with a similar batch of compost is impossible since storage affects the organic material including the microbial communities. The objective of this study was to investigate the effects of 3 types of storage (dry at +20°C; freezing at -20°C and cooling at +4°C) of 8 composts for 12 wk on the suppression of *Fusarium oxysporum* f. sp. *lini* induced by mixes of composts with peat substrate (20/80%, vol./vol.). A significant ($P < 0.0001$) storage method × compost interaction was found with respect to suppression of fusarium wilt of flax, indicating that the effect of storage type on disease suppression is compost-dependent. For 7 composts storage had no (13 cases) or a significantly positive effect (8 cases) on disease suppression and for 1 compost there was a significant negative effect of storage on disease suppression. Significant changes in microbial activity and 16S-rDNA DGGE banding patterns of the composts were observed as a result of all tested ways of storage and these changes could be related to changes in disease suppression: relatively strong changes in microbial activity and bacterial composition due to storage had a relatively strong effect on disease suppression. The cool storage treatment (4°C) resulted in the least deviation in disease suppression from the fresh compost, although the effect of freezing gave the most reliable results with the lowest standard deviation.

Introduction

Composts have been shown to enhance disease suppression against soilborne plant pathogens in peat or soil mixes (Scheuerell et al., 2005; Termorshuizen et al., 2006; reviewed by Hoitink and Boehm, 1999). A factor constraining compost research is that repetition of experiments with the same batch of compost is difficult since during storage further

decomposition of the organic material always occurs. Storage is often necessary if different composts are to be compared, since they usually cannot be collected within a single day. This topic is also relevant for the predictability of disease suppressiveness of commercial composts that are stored in bags until they are used. Storage of compost implies phenomena like drying-rewetting, freezing-thawing, or cooling-returning to room temperature, depending on the type of storage. These have been studied for soil samples (e.g. Pesaro et al., 2004; Sharma et al. 2006; Koponen et al., 2006), but much less so for compost (Mondini et al., 2002; Yang et al., 2004).

Microbial characteristics of compost or soil change as a result of storage. Microbial biomass generally declines during drying, freezing and cooling and microbial activity declines sharply. Only during cooling microbial activity continues to some extent (Jenkinson and Powlson, 1976; Butler et al., 2001). After revival through rewetting, thawing or returning to room temperature, typically the microbial biomass is reduced for a prolonged period of time (Pesaro et al., 2004; Stenberg et al., 1998; Schimel and Clein, 1996). Dead cells are mineralized and non-biomass nutrient sources become available as a result of physical disruption of the substrate, causing a temporary strong increase in microbial activity (Gestel et al., 1993; Scheu and Parkinson, 1994; Pesaro et al., 2004). For example, in experiments on soil, freezing-thawing led to a 47% increase of substrate-induced respiration (Pesaro et al., 2003) after which it returned to reference levels within 10 days, but microbial biomass, as estimated by direct cell counts and soil DNA content, was reduced by 24-33 and 15-23% respectively until at least 42 days after thawing. Such patterns of decrease of microbial biomass during storage and increase of microbial activity after storage have been reported for drying-rewetting (Bottner, 1985; Gestel et al., 1993) and freezing-thawing (Stenberg et al. 1998; Pesaro, 2003; Schimel and Clein, 1996), although in few cases complete recovery of microbial biomass has been reported (Stenrød et al., 2005). For cooling-returning to room temperature a decrease of microbial biomass generally coincides with a decrease of microbial activity (Stenberg et al., 1998; Butler et al. 2001; Jenkinson and Powlson, 1976).

Typically the microbial communities show a significant shift in their composition due to storage. The easily available energy and nutrient sources originating from dead cells and non-biomass sources lead to an increased microbial activity by primarily r-strategists (Gestel et al., 1993, Scheu and Parkinson, 1994; Pesaro et al., 2004). Thus, shifts in microbial composition take place as a result of changes in the substrate and because some species may be more sensitive to stress invoked during drying-rewetting (Scheu and Parkinson, 1994; Pesaro et al., 2004) or freezing-thawing (Sharma et al., 2006; Yang et al., 2004) than others. The active microbial biomass is generally more affected by storage than the dormant component (Bottner, 1985, Gestel et al., 1993). Microbial shifts after revival of stored substrates may continue for a prolonged period of time. For example, Scheu and Parkinson (1994) reported an increase in bacterial biomass during the first 10 days after drying-rewetting, followed by a gradual shift to fungal dominance up to 40 days after storage. Such changes have also been reported as a result of freezing-thawing (Sharma et al., 2006; Yang et al., 2004), although not consistently (Pesaro et al., 2003; Koponen et al., 2006).

As changes in the microbial community as a result of storage are likely to happen and the disease suppressive properties of compost/peat mixes are, at least in part, driven by its

microbial activity and composition (Hoitink and Boehm, 1999), the question arises to what extent compost storage affects disease suppression. The goal of this research was to test the effect of 3 commonly used storage methods, drying, freezing, and cooling, on the disease suppressive properties of 8 composts with respect to Fusarium wilt of flax. Compost-induced suppression of Fusarium wilt of flax, caused by *Fusarium oxysporum* f. sp. *lini*, has been reported by several researchers for a wide range of different composts (Serra-Wittling et al., 1996; Cotxarrera et al., 2002; El-Masry et al. 2002; Borrero et al., 2004; Termorshuizen et al., 2006) and is, at least partly, competition-based (Borrero et al., 2004; Serra-Witling et al., 1996; Termorshuizen et al., 2006). Basal respiration of fresh and revived composts was determined as well as the composition of the dominant 16S-rDNA community using DGGE in order to gain insight in possible causes of changes in disease suppression.

Material and methods

Collection and storage of composts

Composts were collected from six different composting sites (Table 1), sieved over a 10-mm screen and brought to 50% water holding capacity (0.40 g water/g f.w.) using tap water. These composts were tested for disease suppression 2 d after collection and for basal respiration after an incubation period of 11 d as described below. The remainder of the composts was put in 3-l plastic bags. The bags were loosely closed to allow oxygen exchange. During 12 wk, the following three storage treatments were carried out: (1) cooling: +4°C

Table 1. Origin of the composts, used in disease suppression experiments.

Site	Compost sample	Disease suppression experiment	Compost age (wk)[a]	Composting method	Raw materials
1	a	1	2	Static indoors pile	Organic household waste (vegetable, fruit and garden waste)
	b		8		
2	c	1	10-12	Static outdoors pile	Leaves and wood trimmings
3	d	1	10-12	Static outdoors pile	Leaves and wood trimmings, manure, clay
4	e	2	2	Tunnel indoors	Organic household waste (vegetable, fruit and garden waste)
	f		8		
5	g	2	10-12	Tunnel indoors	Organic household waste (vegetable, fruit and garden waste)
6	h	2	10-12	Static outdoors pile	Leaves and wood trimmings

[a] Compost age at time of sampling.

storage, (2) freezing: –20°C storage, and (3) drying: storage at 20°C after drying the composts for 24 h at 60°C. At the end of the storage incubation, frozen and cooled samples were brought back to room temperature and all plastic bags were checked for their moisture content and wetted to 50% water holding capacity (0.40 g water g^{-1} f.w.) if needed.

Chemical analyses

Air-dried pure compost samples (2 g, sieved over a 2 mm sieve), fresh or stored, were added to 100 ml 0.01 M $CaCl_2$ solution and shaken for 2 h. Measurements of pH and EC were carried out with a pH/mV meter (InoLab, Germany). Organic matter content of the pure compost samples was determined by loss-on-ignition. The organic matter of the samples was assessed gravimetrically by dry combustion of the organic material in a furnace at 500-550°C. Total N and C were measured with a Fisons Type EA 1108 Element Analyzer (Milan, Italy) according to the method of Dumas (Anonymous, 1997).

Microbial activity

Plastic bags with fresh compost or compost that was rewetted or returned to room temperature were placed for 11 d at 20°C to restore microbial activity. Basal respiration of the composts was determined with an automated system in which a continuous air flow of 50 ml min^{-1} was led over 30.0 g f.w. of compost in glass tubes (length 24 cm, diam. 3.5 cm) incubated at 20 ºC for 24 h. The CO_2-concentration in this air stream was measured by means of a computer-controlled switching device and an infrared CO_2-analyser (ADC 7000, Analytical Development Corporation, Hoddesdon, UK) which allowed hourly measurements. For calculation of the basal respiration the readings of the first ten h of incubation were omitted. Basal respiration was expressed as μg CO_2 g^{-1} d.w. substrate h^{-1} and determined in triplicate for all compost treatments.

Bioassays

Disease suppression of the fresh and the differently stored composts was determined for the *Fusarium oxysporum* f. sp. *lini* (Fol) / flax pathosystem. Talcum inoculum of Fol (isolate Foln3, kindly provided by P. Lemanceau, INRA, Dijon, France) was produced on a malt extract broth (Blok et al., 2000). Erlenmeyer flasks with malt extract broth were autoclaved (121°C, 20 min) and closed with a cotton plug, inoculated with three plugs of malt extract agar containing growing mycelium of Fol, and incubated on a shaking device for 7 d at 25°C. Fungal cultures were comminuted with a blender (1 min at high speed) and growing medium was removed by centrifuging for 30 min at 4000 rpm. The fungal biomass was resuspended in sterile distilled water and was added to talcum powder (2:1, wt:wt). This mix was air-dried for 3 wk under sterile conditions to allow the formation of microchlamydospores. The air-dried inoculum was passed through a sieve with 0.36 mm mesh size. Inoculum was stored at 4°C until use. The density of the inoculum thus obtained was about 10^7 propagules g^{-1} talcum.

One wk before sowing (2 d after collection of the fresh samples and 1 d after rewetting/thawing/returning to room temperature of the stored samples), compost/peat-based (Lentse potgrond no. 4, Lent, the Netherlands) substrate mixes (20/80%, vol./vol.) were made. Water was added to the mixes to reach a pF of 1.9. One day before sowing the inoculum was thoroughly mixed with the substrate mixes in densities of 0, 10^4, and 10^5 propagules ml^{-1} mix. The control consisted of a substrate of 100% potting soil (Lentse potgrond no. 4, Lent, the Netherlands). Seeds of flax (*Linum usitatissimum* L. cv. Opaline; Institute Technique du Lin, Paris, France) were disinfested by washing them for 1 min in 1% NaOHCl followed by thorough rinsing in running tap water for 5 min. The disinfested seeds were pre-germinated for 2 d at 20°C in the light. Sowing took place 1 d after filling the pots. In each pot, 13 seeds were sown 7 d after preparing the substrate mixes, and 10 d after sowing the number of seedlings was reduced to 10. The pots were placed on saucers in the greenhouse (20°C, 60-80% RH) in a randomized blocks design (5 replicates per treatment). Pots were watered daily with a soft water stream on the pot.

Disease severity of the flax plants was recorded twice a week from 17 to 48 d after sowing. Rating took place using the following index: 1 = < 10% yellow leaves; 2 = 10-40% yellow leaves, < 10% wilted leaves; 3 = 40-75% yellow leaves, 10-40% folded and dead leaves; 4 = almost dead plant; 5 = dead plant. The Area-Under-the-Disease-Progress-Curve (AUDPC; Campbell and Madden, 1990) was calculated per pot and the percentage disease suppression was calculated as: $100\% - 100\% \times (AUDPC_{\text{compost-amended potting mix}} / AUDPC_{\text{non-amended potting mix}})$.

Sample collection, DNA extraction and PCR amplifications

After the differential storage treatments for 12 wks and an incubation period of 11 d at 20°C as described above, samples of 5 g were collected and stored at -20°C before DNA extraction took place 12 wk later. DNA extraction was carried out with the MoBio Ultraclean soil DNA extraction kit (Biozym TC, Landgraaf, the Netherlands), in accordance with the protocol furnished by the manufacturer except that an extra glas bead beating step during 30 sec was performed using a Ribolyser (Hybaid, Middlesex, United Kingdom). PCR was performed in a PTC-100 thermal cycler (Mj Research, Inc., Tilburg, the Netherlands).

PCR amplification of bacterial 16S rDNA genes was performed according to Heuer et al. (1997). Primers, F984GC (CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G AAC GCG AAG AAC CTT AC; 5'→3') and R1378 (CGG TGT GTA CAA GGC CCG GGA ACG; 5'→3'), were used for the specific amplication of 16S rDNA fragments of eubacteria (Heuer et al., 1997). The reaction mix (final vol. 50 µl) consisted of 200 µM dNTP, 3.75 mM $MgCl_2$, 1x Stoffel buffer (Applied Biosystems, Foster City, USA), 0.4 µM primer F984GC, 0.4 µM primer R1378, 1.0% formamide, 2 ng μl^{-1} T4 gene 32 protein (Boehringer, Mannheim, Germany) and 0.1 U μl^{-1} Amplitaq Stoffel polymerase. 1.0 µl DNA extract was used as a template. A touchdown PCR was performed and included 4 min at 94°C followed by 36 cycles of 1 min at 94°C, 1 min starting at 60°C, and 2 min at 72°C. The annealing temperature was shifted from 60 to 55°C during the first 10 cycles and remained at 55°C during the remaining 26 cycles. Final extension step was at 72°C for 10 min, followed

by 10°C for 5 min.

Denaturating gradient gelelectrophoresis (DGGE) was performed with the phorU2 system (Ingeny, Leiden, the Netherlands). PCR products (15 to 20 ml) were applied directly onto 6% (wt/vol.) polyacrylamide gels in 0.53 TAE buffer (20mM Tris-acetate (pH 7.4), 10 mM sodium acetate, 0.5 mM disodium EDTA) containing a linear denaturing gradient (a 45-65% gradient (eubacteria and actinomycetes) or a 30-80% gradient (fungi) of urea and formamide). The gradients were formed with 6% (wt/vol.) acrylamide stock solutions that contained no denaturant and 100% denaturant (the 100% denaturant solution contained 7 M urea and 40% (vol./vol.) formamide deionized with AG501-X8 mixed-bed resin (Bio-Rad, Veenendaal, the Netherlands)). The gels were electrophoresed for 15 h at 60°C and 100V. After electrophoresis, the gels were stained for 30 min with SYBR Gold I nucleic acid gel stain (Molecular Probes Europe, Leiden, the Netherlands) and were photographed under UV light by using a SYBR Green gel stain photographic filter (Molecular Probes) and a Docugel V system apparatus (Biozym, Landgraaf, the Netherlands).

Statistical analysis

Banding pattern analysis and comparison of gels was processed by GelcomparII software (version 4.5; Applied Maths, Woluwe, Belgium). Gels were analysed using the instructions from the manual. Each gel contained 4 marker lanes for reference purposes. Background correction was adapted to gel quality. Identification of bands was performed with settings of 5%. Correspondence of bands between different samples was performed with 1% dynamic range settings. Distance matrices, based on the presence/absence of bands, were calculated using Pearson correlation and clustering was performed using the UPGMA algorithm. Statistical analysis of disease suppression was carried out by analysis of variance using untransformed AUDPC-data since the standard variations were homogeneous among treatments. Means were separated according to the Duncan's multiple range test ($P < 0.05$). All statistical analyses were carried out with SAS version 9.1 (SAS Institute Inc., Cary, USA).

Results

Effects of storage on disease suppression

Out of the 32 disease suppression assays performed, 24 (75%) showed significant ($P < 0.05$) disease suppression in the compost-amended treatments. Significant disease stimulation in the compost-amended treatments did not occur. Before storage, composts a, e, f, g and h showed significant disease suppression (Fig. 1; Table 2). Disease suppression was significantly affected by storage method ($P = 0.0292$) and compost sample ($P < 0.0001$) as well as by their interaction ($P < 0.0001$; Table 3), indicating that effect of storage on disease suppression is compost-dependent. Storage did not affect disease suppression (13 cases) or had a significantly positive effect on disease suppression (8 cases) (Table 2), except for compost h, where all storage methods eliminated the 24% disease suppression observed for

the fresh compost. For compost a, all storage methods significantly ($P < 0.05$) increased disease suppression. For composts e and g there was no effect of any type of storage on disease suppression.

Considering all composts, the cooling storage resulted in the least deviation in disease suppression from the fresh compost, although the effect of freezing gave the most reliable results with the lowest standard deviation (change in disease suppression by 4.4 ± 21.0 and 10.5 ± 15.6 percentage points for cooling and freezing respectively). Drying composts showed an intermediate change in disease suppression with a high standard deviation (6.6 ± 27.9 percentage points).

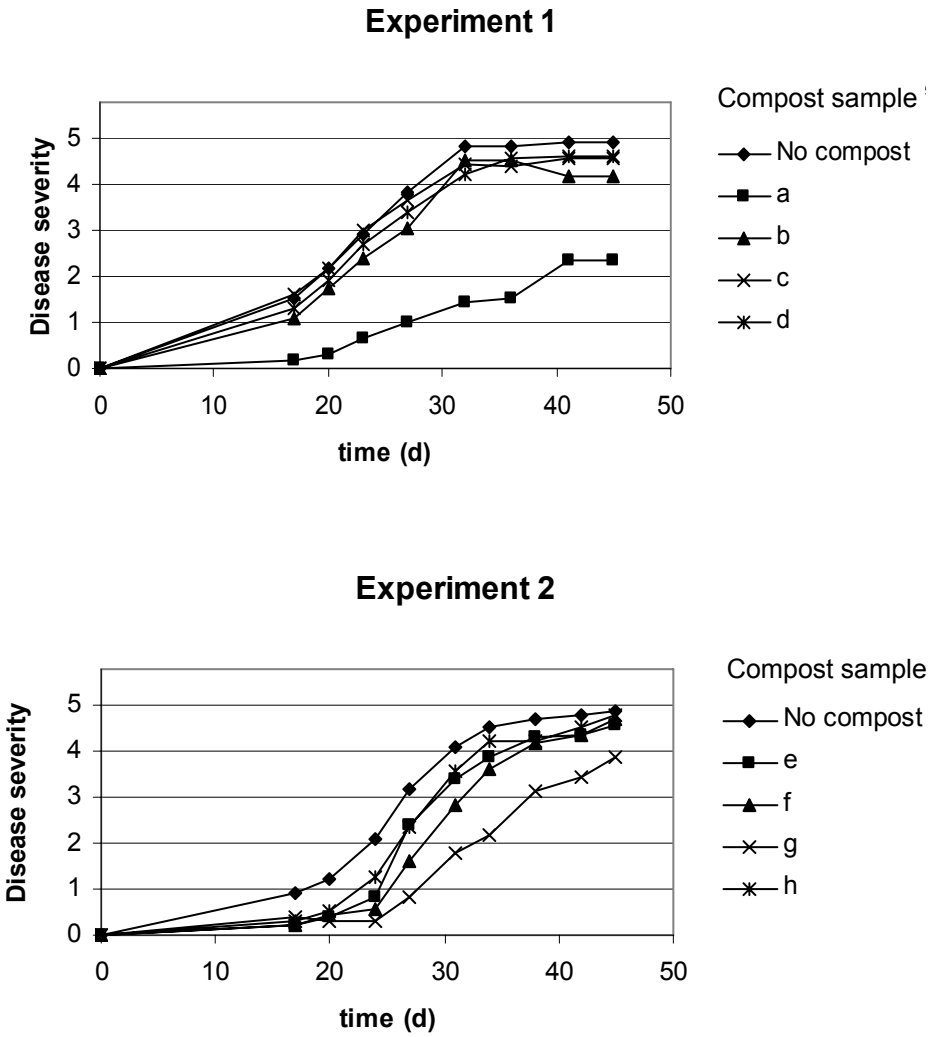

Figure 1. Development of flax wilt caused by *Fusarium oxysporum* f. sp. *lini* over time of non-amended and fresh compost-amended peat-based mixes (20/80, vol./vol.). Composts are described in Table 1. Rating of disease severity is described in the materials and methods.

Table 2. Suppression[a] of flax wilt caused by *Fusarium oxysporum* f. sp. *lini* in compost-amended peat-based mixes (20/80, vol./vol.) with fresh compost (no storage) and the same composts stored for 12 weeks dried and stored at 20°C, frozen at -20°C, or cooled at +4°C. Before the disease suppression assay was started stored composts were rewetted or returned to 20°C and incubated for 8 days at 20°C.

Experiment	Compost sample	Storage method				Average
		Fresh (no storage)	Dry (+20°C)[b]	Freeze (-20°C)	Cool (+4°C)	
1	a	67c *[c,d]	97a*	85b*	90ab*	85
1	b	14bc	37a*	22ab*	1c	19
1	c	4b	27a*	24a*	12ab	17
1	d	8b	42a*	27ab*	22ab*	25
2	e	29a*	32a*	45a*	42a*	37
2	f	37b*	46ab*	58ab*	68a*	52
2	g	53ab*	29b*	61a*	45ab*	47
2	h	24a*	-21b	-2b	-9b	-2
	Average	30	36	40	34	

[a] Disease suppression was calculated as: 100% – 100% × ($AUDPC_{amended\ potting\ mix}$ / $AUDPC_{non\text{-}amended\ potting\ mix}$), where AUDPC = Area-Under-the-Disease-Progress-Curve (Campbell and Madden, 1990).

[b] Storage temperature.

[c] *: Significant disease suppression ($P < 0.05$) is indicated with an asterisk. Significant disease stimulation did not occur.

[d] Within each row, values followed by the same letter are not significantly different according to the Duncan's multiple range test ($P < 0.05$).

Table 3. Analysis of variance of disease suppression of flax wilt caused by *Fusarium oxysporum* f. sp. *lini* induced by compost-amended peat as affected by compost sample and compost storage method (Table 1).

Effect	Disease suppression			
	df	Mean Square	F-value	Pr > F
Compost sample	7	13896	55.66	< 0.0001
Storage method	3	773	3.1	0.0292
Interaction	21	951	3.81	< 0.0001

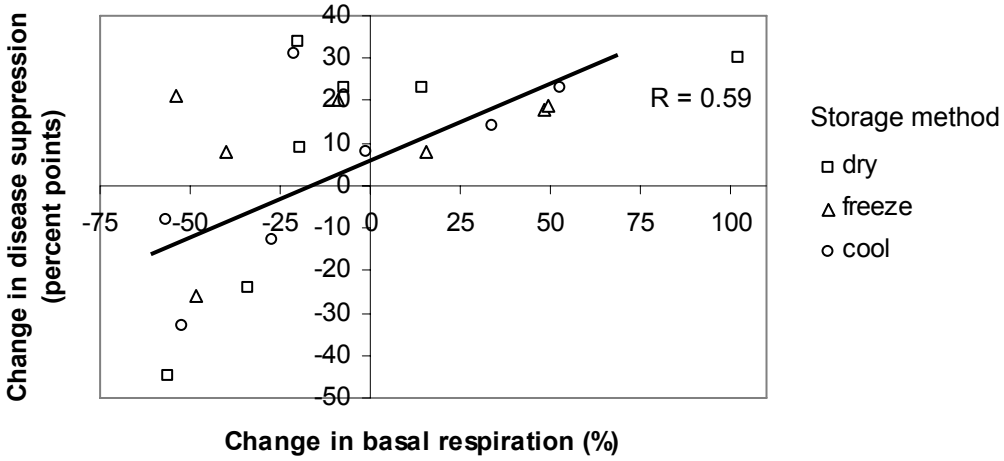

Figure 2. Correlation between change in basal respiration (%) and change in disease suppression (percentage points). Compost samples were stored in three different manners: dried (stored at +20°C), cooled (+4°C) and frozen (-20°C). The bioassay and basal respiration was started and measured 8 and 11 d respectively after rewetting or returning to 20°C.

Table 4. Basal respiration rate (10^{-6} g CO_2 g^{-1} d.w. h^{-1}) of 8 compost samples (Table 1), fresh (no storage) and stored for 12 weeks under 3 different storage conditions. Respiration of the stored composts was measured 11 d after rewetting or returning to 20°C.

		Storage method			
Experiment	Compost sample	Fresh (no storage)	Dry (+20°C)[a]	Freeze (-20°C)	Cool (+4°C)
1	a	344.6a[b]	697.8b	510.5ab	526.3ab
1	b	120.3a	138.2a	138.8a	87.8a
1	c	57.5a	53.5a	52.4a	57.0a
1	d	14.5a	11.6a	21.7a	19.4a
2	e	n.d.[c]	57.0a	61.4a	50.0a
2	f	35.4a	28.6a	16.3b	27.9ab
2	g	45.7a	30.1b	27.5bc	19.9c
2	h	38.6a	16.9c	19.9b	18.4bc
	Average	92.3 a	129.0b	104.7ab	101.8ab

[a] Storage temperature.
[b] Within each row, values followed by the same letter are not significantly different according to the Duncan's multiple range test ($P < 0.05$).
[c] n.d. = not determined.

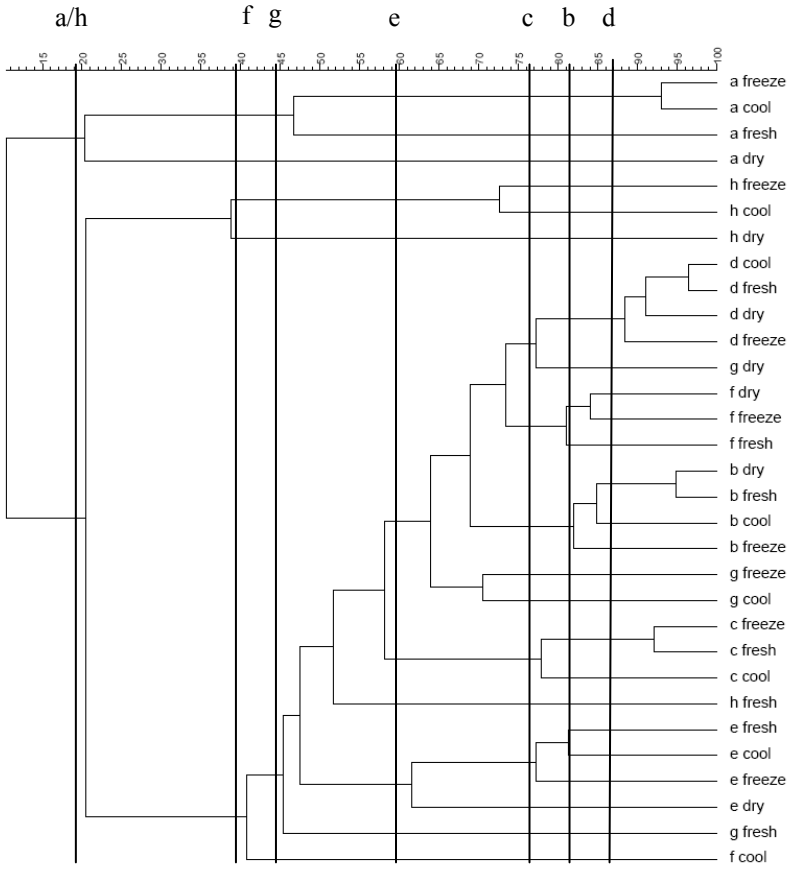

Figure 3. Dendrogram based on 16S-rDNA DGGE banding patterns in fresh compost samples and stored compost samples of 8 tested composts stored in three different manners: dried and stored at +20°C, frozen at –20°C or cooled at +4°C and frozen (-20°C). Samples of the stored composts were taken after rewetting or returning to 20°C and an incubation for 11 d at 20°C. Additional vertical lines indicate the minimal similarity of the different treatments for each compost sample.

Changes in microbial activity and bacterial DGGE patterns

Storage did not affect basal respiration (13 cases), had a significantly positive effect (1 case) or a significantly negative effect (7 cases: composts g and h for all storage methods and compost f for freezing) on basal respiration (Table 4). Change in basal respiration after storage was positively correlated with change in disease suppression (Fig. 2; r = 0.59, P = 0.005). Any storage type of compost h led to a decrease of the basal respiration by about 50% (Table 4), which coincided with a significant decline in disease suppression.

16S-rDNA DGGEs were grouped mainly according to compost sample, indicating an overriding effect of compost sample over method of storage (Fig. 3). Similarities of 16-rDNA DGGE patterns between fresh compost samples and stored samples (Table 5) were significantly affected by compost sample ($P < 0.0001$) but not by storage method ($P = 0.695$). For composts a and h, all storage conditions led to major changes in bacterial composition (similarities between fresh and stored compost < 60%) (Table 5, 6). A positive correlation was observed between change (in percentage points) in disease suppression after storage and

Table 5. Similarity (%) in bacterial composition based on 16S-rDNA DGGE banding patterns between fresh (no storage) compost samples and compost samples stored for 12 weeks under 3 different storage conditions. Samples of the stored composts were taken 11 d after rewetting or returning to 20°C.

Exp.	Compost sample	Dry (+20°C)[a]	Freeze (-20°C)	Cool (+4°C)	Average	
1	a	27	39	55	40	c[b]
1	b	95	87	84	88	a
1	c	n.d.[c]	92	78	85	a
1	d	91	87	96	92	a
2	e	54	75	81	70	ab
2	f	79	83	50	71	ab
2	g	57	66	52	59	bc
2	h	32	50	29	37	c
	Average	62 A[d]	72 A	66 A		

[a] Storage temperature.
[b] Within each column, values followed by the same lower case letter are not significantly different according to the Duncan's multiple range test ($P < 0.05$).
[c] n.d. = not done.
[d] Within each row, values followed by the same upper case letter are not significantly different according to the Duncan's multiple range test ($P < 0.05$).

Table 6. Total number of 16S-rDNA DGGE bands in fresh composts, and the number of lost and novel bands respectively in the stored composts. Samples of the stored composts were taken 11 d after rewetting or returning to 20°C.

Compost sample	Fresh (no storage)	Storage method					
		Dry (+20°C)[a]		Freeze (-20°C)		Cool (+4°C)	
		lost	novel	lost	novel	lost	novel
a	18	14	8	12	4	15	8
b	24	8	9	4	8	8	9
c	20	n.d.[b]	n.d.	7	6	7	7
d	21	8	10	3	7	5	7
e	20	5	10	5	8	5	13
f	18	6	10	6	7	7	11
g	20	10	9	4	13	7	13
h	38	24	1	14	16	17	10

[a] Storage temperature.
[b] n.d. = not done.

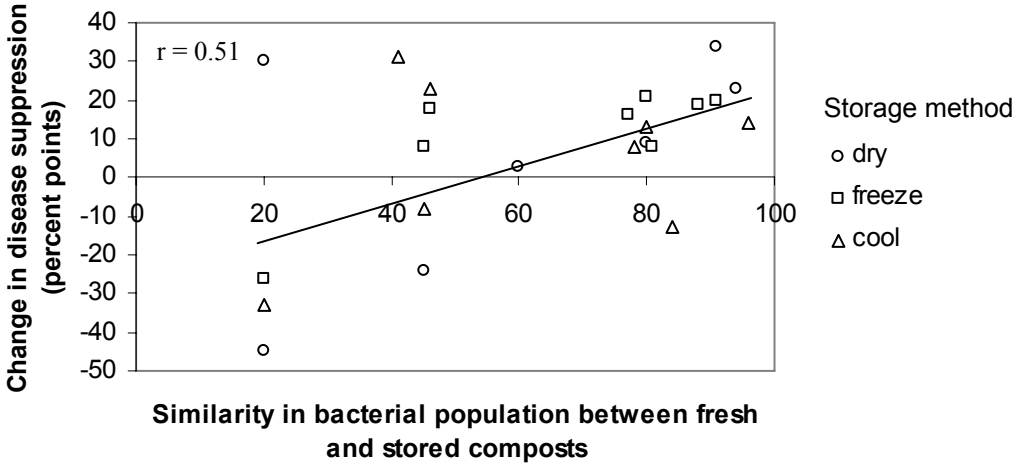

Figure 4. Correlation between the similarity 16S-rDNA DGGE banding patterns between fresh and differently stored composts and change (in percentage points) in disease suppression (r = 0.51, $P < 0.05$). Compost samples were stored in three different manners: dried (stored at +20°C), cooled (-4°C) and frozen (-20°C). Samples for DGGE analysis of the stored composts were taken 11 d after rewetting or returning to 20°C. The bioassay was performed with the composts, 8 d after rewetting or returning them to 20°C.

Table 7. Physico-chemical characteristics of 8 different fresh[a] composts.

Compost sample	Organic matter (% of total bulk)	pH	EC (mS cm^{-1})	Total C (% of total bulk)	Total N (% of total bulk)
a	32.6	7.56	2.25	20.1	1.15
b	41.1	8.10	3.40	21.2	1.66
c	26.0	7.93	0.14	14.3	0.89
d	36.8	6.95	4.69	21.6	1.55
e	28.1	7.34	2.59	14.1	1.05
f	25.1	7.46	3.30	15.1	1.23
g	34.2	7.47	3.28	18.5	1.39
h	22.9	7.93	0.80	12.0	0.60

[a] No significant effects of storage on physico-chemical characteristics were observed.

the similarity of 16S-rDNA DGGE banding patterns between fresh and differently stored composts (r = 0.51, $P < 0.05$; Fig. 4). Strong reductions in disease suppression due to storage were observed only for those stored composts that showed low similarity of 16S-rDNA banding patterns with the fresh composts. Conversely, the stored composts that showed high similarity in 16S-rDNA banding patterns with the fresh composts showed only little change in disease suppression.

Physico-chemical characteristics

On average over all composts, no significant changes in organic matter, pH, EC, total C or total N were observed as a result of storage (Table 7). When only the composts are included that in their fresh state induced significant disease suppression (composts a, e, f, g, h; Table 2), positive correlations were observed between disease suppression and organic matter ($r = 0.84$; $P = 0.073$), total C ($r = 0.99$; $P = 0.002$) and total N ($r = 0.63$; $P > 0.15$). Compost h, the compost that showed negative effects of storage on disease suppression, showed remarkably lower organic matter, total C and total N contents as compared to the other composts (Table 7).

Discussion

The different storage methods of composts, dried and stored at 20°C, frozen at -20°C or cooled at 4°C, had in general only relatively small, and frequently insignificantly positive, effects on their properties to suppress Fusarium flax wilt, and resulted in several cases in a significant increase in disease suppression. The significant compost sample × compost storage interaction on disease suppression indicates that storage effects depend on compost characteristics. For the composting practice this means that storage does not necessarily diminish disease suppression, although exceptions (compost sample h) can occur.

Prediction on the disease suppression properties of a given stored compost seems possible by determing the effect of storage on basal respiration (Fig. 2). The observation that basal respiration of the fresh composts did not correlate with disease suppression should be ascribed to the wide differences in the composts studied.

The duration of revival before determining parameters such as basal respiration may affect the results since the strong disturbance brought about by storage-revival result in a flush in easily degradable lysed cells, which lead to a temporary strong increase in respiratory activity (Stevenson, 1956; Scheu and Parkinson, 1994; Pesaro et al., 2004). Pesaro et al. (2004) observed an increase of 38% in substrate-induced respiration 2 d after rewetting dried soil, which decreased until it reached reference levels from day 6 onwards. Stevenson (1956) showed that the temporary increase in respiration can sustain for at least 7 days, depending on soil type. In our study, no significant positive changes of basal respiration were observed (except for the dry-stored compost sample a), which is likely due to the fact that composts were allowed to revive for 11 days at 20°C before basal respiration measurement took place. Stenberg et al. (1998) reported a reduction in basal respiration of 30 and 38% for soil samples stored for 3 months cool (+5°C) or frozen (-15°C) respectively measured 8 days after revival.

The consequence of any type of storage is most likely that multiple organisms die, leading to a flush of readily available nutrients after returning to room temperature or rewetting, resulting in fast colonization by r-strategists which may be quite successful in reducing the rhizosphere effect with respect to *F. oxysporum* f. sp. *lini*. This may explain the small positive changes in disease suppression that were found for several composts. The observation that changes in the bulk compost 16S-rDNA banding patterns were more compost

than storage method dependent point in the direction of a strong involvement of the substrate quality and its associated microbial community. Moreover, potential differences as a result of storage method may have been reduced by the freezing storage for 12 wks, which took place on all samples before the DNA extraction was carried out. The correlation between microbial activity and disease suppression, and the DGGE results both indicate an aspecific nature of disease suppression of flax wilt which has also been suggested by Borrero et al. (2004), Serra-Wittling et al. (1996) and Termorshuizen et al. (2006).

The changes in bacterial composition as a consequence of drying-rewetting and cooling-returning to room temperature we report confirm the conclusions of Pesaro et al. (2004) and Jenkinson and Powlson (1976) that shifts in microbial populations take place after drying-rewetting and cooling-returning to room temperature. In experiments on drying-rewetting, Pesaro et al. (2004) report on significant differences in 16S-rRNA analysis of bacteria between reference and dried-rewetted soil samples 2, 6 and 34 days after rewetting. Significant differences were also found between 16S-rRNA analyses of the different sampling days. 21% of the 16S-rRNA fragments found in untreated soils were not found in dried-rewetted soils and 25% of the 16S-rRNA fragments appeared new in the treated soil (Pesaro et al., 2004). In the current study, we observed losses of 25-78% of 16S-rDNA bands and 3-56% novel bands as a result of drying-rewetting. Reported effects of freezing-thawing on the microbial composition are not consistent. While we found significant changes in bacterial composition for several composts, Pesaro et al. (2003) demonstrated with *Hae* III RFLP analyses of bacterial communities that no significant changes in bacterial composition occurred due to freezing–thawing of soil samples (analysed 0-42 d after thawing). On the other hand, Sharma et al. (2006) observed strong changes in bacterial community structure and function during the progress of a freezing-thawing event in soil: the profiles derived after 1 d of freezing and 9 d after subsequent thawing had a similarity of only 70% to the control profiles. Fewer bands (50% of those present in the control) were obtained for samples that were exposed to a freezing-thawing treatment than for the control samples, which could be attributed to a decrease in microbial members that was counterbalanced by the proliferation of survivors. In the current study, we observed losses of 14-67% of the total detected 16S rDNA bands in the fresh compost and 22-65% novel detected 16S rDNA bands as compared to the fresh compost after freezing-thawing.

Contrary to the results of Mondini et al. (2002), no relation between compost age and the effect of storage method was observed. Mondini et al. (2002) found that compost material that was sampled after 19 and 60 days of composting and subsequently air-dried for 7 days showed a reduction in microbial biomass 2 days after rewetting of 40 and 0% respectively. Probably the death of microorganisms in compost samples by desiccation was balanced by the growth of survivor microorganisms due to the relatively high amount of decomposable substrate released after rewetting (Mondini et al., 2002).

In conclusion, we demonstrated that significant changes in bacterial composition and microbial activity occur as a result of all tested ways of storage. Small changes in bacterial composition and microbial activity due to storage have a limited effect on disease suppression. The degree of change in bacterial composition appeared to be compost-dependent. The cooling storage resulted in the least deviation in disease suppression from the

fresh compost, although the effect of freezing gave the most reliable results with the lowest standard deviation.

Acknowledgements

This study was undertaken as part of the first author's PhD program and was funded by the EU project QLK5-CT-01442. We thank W.J. Blok for discussions and M. Caffort for technical assistance in the greenhouse.

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Section II

Phytosanitary aspects of composting

Chapter 6

Phytosanitary risk assessment of composts

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Compost Science & Utilization 13(2005): 108-115

Abstract

Assessment of phytosanitary risks associated with application of composts in agriculture generally has focused on the sanitation (self-heating) phase during composting when most plant pathogens are inactivated due to lethal temperatures. However, a few plant pathogens are heat resistant and they may survive a properly monitored and controlled composting process. To assess the phytosanitary risks associated with compost utilization, several additional factors need to be considered which all relate to a tracing-and-tracking principle. It includes the composition of the original waste and several aspects related to compost utilization. The following parameters are considered to be key-factors: 1) the proportion of host biomass relative to the total quantity of biowaste, 2) the proportion of host infected with a pathogen, 3) the density of infected host material, 4) the proportion of propagules of a pathogen that survived the process, and 5) the threshold density of a pathogen in soil above which disease of the host is expected to develop. While the first two parameters may be rather easy to estimate and information on survival of many plant pathogens can be obtained from the literature, little knowledge exists on the density of the pathogens in host materials or on threshold values. This applies particularly to virus diseases. The phytosanitary risk of several types of plant pathogens is discussed in some detail in this paper. Recommendations are given for testing of a composting process for phytohygienic safety.

Introduction

During proper composting, high temperatures reached during the heating phase all but guarantee the inactivation of the majority of plant pathogens residing in biowastes (Bollen, 1989, 1993; Bollen and Volker, 1996; Ryckeboer, 2001). Notwithstanding this fact, the use of compost in some regions is regarded as a potential hazard because of the potential presence of pathogens in composts. Indeed, survival of a few soilborne plant pathogens during composting has been reported (Table 1; Noble and Roberts, 2003). However, phytohygienic

risks of compost use should be balanced against the advantages of applying compost as disease suppressive material as well as against the risks associated with other farming practices that may enhance the activity of plant pathogens. This review aims to explore the feasibility of reliably estimating phytohygienic risks associated with compost applications in agriculture. For this risk assessment, the entire production chain from generation of the biowaste to its application as a compost should be taken into consideration. In the following discussion three components will be distinguished and discussed: 1) the composition of the biowaste, 2) the degree of sanitation achieved during composting, and 3) utilization aspects of the compost.

Impacts of biowastes composition on pathogen survival

When the composition of biowastes is well-defined, the pathogens that are likely to be present are well-known also. However, when the biowaste is highly variable, it is difficult to predict the presence of pathogens.

Well-defined biowastes typically originate from agriculture, horticulture or arboriculture. Generally, woody materials impose negligible phytohygienic problems. The most common plant pathogen occurring in wood is *Armillaria*, which is killed readily at moderate temperatures (Yuen and Raabe, 1984). Residues of solanaceous plants generally are recommended to be excluded from compost heaps since they contain various highly persistent pathogens such as tobacco mosaic virus. It rates among the most notorious heat resistant viruses. However, in other cases, risks may be lower than expected. For example, cucumber green mottle mosaic virus (CGMMV) is resistant to composting temperatures of up to 70-72°C for 3 days and thus it may survive composting. Nevertheless, cucumber biowaste is of little risk in regions where cucumbers are grown in glasshouses on rockwool or on soils where other hosts of this virus (melon, watermelon) are not cultivated. Farmers who compost their own biowaste represent a special case. The risk that pathogens are introduced here to a susceptible crop could be greater because the windrow process used on small farms may lead to more survival of plant pathogens compared to the higher temperature of the composting process utilized at large composting plants (see below). We recommend more research on this topic. An other extreme represents compost made from vegetable, fruit and garden (VFG) biowastes collected from households. This material is quite diverse in composition, varies between different locations and in time, which leads to lower densities of specific pathogens but an increased diversity of pathogens.

Impacts of sanitation during composting

The fate of plant pathogens during composting was reviewed several times recently (Bollen, 1993; Bollen and Volker, 1996; Ryckeboer, 2001; Noble and Roberts, 2003) and therefore we will not deal with this topic in detail here.

The type of composting process used is crucial. Proper aerobic composting removes

Table 1. Summary of the literature on the fate of plant pathogens during composting.

Species	Group	Disease	Host	References
Pathogens that are destroyed during composting				
Airborne fungi	Fungi, Chromista	various	various	cf. Bollen and Volker, 1996
Nematodes	Nematoda	various	various	cf. Bollen and Volker, 1996
Bacteria	Bacteria	various	various	e.g. Bruns et al., 1993; Hoitink et al., 1976; López-Real and Foster, 1985; Elorietta et al., 2002
Armillaria spp.	Fungi	root and butt rot	woody plants	Yuen and Raabe, 1984
Colletotrichum coccodes	Fungi	black dot	Solanaceae	Bollen, 1985
Cylindrocarpon destructans	Fungi	root rot	various	Bollen, 1985
Fusarium solani	Fungi	root rot	many	Bollen, 1985
Phomopsis sclerotioides	Fungi	black rot	cucumber	Bollen, 1985
Phytophthora and *Pythium* spp.	Chromista	root rot, damping-off	many	e.g. Bollen, 1985
Rhizoctonia solani	Fungi	root rot, damping-off	many	Bollen, 1985; Bollen et al., 1989
Sclerotium cepivorum	Fungi	white rot	onion	Bollen et al., 1989
Sclerotium rolfsii	Fungi	various	many	Yuen and Raabe, 1984
Sclerotinia sclerotiorum	Fungi	soft and root rot	many dicots	Herrman et al., 1984
Sclerotinia trifoliorum	Fungi	leaf spot	legumes	Dittmer and Weltzien, 1988
Stromatinia gladioli	Fungi	dry rot	Gladiolus	Bollen et al., 1989
Verticillium dahliae	Fungi	wilt	many dicots	Bollen et al., 1989
Pathogens that may survive during composting				
Cucumber green mottle mosaic tubamovirus	Virus	various	cucumber, (water)melon	Lecoq, 1988; Avgelis and Manios, 1992
Fusarium oxysporum	Fungi	root rot, wilt	various dicots	Bollen, 1985, but see also Suárez-Estrella et al., 2003
Olpidium brassicae	Fungi	vector of LBVV and TNV	lettuce, beans, cucumber	Bollen, 1985
Potato mop top virus	Virus	dwarfing, stunting	potato	Nielsen and Molgaard, 1997
Synchytrium endobioticum	Fungi	wart disease	potato	Bollen, 1985
Tobacco necrosis virus	Virus	necrosis	beans, cucumber, potato, tulip	Bollen and Volker, 1996; López-Real, 1985
Tobacco rattle virus	Virus	various	potato, bulbs	Bollen and Volker, 1996
Tomato and tobacco mosaic virus	Virus	various	many, notably Solanaceae	Herrman et al., 1994; Matthews, 1970; Ryckeboer, 2001
Macrophomina phaseolina	Fungi	Dry root rot	many	Bega and Smith, 1962; Mihail and Alcorn, 1984; Lodha et al., 2002

Table 1 (continued).

Survival capability unknown				
Beet necrotic yellow veins virus	Virus	Rhizomania	beets	-
Lettuce big vein virus	Virus	stunting	lettuce	-
Olpidium radicale	Fungi	vector of cucumber necrosis virus and melom necrotic spot virus	cucumber	-
Polymyxa betae	Protoctista	vector of BNYVV	beets	-
Spongospora subterranea	Protoctista	powdery scab and vector of PMTV	potato	-

the majority of plant pathogens (Table 1). Professional composters check temperatures routinely and they control the moisture content of the biowaste to maintain optimum conditions for pathogen destruction. They also turn the compost heaps regularly so that all parts become exposed to high temperatures. On the other hand, hobbyist gardeners who compost biowastes for themselves may perform barely any checks on composting conditions. In addition, the quantity of compost produced usually is small relative to professional composters. This means that the effects of low temperature outer edge of windrows is significant (Ryckeboer, 2001) especially when piles are not turned. Nevertheless, even in small composting vessels of 0.2 m^3, strong reduction of persistent pathogens such as TMV and *Plasmodiophora brassicae* has been reported (Ryckeboer, 2001), probably due to the much longer composting time which may compensate for the poor conditions relative to those existing at larger properly managed facilities where the composting period can be as short as 3-6 weeks (cf. Katan and DeVay, 1991).

Impacts of surviving pathogens during compost utilization

Where a risk of presence of plant pathogens in compost exists, its potential impact should be balanced against the population density of these plant pathogens and their hosts on the soil where the compost is to be applied. As explained above, heat resistant CGMMV poses acceptable phytosanitary risks in the Netherlands because it is not likely to become in contact with a host plant there. On the other hand, application of compost which may be contaminated with tobacco rattle virus should be discouraged on ploughed grassland where a bulb crop is grown afterwards (see below). Stated more generally, *tracing and tracking* of composts in terms of raw materials composition and source is necessary for low risk beneficial utilization in agriculture.

Towards phytohygienic risk assessment of compost

Pathogen density in the biowaste is calculated as follows (see Table 2 for an explanation of the symbols used below):

Table 2. Summary of symbols used in equations describing the fate of pathogens.

Symbol	Explanation	Units
$H_{biowaste}$	proportion of host biomass relative to the total quantity of biowaste	[-]
$H_{pathogen}$	proportion of host material infected with the pathogen	[-]
$P_{biowaste}$	pathogen density in biowaste	[propagules g^{-1} biowaste]
$P_{compost}$	pathogen density in compost	[propagules g^{-1} compost]
P_{host}	pathogen density in infected host material	[propagules g^{-1} host material]
P_{soil}	pathogen density in compost-amended soil	[propagules g^{-1} soil]
$P_{surviving}$	proportion of surviving pathogen propagules relative to $P_{biowaste}$	[-]

$$P_{biowaste} = P_{host} \times H_{pathogen} \times H_{biowaste} \quad (1),$$

where $P_{biowaste}$ and P_{host} are the density of pathogens in biowastes [propagules g^{-1} biowaste] and in infected host materials [propagules g^{-1} host material], respectively; $H_{pathogen}$ the proportion of host materials infected with the pathogen, and $H_{biowaste}$ the proportion of host biomass relative to the total quantity (amount) of biowaste.

In the absence of sanitation, pathogens present in biowastes would be concentrated during the composting process due to mass reduction (= biowaste weight before / biowaste weight after composting). After composting, the density of the pathogen in the compost can be defined as:

$$P_{compost} = P_{biowaste} \times \text{mass reduction} \times P_{surviving} \quad (2),$$

where $P_{compost}$ is the density of pathogens in compost [propagules g^{-1} compost] and $P_{surviving}$ is the proportion of propagules of the pathogen that survived relative to $P_{biowaste}$. Prediction of $P_{surviving}$ naturally depends on the type of composting process applied. This can be complex since inactivation mechanisms specific pathogens are usually not known precisely. Most publications emphasize effects of temperature on inactivation. Katan and DeVay (1991) showed that effects of temperature are linearly related to the log of incubation time. However, additional or interacting effects of temporary anaerobiosis on heat treatment during composting cannot be excluded.

Neglecting the contribution of compost to the bulk volume of the amended soil, the amount of pathogen present in soil after compost application can be defined as:

$$P_{soil} = P_{compost} \times \text{compost addition} / \text{soil volume} \quad (3),$$

where P_{soil} is the density of the pathogen in soil [propagules cm^{-3} soil].

Assuming that 40 tonnes ha^{-1} ($= 4 \times 10^7$ g) of compost is applied evenly to the top 20

cm layer (= 2×10^9 cm^3), P_{soil} would equal to $P_{compost} \times 4 \times 10^7 / (2 \times 10^9) = 2 \times 10^{-2}$ g cm^{-3} or

$P_{soil} = P_{compost} \times 2 \times 10^{-2}$ propagules cm^{-3} soil (4).

By substituting (1) into (2) and (2) into (4) we obtain:

$P_{soil} = H_{biowaste} \times H_{pathogen} \times P_{host} \times P_{surviving} \times$ mass reduction $\times 2 \times 10^{-2}$ (5).

The value P_{soil} should be evaluated against the soil inoculum density of the pathogen prior to compost application, presence of other sources of inoculum such as infected planting material and the threshold level of inoculum density below which the pathogen does not incite disease. It could, for example, be proposed that P_{soil} should be smaller than 0.001× the abovementioned threshold level. It may be difficult to estimate threshold levels as these may be soil type dependent, and typical densities causing losses are not well defined for soilborne viral pathogens.

In eight examples listed below, we investigate the risk analysis for pathogens that generally are considered to be problematic (Table 1), assuming that a temperature of 60°C can be obtained for at least 3 days during composting with a mass reduction of 2.5. These assumptions lead to:

$P_{soil} = H_{biowaste} \times H_{pathogen} \times P_{host} \times P_{surviving} \times 5 \times 10^{-2}$ (6).

Furthermore, the risk assessments apply to conditions in the Netherlands and to VFG-compost because this type of compost is generally considered as most problematic from a phytosanitary point of view.

Example 1: Cucumber green mottle mosaic virus (CGMMV). This non-vectored virus can resist temperatures of up to 70-72°C for 3 days (Avgelis and Manios, 1992; Lecoq, 1988). It is able to infect host roots without the aid of a vector.

In the Netherlands, household biowaste contains low quantities of hosts of this virus (i.e. cucumbers, melons and watermelons). The disease is quite rare in the Netherlands, leading to low estimates for $H_{biowaste}$ and $H_{pathogen}$. However, pathogen density in infected host material (P_{host}) is unknown as are its threshold loss levels. For the Netherlands, hosts (mainly cucumbers) of CGMMV are cropped mainly on rockwool and thus it cannot become exposed to CGMMV-containing compost. Given the low incidence of hosts in biowaste material and of CGMMV itself in the Netherlands, the risk for organic farmers who use compost instead of rockwool is estimated to be low.

Example 2: *Olpidium brassicae*. The inactivation temperature of this pathogen is 30 min. at 55.0-62.5°C (Bollen, 1985), 2 weeks at 50°C or 8 weeks at 40°C (Asjes and Blom-Barnhoorn, 2002). We may assume that the great majority of propagules of this pathogen will be inactivated during composting at 55°C for 4 days ($P_{surviving} = 1 \times 10^{-5}$). The pathogen poses a "double threat" because it serves also as vector of tobacco necrosis virus (TNV) and lettuce

big vein virus (LBVV). It can vector these viruses into roots.

In the Netherlands, the pathogen is rare on lettuce, beans and cucumber. The pathogen occurs only in the root system, which is unlikely to be incorporated in biowaste in significant quantities, leading to a very low estimate for $H_{biowaste}$ (1×10^{-6}). Furthermore, if infected host material were to be included in biowaste, the probability that it would contain *O. brassicae* is considered to be very low. Here we tentatively estimate $H_{pathogen}$ at 1×10^{-5}. P_{host} is estimated (100) based on personal observations. Although damage threshold values for *O. brassicae* are not known, it is extremely unlikely that the P_{soil} level of 5×10^{-16} propagules g^{-1} (2.5×10^{-7} propagules ha^{-1}) may incite a phytosanitary problem, given that the composting process is performed properly. We stress, however, that the abovementioned estimates are rough and they need confirmation based on detailed observations.

TNV and LBVV, as mentioned above, are transmitted by *Olpidium brassicae* but, in contrast to the fungal pathogen, they usually infect the shoot. TNV is inactivated when incubated for 4 days at 54°C, thus making rigorous composting crucial. In contrast to tobacco rattle virus (TRV), TNV may be transmitted as an externally attached particle to zoospores of the vector. Thus, TNV that survived composting may lead to plant infection if *O. brassicae* is resident in the amended soil. In the Netherlands, the pathogen is very rare except for ploughed grassland on which bulb crops are grown where it can be fairly common (pers. comm. D. Peters, Wageningen University). Hosts of TNV include potato, bean, cucumber and tulip. Although damage threshold levels are not known, it seems unlikely that TNV can incite problems except on ploughed grassland where hosts are grown commonly.

LBVV occurs rarely as infections in the Netherlands and lettuce is the only host. The inactivation conditions of this virus are unknown, but if the vector is reduced (see above) during composting, then we have to assume that risks associated with mechanical transmission pose a significantly greater risk than that associated with utilization of infested composts.

Example 3: ***Plasmodiophora brassicae*** (the causal agent of club root). Although considerable quantities of host tissues (i.e. cruciferous plant species) may be delivered to composters by households and the processing industry, infected host tissue (i.e. the root system) should only originate from hobbyist gardeners who discard entire plants if they grow poorly. This seems to be quite a rare event because only few gardeners might do this. The more experienced gardeners prevent clubroot by liming of the soil. Thus, for VFG-waste, $H_{biowaste}$ (constricted to roots) like *O. brassicae*, is quite low. On the other hand, even a few infected cabbage roots would yield a high density of the pathogen. Accurate data appear to be nonexistent. Most literature indicates complete inactivation of *P. brassicae* when treated for at least 4 days at 60°C. In some cases, however, survival at higher temperatures has been reported (Martin, 1963; Ylimäki et al., 1983; Marciniszyn and Gotschall, 1995; Anonymous, 1996) although even then only a fraction survived. Clearly more research on the mechanism of inactivation has to be done (Ryckeboer, 2001). Given the ubiquity of this pathogen, and its long-term survival in soil we expect that its soilborne inoculum density normally is greater than the density in compost. We tentatively conclude that the phytohygienic risk associated with *P. brassicae* is acceptable at locations where this pathogen is common.

Example 4: *Polymyxa betae*. This parasite causes no direct damage but it vectors beet necrotic yellow veins virus (BNYVV), which causes rhizomania in beets. The fungal propagules occur only in the root system. It has been estimated that 10-15% of the fungal propagules is virus-infected (Tuitert, 1990). The inactivation temperatures for the virus and its vector are unknown, but reduction to 'negligible populations' of *P. betae* has been reported after heating of an infested soil/water suspension to 65°C for 30 min. (Dickens et al., 1991).

In the Netherlands this pathogen occurs mainly in sugar beets where it is not rare. Composted beet material from farmers or the beet processing industry should therefore be refused until more is known about the fate of this pathogen during composting and beet processing. Not much is known about the frequency of *P. betae* in beet vegetables used for human consumption (e.g. red beets) or in allotment gardens. Nevertheless, as for *Olpidium brassicae* and *Plasmodiophora brassicae*, it seems unlikely that significant numbers of beet roots are delivered to facilities that compost VFG-waste. However, more research is clearly needed on this disease since Tuitert and Hofmeester (1992) reported that the pathogen rapidly increased in populations over the years independent of the initial inoculum density. This indicates that very low levels indeed may lead to disease in this case.

Example 5: *Spongospora subterranea*. This pathogen causes powdery scab in potatoes. It also is a vector of potato mop top virus (PMTV). It forms resting spores in the potato peel and thus may be quite common in VFG-waste. MacKay and Shipton (1983) reported reduction of powdery scab of 80% after treatment of infected seed tubers in a water bath at 55°C for 10 min., but the inactivation temperature of PMTV is 90°C for an incubation time of 15 min. (Nielsen and Molgaard, 1997). However, free-living PMTV is probably unable to infect the host (Jones and Harrison, 1969). Jones and Harrison (1969) reported that not all provenances of the pathogen are viruliferous and of those that are viruliferous only a small proportion of the zoospores is so (Torrance in Harrison et al., 1997). Little quantitative data exists about this pathogen, which is related to difficulties associated with its detection. The damage threshold level for this pathogen is not known. Harrison et al. (1997) argued that reducing seed tuber-borne inoculum has little value if the soil is infested. Therefore, it is difficult to estimate the possible contribution of surviving propagules in compost on the impacts of the soilborne inoculum on this disease.

Example 6: *Synchytrium endobioticum*. This pathogen which causes potato wart disease, is a very rare and quarantined pathogen in the Netherlands. Locations that are infested are restricted by severe quarantine measures. Bollen (1985) reported inactivation after incubation in water at 50-60°C for 30 min., indicating that proper composting may eliminate the pathogen completely. Thus, although potato material is common in VFG-waste, it is highly unlikely that composts can serve as carriers for this pathogen as long as the process is monitored properly.

Example 7: Tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV). Several properties of these viruses cause them to pose a serious threat to composting of VFG biowaste. The inactivation temperature has been reported to be above 60°C. The viruses do

not need vectors to infect roots from the soil. Furthermore, the mechanism of inactivation during composting is unknown. Quite some controversy exists about the inactivation temperature, as reviewed by Ryckeboer (2001). The situation is further complicated by the possibility that the virus can occur in a reversible inactive state (Bartels, 1956), probably by binding to soil particles. Nevertheless, increased temperatures consistently cause a reduction in virus titer (Ryckeboer, 2001). In the Netherlands, the actual risk of the occurrence of both viruses in compost is low in spite of their wide host ranges because ToMV is extremely rare and TMV is rare (D. Peters, Wageningen University, pers. comm.). In fact, the phytosanitary risk associated with TMV-contaminated cigarettes may be significantly higher.

Example 8: Tobacco rattle virus (TRV). Although it has been demonstrated that TRV may well survive rigorous composting conditions, it is very unlikely that problems will develop with this virus since it can only infect hosts when it is transmitted by nematodes (*Paratrichodorus* and *Trichodorus* spp.) which are readily killed during composting (Bollen and Volker, 1996). Furthermore, uptake of TRV by these nematodes in soil is thought to not occur (Bollen and Volker, 1996).

Phytohygienic safety testing of composts

Two approaches for testing the phytohygienic safety of composts are in common use. The first test relies on testing of the compost itself for the density of pathogens. The second approach relies on time/temperature exposure of pathogens during the process. Each is reviewed below.

1. **Testing for the presence or survival of pathogens:** Christensen et al. (2001) used the terms "direct process evaluation" when survival of pathogens was tested by incorporating bags containing inoculum into the compost (infested) and "spot test analysis" when (uninfested) samples of composts were tested for the presence of pathogens. The latter method is only advisable if common pathogens are to be tested, such as the human pathogenic Enterobacteriaceae (Christensen et al., 2001). Most plant pathogens are relatively rare and, if present in significant quantities, heterogeneously distributed in biowastes. Therefore, spot test analysis is not advisable for evaluation of the phytosanitary aspects of a composting process. An exception would be biowaste consisting of a single source of input, for which the presence of specific pathogens would be fairly well-known also.

   "Direct process evaluation" is used regularly when a specific composting process is evaluated. If the pathogen does not survive in this test, then the composting process is considered to be safe with regard to this pathogen. This yields useful information on the fate of these pathogens during composting, but it gives no information whether the composting process of other batches of biowaste would lead to inactivation of pathogens. This may be particularly so if the composition of biowaste varies according to season. There are two additional problems associated with testing for the presence or survival of a

specific set of plant pathogens. First, if these pathogens do not survive, would this be a guarantee that other pathogens also are inactivated? Second, the choice of pathogen is crucial. Frequently, TMV, *Plasmodiophora brassicae* and *Polymyxa betae* are used as test organisms but one may wonder whether these pathogens are suitable. TMV may have an undetectable but viable state which may give rise to false-negatives in the detection assay. The mechanism of inactivation of *P. brassicae* is not yet known, and a broad range of inactivation temperatures have been reported for this pathogen. Furthermore, the detection method, involving a bioassay with susceptible cabbage seedlings is cumbersome, and the detection limit is not known. Finally, inactivation conditions of *P. betae* are unknown; the pathogen is quite rare, and as for *P. brassicae*, the detection procedure is cumbersome.

2. **Monitoring of composting process parameters that are related to pathogen inactivation.** During aerobic composting the temperature is usually regarded as the major factor causing inactivation. Usually the exposure of compost to a minimum high temperature (e.g. 55-60°C) for a specified time interval (e.g. 2 wk) is required (summarized by Ryckeboer, 2001). Christensen et al. (2001) reported that properties such as organic matter content, C:N ratio and pH of mature composted biowaste did not correlate with pathogen inactivation. However, it is very well possible that temporary anaerobiosis significantly contributes to inactivation of some pathogens (Ryckeboer et al., 2002; Termorshuizen et al., 2003) and that its interaction with temperature is synergistic (Kumar et al., 1999). Thus, survival conditions based on temperature exposure alone during aerobic composting may lead to underestimations. This field clearly needs more research.

   Apart from the composting conditions, checks should also be carried out on the operating conditions of the composting plant. Thus, equipment such as front-end-loaders may not be used simultaneously for raw biowaste and compost because pathogens could contaminate the compost. Furthermore, movement and turning of wastes at a composting site should be organized in such a manner that opportunities for contamination of compost by shovels, transport cars etc. are minimized.

Conclusions

Most plant pathogens are inactivated during proper aerobic composting. Conditions which should be monitored include composting temperatures and the general working conditions at the plant. For pathogens that may survive these conditions, however, knowledge on their occurrence in the biowaste and of the crops grown on the sites where the compost is to be applied is required. Quantification of phytosanitary risks is still difficult since predictive data are lacking for the pathogens considered in this review. Especially for viral pathogens, data on the density of pathogens in infected plant materials and on damage threshold levels is lacking. Quantification of phytosanitary risks of composts is not always necessary, however. For example, although TRV can resist composting conditions, the vector of TRV is readily inactivated during composting and the virus therefore cannot infect plants without the vector.

In general, it seems safe to use even composts produced from VFG-compost albeit the fate of some pathogens still is not understood.

Acknowledgements

We thank D. Volker for critically reading the manuscript. This work was supported by the European Commission (Framework Programme 5; The Compost Management Project; no. QLK5-CT-2001-01442).

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Chapter 7

Conditions required for eradication of *Polymyxa betae* during composting

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Journal of Phytopathology, accepted

Abstract

The composting conditions required for eradication of *Polymyxa betae,* the vector of beet necrotic yellow vein virus (BNYVV) in sugar beet, were investigated. Survival of resting spores of *P. betae* was determined under aerobic (30 min, 4 and 21 days) and anaerobic (4 days) conditions under several temperature regimes in a water suspension and in leachate extracted from an aerobic compost heap. In water under aerobic conditions the lethal temperature was 60, 55, and 40°C for exposure times of 30 min, 4, and 21 days respectively. The effect of compost leachate and/or anaerobic conditions on survival of *P. betae* depended on temperature. After incubation for 4 days at 25°C, no significant effects of anaerobic conditions or leachate on the survival of *P. betae* were found. However, at 40°C for 4 days under anaerobic conditions, survival of *P. betae* was 0.3% compared to survival under aerobic conditions in water at the same temperature. In leachate taken from an aerobic compost heap, aerobically incubated at 40°C for 4 days, survival of *P. betae* was 6% compared to survival in water at the same temperature. Since anaerobic spots are prevalent in aerobic compost heaps, especially during the thermophilic phase, actual inactivation temperatures under composting conditions are likely to be lower than the temperatures we found for eradication in water under aerobic conditions.

Introduction

One of the test organisms to determine the phytosanitary status of a composting process that has been used extensively in the Netherlands is *Polymyxa betae.* Remarkably, its exact inactivation conditions are not known. *P. betae* transmits the beet necrotic yellow vein virus (BNYVV) into sugar beet. Although resistance is present against the most commonly occurring pathotypes A and B, pathotype P, that has been reported only from France (Koenig

et al., 1997) and the UK (Harju et al., 2002), is able to infect partially resistant sugar beet cultivars (Tamada et al., 1996). Recently, resistance-breaking isolates of BNYVV of the A-type have been reported too (Liu et al., 2005).

Temperature is considered a major factor determining the inactivation of plant pathogens during composting. Composting conditions to eradicate pathogens have been defined on the basis of standard experimental conditions of 30 minutes of incubation in aerated water (Bollen, 1993). For phytosanitary reasons, composting conditions are defined to reach a temperature of > 60°C for at least 3 days (Noble and Roberts, 2004). The conditions prevailing during the thermophilic phase of composting are different from the aerated water suspensions used in standard test conditions. During the thermophilic phase of composting, which may last several days, temporal and local anaerobic conditions widely occur (Fernandes and Sartaj, 1997; Veeken et al., 2002; Beck-Friis et al., 2003), leading to decomposition products that may be toxic to multiple organisms. For *Plasmodiophora brassicae*, the causal agent of clubroot, it has been shown that the lethal temperature decreases from > 55°C under aerated conditions (Ylimaki et al., 1983) for one day to 45°C (Horiuchi et al., 1983) when incubated anaerobically. As *P. betae* is taxonomically closely related to *P. brassicae* (both Plasmodiophoromycetes, kingdom Protoctista), forming comparable types of resting spores, it is well possible that the eradication temperature of *P. betae* is influenced by the presence of leachate or anaerobic conditions. The formation of volatile organic acids during anaerobic circumstances has been suggested to play a key role in the inactivation process of several soil borne pathogens (Okazaki and Nose, 1986; Tenuta et al., 2002).

The goal of the present research was to determine the survival of resting spores of *P. betae* under aerobic and anaerobic conditions under several temperature regimes in a water suspension and in leachate from a compost heap. *P. betae* is only one of the few pathogens of which the inactivation temperature has not been determined, the reason for it being that the available quantification techniques are quite time-consuming. In this study, we used the most probable number (MPN) method of serially diluted soils using a bioassay developed by Tuitert (1990). In this method quantification was done with serial dilutions of the infective inoculum and the presence or absence of *P. betae* in the sugar beet roots was determined.

Materials and Methods

Overview

The survival temperature of *P. betae* Keskin in aerated water was determined at 25, 45, 50, 55, and 60°C for incubation times of 30 min and 4 days. Based on these results, a similar experiment was carried out at 25 and 40°C with 21 days of exposure. The survival of *P. betae* in leachate derived from a young compost heap, incubated for 4 days at 25 and 40°C, under aerobic or anaerobic conditions, was compared with survival in water under the same conditions.

All temperature treatments were carried out in a shaking water bath (120 rpm) with high-precision temperature control. During incubation, temperatures were recorded constantly during the 30 min treatment and daily for the 4 and 21 days treatments. Temperature was recorded using two thermocouples of which one was situated in the heated water bath and the other inside one of the erlenmeyers.

Collection of P. betae infested soil

Clayey soil known to be naturally infested with resting spores of *P. betae* was collected in May 2002 from the upper 10 cm of a sugar beet field at an arable farm at Nagele (Flevoland, the Netherlands). The samples were air-dried, ground, mixed thoroughly and stored at 20°C. Tuitert (1990) reported that such storage maintained the inoculum potential unaffected for at least 28 months.

Experiment A: aerobic temperature treatments in water for 30 min, 4 days and 21 days

Per temperature treatment three 100-ml erlenmeyers were filled with each 60 g (= 50 ml) of soil inoculum and 50 ml demineralised water. The suspensions were thoroughly mixed and loosely covered with aluminum foil to reduce evaporation. The soil-water suspensions were exposed to the following exposure / temperature treatments: 30 min: 25, 45, 50, 55, 60°C (run 1, Table 1); 4 days: 25, 45, 50, 55, 60°C; 21 days: 25, 40°C (Table 1). The 30 min experiment was repeated with another sample of soil inoculum for the 50, 55, and 60°C temperatures (run 2; Table 1). The erlenmeyers were completely randomized in the incubator.

Experiment B: aerobic and anaerobic temperature treatments in water and in leachate for 4 days

Leachate of 3-days-old compost consisting of vegetable, fruit and garden waste was collected at a tunnel composting plant (Essent Milieu AVL, Venlo, the Netherlands). Half of the leachate was collected anaerobically, in six 100-ml glass erlenmeyers that were each filled with 60 g of soil inoculum, closed with an air-tight plug and immediately flushed with N_2 to create anaerobic conditions. As control served six 100-ml erlenmeyers, each filled with 60 g of soil inoculum and 50 ml water, closed with an air-tight plug and flushed with N_2. The anaerobic erlenmeyers were stored in the dark at 4°C until temperature exposures were carried out the next day. For the aerobic-leachate incubation, a 1-l-glass bottle was filled with the leachate, closed with an air-tight plug and stored in the dark at 4°C until temperature exposures were carried out the next day. The aerobic temperature exposures were carried out in six 100-ml erlenmeyers, each filled with 60 g of soil inoculum and 50 ml water or leachate, loosely covered with with aluminum foil to reduce evaporation.

Eight temperature exposures were carried out. The anaerobic soil-leachate suspensions, the anaerobic soil-water suspensions, the aerobic soil-leachate suspensions and aerobic soil-water suspensions were exposed to a 4 days temperature treatment at 25 or 40°C (Table 2) in a completely randomized design.

Determination of inoculum density

Tuitert's (1990) Most Probable Number (MPN) bioassay was used to determine the density of infective inoculum. After the heat treatment, 180 g (= 150 ml) soil inoculum was mixed with 1350 ml autoclaved dry coarse sand (dilution 10^{-1}). Subsequently, dilutions with autoclaved dry coarse sand of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made after thoroughly mixing, and six 200-ml pots were filled with each dilution. One 3-weeks-old seedling of *Beta vulgaris* cv. Helsinki (experiment A) or cv. Auris (experiment B) (kindly provided by dr. J.H.M. Schneider, Institute for Rational Sugar Production, Bergen op Zoom, the Netherlands) was planted per pot. Saucers were placed under each pot and the pots were incubated in a completely randomized block design in a greenhouse at 23°C, 60% RH and a light period of 16 h. After 6 weeks the plants were harvested and their root systems examined for presence of resting spores of *P. betae* under a light microscope (magn. 200×). The detection limit of the used method was 0.01 cfu g^{-1} soil, representing one resting spore in the root system of one plant.

Chemical analysis

For the determination of short-chained volatile fatty acids (VFA) in the leachate, 5 test tubes were filled with 4 ml leachate and 1 ml 10% formic acid. The tubes were closed with an air-tight plug and were stored in the dark at 4°C until further analysis. Volatile fatty acids (acetic, propionic, butyric and valeric acid) were determined by gas chromatography. The samples were centrifuged (10,000 rpm) and acidified to pH 2. One µl per sample was injected into a Hewlett Packard 5890 series II gas chromatograph equipped with a 30 m × 0.32 mm, df = 0.25 µm Alltech AT-Aquawax-DA column (Deerfield, USA) and a flame ionization detector (T = 300°C). A pre-column (not activated) (2.5 m × 0.32 mm) (Deerfield, USA) was used to prevent the main column from wearing. The initial temperature was 80°C and the temperature rise was set at 25°C min^{-1} to 210°C. The carrier gas, nitrogen, was set at a flow rate of 2.6 ml min^{-1}.

Statistical analysis

Numbers of infected plants in the dilution series of the bioassays were used to estimate the most probable numbers (MPN) of infective units of *P. betae* ml^{-1} of soil inoculum per treatment. Most probable numbers and 95% confidence intervals were calculated with a MPN Excel spreadsheet obtained from Blodgett (2003).

Results

Survival of *P. betae* in aerated water decreased with increasing temperature and exposure time (Table 1). At an exposure time of 30 min, complete inactivation was found at

Table 1. Most Probable Numbers (MPN) of infective units and 95% confidence limits of infection of roots with *Polymyxa betae* after incubation at various temperatures for 30 min, 4d or 21 days in aerated water (experiment A).

			95% confidence intervals	
Exposure time	T (°C)	MPN (cfu g^{-1})	Lower limit	Upper limit
30 minutes, run 1	25	53.46a[a]	21.35	134.02
	45	1.15b	0.52	2.58
	50	0.65b	0.28	1.52
	55	0.01c	0.00	0.05
	60	0.00c	0.00	0.00
30 minutes, run 2	50	144.76a	51.88	404.63
	55	0.00c	0.00	0.00
	60	0.00c	0.00	0.00
4 days	25	79.92a	32.82	194.89
	45	2.73b	0.94	7.92
	50	0.01c	0.00	0.05
	55	0.00c	0.00	0.00
	60	0.00c	0.00	0.00
21 days	25	46.52a	17.25	129.14
	40	0.00c	0.00	0.00

[a] Lower case letters in the columns of Most Probable Numbers indicate significant ($P < 0.05$) differences between the treatments.

Table 2. Most Probable Numbers (MPN) of infective units and 95% confidence intervals of infection of roots with *Polymyxa betae* after incubation in water or compost leachate under aerated or anaerobic (N_2-flushed) conditions at 25 and 40°C for 4 days (experiment B).

		Incubation temperature							
		25°C				40°C			
Incubation conditions				95% c.i.[a]				95% c.i.	
Aeration	Incubation medium	MPN (cfu g^{-1})		lower limit	upper limit	MPN (cfu g^{-1})		lower limit	upper limit
Yes	Water	25.91	ab[b]	8.58	78.31	8.00	b	3.25	19.70
No	Water	128.76	a	41.40	401.18	0.02	d	0.00	0.07
Yes	Compost leachate	14.75	b	6.71	32.47	0.45	c	0.27	0.76
No	Compost leachate	48.12	ab	16.85	137.63	0.01	d	0.00	0.05

[a] c.i. = confidence interval.

[b] Lower case letters in the columns of Most Probable Numbers indicate significant ($P < 0.05$) differences between the treatments.

60°C for both runs. At 55°C, nearly complete inactivation was found: > 99.9% in run 1 and 100% in run 2 as compared to incubation at 25°C. At an exposure time of 4 days, complete

inactivation was found at 55°C and at 50°C inactivation was > 99.9% (Table 1). After both 30 min and 4 days of incubation, a reduction > 96% was observed at 45°C incubation. After 21 days of incubation complete inactivation was found at 40°C.

The effect of leachate or anaerobic incubation depended on temperature of incubation. At 25°C, survival of *P. betae* was not affected by leachate or anaerobic incubation (Table 2). However, at 40°C, survival of *P. betae* in anaerobically incubated water was 99.7% less than in aerated water (Table 2). In aerobically incubated leachate, reduction was 94.4% compared to aerobically incubated water. Incubating the leachate anaerobically lead to a further reduction by 97.8% compared to incubation in leachate aerobically. VFA concentrations measured in the leachate incubated at 25 and 40°C directly after sampling (acidified to pH 2) were 164.8 mg l^{-1} acetic acid, 5.74 mg l^{-1} propionic acid, 2.61 mg l^{-1} butyric acid and 0.0 mg l^{-1} valeric acid.

Discussion

Literature on the composting conditions required for eradication of *P. betae* is scanty. Only two unrefereed reports cited by Sansford (2003) were found, which are difficult to interpret as experimental details are not known. One may wonder why the conditions to eradicate *P. betae* are so poorly known, as there is a desire to compost organic residues originating from sugar beet factories. A major reason is the difficulty involved in quantifying the density of resting spores of which a MPN-based bioassay combined with microscopic examination of the whole root system is the most precise method.

In our study, no survival was observed in aerated water at 60°C for incubation times of 30 min or longer. Since temperatures of 60°C are usually reached in Dutch composting plants for several days (pers. comm. J. Nooteboom, AVR AVIRA, The Netherlands 2005; T. Brethouwer, Essent, The Netherlands, 2005) we consider the current composting circumstances safe to eradicate *P. betae*. Although the lethal temperature of microorganisms usually is determined under aerated conditions in pure water for 30 min, testing for longer periods of incubation in the presence of compost leachate is more realistic. In addition, temporary anoxia occurs commonly during aerobic composting (Fernandes and Sartaj, 1997; Veeken et al., 2002; Beck-Friis et al., 2003), and then the lethal temperature is close to 40°C (this paper). Overall, our results indicate that complete eradication of *P. betae* is well guaranteed under the German norms for composting (i.e. 2 weeks > 55°C or 1 week > 65°C for open air composting, or 1 week > 60°C for closed facilities; Hogg et al., 2002). A decrease of the lethal temperature under conditions of increased salt concentration, acidified tap water and anoxia (in a mesophilic digester) as compared to sterile tap water was also reported for *Salmonella enterica* W775, *Escherichia coli* , and *Ralstonia solanacearum* (pers. comm. D. De Clercq, Katholic University Leuven, Belgium). Conditions of anoxia or presence of compost leachate at 35-37°C also led to inactivation of *Fusarium oxysporum*, *Salmonella typhimurium*, *R. solanacearum*, and *P. brassicae*, but not of *Sclerotium cepivorum* (Termorshuizen et al., 2003).

Increased inactivation under anoxic conditions or in the presence of compost leachate has been related to presence of volatile fatty acids (VFA). Tenuta et al. (2002) found 95% reduction in viable microsclerotia of *Verticillium dahliae* after exposure to 300 mg l^{-1} acetic acid for 4 days. The concentrations of VFAs we found were about half of those found by Tenuta et al. (2002) at pH 7.7. In our case the leachate was acidified after collection because at the prevailing high pH levels (7-8) of the leachate, the majority of volatile fatty acids occurs in non-toxic, dissociated form. Thus, at pH 7-8, the organic acid concentrations were likely much lower than at pH 2. Therefore, organic acids seem not to be the predominant factor causing inactivation of resting spores of *P. betae* in leachate and/or anaerobic circumstances in our experiments.

The single risk of survival of *P. betae* during compost consists of occurrence of dry pockets in compost heaps which may well develop if water channels are formed in the composts (Bollen and Volker, 1996). This risk could be minimized by regularly converting the compost heap.

In conclusion, we report here that resting spores of *P. betae* do not pose a threat under proper composting conditions. We demonstrated that the presence of leachate or anoxic conditions may lead to significant reductions in survival of *P. betae*. These effects are temperature dependent and may lead to considerably lower inactivation temperatures under compost heap conditions where temporal and local anaerobic conditions and toxic decomposition products widely occur.

Acknowledgements

This study was undertaken as part of the first author's PhD program and was funded by the EU project QLK5-CT-01442. We thank Prof. Dr. A.H.C. van Bruggen for critically reading this manuscript and D. Volker for technical assistance.

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Chapter 8

General discussion

How can compost as a tool to manage soilborne pathogens and as a substitute of peat be achieved? For this, a compost needs to be free of pathogens itself (Section II) in the first place. It should be kept in mind that a primary reason for composting organic material is the inactivation of plant pathogens. Secondly the effect of compost on plant pathogens resident in the substrate needs to be reliable and at least not disease conducive, and, preferably, disease suppressive (Section I). The focus in this study was primarily on predicting disease suppressive characteristics of compost in peat-based substrates.

Often disease suppression induced by compost is regarded as a general phenomenon. The implicit assumption often present in communications about the positive aspects of compost with respect to disease suppression is that it will be effective for multiple plants and for multiple composts, irrespective of the growing conditions such as soil type. However, it is widely known that compost quality is in no way a constant factor, being dependent of organic matter composition and composting conditions, including composting duration (Hoitink and Boehm, 1999). Furthermore, pathogens differ widely in their ecology; for example, some species are competition-sensitive, such as *Pythium ultimum* causing damping-off, some species are much less so (e.g. *Verticillium dahliae* causing wilt), and some species can be strongly affected by specific antagonists (e.g. *Gaeumannomyces graminis* affected by strains of *Pseudomonas fluorescens*). The idea behind this research thus was that different composts constitute substrates for different (groups of) microorganisms and hence may affect various plant pathogens differently. We also hypothesized that host species can be important: the chemical and biotic composition of the rhizosphere varies according to the host species involved and the ability of compost to influence the rhizosphere may vary accordingly. Variation between different batches of compost as well as their effects on the disease suppression of different pathosystems is dealt with in Chapters 2 and 3. A specific question was to what extent composts are able to affect the microbial rhizosphere composition of various plant species (Chapter 4). Because of the variable effects, showing a significant compost × pathosystem interaction with respect to disease suppression, an experiment was undertaken with the same basic questions but with only one pathogen, *Pythium ultimum*, tested on five different hosts.

A problem with the interpretation of results of compost research often arises with respect to the consistency of disease suppressiveness at a certain composting facility. Can results of one, or a few, experiments that quantified disease suppression be extrapolated to all composts with the same organic material and the same composting conditions? This problem is also apparent because true repetition of experiments is not possible in compost research. If compost is collected for repetition, the material will never have exactly the same conditions as the compost collected for the first experiment. An alternative is to store the collected compost, but it is likely that storage itself, together with the subsequent reactivation by rewetting or defrosting, depending on the type of storage, has an effect on compost characteristics. This

has been studied to some extent for soils (Tzeneva, 2006) and less so for composts, but the consequences of storage on the disease suppressive characteristics were not known. We compared three types of storage of eight composts with respect to wilt in flax caused by *Fusarium oxysporum* f. sp. *lini* (Chapter 5).

As stated above, the phytohygienic status of the composting process should be such that pathogens are absent in the compost. The material that is to be composted originates primarily from plant materials, and a variety of pathogens is likely to occur in them. Phytosanitation research and practice often focus on those pathogens that might be able to survive the temperature developed during the composting process. In Chapter 6, we consider that other factors need to be weighed also in order to arrive at a realistic estimation of risk. In Chapter 7, the conditions for eradication of *Polymyxa betae*, the vector of Beet Necrotic Yellow Vein Virus, causing rhizomania in sugar beet, are studied. We hypothesized that not only temperature but also anaerobic conditions and the presence of compost leachate determine the rate of inactivation of this pathogen.

Section I. Compost-induced suppression of soilborne plant pathogens

A wide range of studies report on compost-induced suppression of soilborne plant diseases (e.g. Hoitink and Fahy, 1986; Craft and Nelson, 1996; Diab et al., 2003; Scheuerell et al., 2005). In many of these studies, suppressiveness of one type of compost has, however, been tested against a single or a few pathogens only, while in reality a host plant may face infection by multiple pathogens. We investigated whether disease suppressive characteristics of composts are pathosystem-specific and whether these characteristics are correlated with physico-chemical, microbiological and faunal characteristics of the composts and compost-amended peat substrates (Chapter 2). Suppressiveness of compost-amended peat-based substrate was determined against *Verticillium dahliae* on eggplant, *Rhizoctonia solani* on cauliflower, *Phytophthora nicotianae* on tomato, *Phytophthora cinnamomi* on lupin, and *Cylindrocladium spathiphylli* on *Spathiphyllum* sp. and of compost-amended loamy soil against *Rhizoctonia solani* on *Pinus sylvestris* and *Fusarium oxysporum* f. sp. *lini* on flax.

In 54% of the bioassays significant disease suppression was observed while only 3% of the cases showed significant disease enhancement. We observed a strong compost × pathosystem interaction: not a single compost was suppressive to all diseases, while for each disease there was at least one compost that induced > 70% suppression. So, to achieve optimal disease control it would be best to first determine the most likely diseases in a given cropping system at a given location or country, followed by optimization of the compost for that particular pathosystem. For peat-based cultivations in greenhouses the range of possible soilborne pathogens is for several hosts not so large. It appeared difficult to relate disease suppression in specific pathosystems to biotic or abiotic parameters of the compost or of the compost/peat mix. Disease suppression induced by compost was not related to the traditional ecological groupings of plant pathogens. Regression analyses of disease suppression of the individual pathogens with parameters of compost-amended peat-based mixes revealed the following groupings: (1) competition-sensitive: *Fusarium oxysporum* and *Rhizoctonia solani* /

cauliflower; (2) rhizosphere-affected: *Verticillium dahliae*; (3) pH-related: *Phytophthora nicotianae*; and (4) specific/unknown: *R. solani* / pine, *P. cinnamomi*, and *Cylindrocladium spathiphylli.* It should be stressed that these regressions need confirmation in additional experiments. For group (1) it would be needed to select a range of composts that lead to differences in respiration after mixing with peat; for group (2) DGGE or related methodologies would need to be used; and for group (3) composts differing in pH could be used, but also one compost could be tested with a range of pH levels using, e.g., lime. Different mechanisms underlying compost-induced disease suppression for different pathosystems were also suggested by Scheuerell et al. (2005). In our study, soil was used for the pathosystems *F. oxysporum* / flax and *R. solani* / pine, and here a soil effect cannot be excluded. In all other studies in this thesis only peat was used. Indeed further studies should elucidate the role of soil type in the effects of compost on plant diseases (see for a recent review Janvier et al., 2006).

Disease suppression appeared to be better predicted by using characteristics of compost-amended peat substrates than by using those of pure composts (Chapter 2). Physico-chemical and biotic characteristics (culturable microorganisms and mesofauna components), however, could not explain the observed compost × pathosystem interaction. In Chapter 3 we therefore elaborated further on 12 of the composts and 5 of the pathosystems. We investigated the effects of compost amendments to peat-based substrate on the bacterial, actinomycete and fungal communities in relation to disease suppression against *Cylindrocladium spathiphylli*, *Phytophthora cinnamomi*, *P. nicotianae*, *Rhizoctonia solani*, and *Verticillium dahliae*. Suppression of the pathosystems *C. spathiphylli*, *P. cinnamomi* and *V. dahliae* was significantly lower for compost/peat mixes that had 16S-rDNA bacterial and/or actinomycete DGGE banding patterns that had higher similarity with the respective peats (denoted as 'peat-like composts') than with the respective composts. This was however reversed for *P. nicotianae* and *R. solani.* These results suggest that *C. spathiphylli*, *P. cinnamomi* and *V. dahliae* are controlled by introduced compost-specific bacteria and actinomycetes. Possibly the opportunistic pathogens *P. nicotianae* and *R. solani*, which cause seedling damping-off, took more advantage of the more nutrient-rich conditions of the compost/peat mix than the other pathogens which are more specialized in attacking older plants. However, more research needs to be done on this. In addition, our results suggest that suppression of *R. solani* may be related to low pH values. Compost/peat mixes that were peat-like with respect to bacteria or actinomycetes showed lower pH levels than the other compost/peat mixes. In line with this, positive correlations were found between disease suppression for the pathosystems *C. spathiphylli*, *P. cinnamomi* and *V. dahliae* respectively pH in the compost/peat mix. We recommend to carry out experiments where compost is manipulated in various ways to achieve a range of different levels of pH in the compost/peat mix and to test for pH effects on disease suppression and microbial composition of the bulk mix and rhizosphere.

The results of Chapter 3 may lead to a novel way to consider effects of compost on disease suppression. As the determination of disease suppression is prone to variation (because there are, inherent to the bioassays, many sources of variation, such as the pathogen used and growing conditions), it may be worthwhile first to screen composts on their ability to affect the microbial composition in the rhizosphere, since all root pathogens do need to pass

this zone in order to reach the plant. The hypothesis, then, would be that only composts that are able to change significantly the rhizosphere are good candidates for successful suppressors of disease.

Compost × pathosystem interactions are likely the result of a combination of a pathogen effect and an effect of plant species (Chapters 2 and 3). We aimed to gain more insight in the importance of plant species in this interaction (Chapter 4). Therefore, we investigated the interactions between plant species and compost type on the microbial population of the rhizosphere in relation to disease suppression of damping-off caused by *Pythium ultimum* on five host species. In 57% of the bioassays significant disease suppression was observed and there was no compost significantly stimulating disease. We observed significant effects of plant and compost and a significant compost × plant interaction, indicating that disease suppressiveness is compost and plant species dependent. We distinguished plants and composts that exerted a strong or weak influence on the rhizosphere microbial community, in accordance with the model of Garbeva et al. (2004). Relatively low disease suppression rates (< 20%) were observed for rhizospheres of plant species that showed high (> 70%) similarity between the rhizosphere bacterial DGGE banding patterns of a compost-amended mix and the non-amended peat (assigned as 'strong' plant species; cf. Chapter 4), while high or low disease suppression rates were observed for rhizospheres of plant species that showed low similarity ('weak' plant species). Composts that had high bacterial DGGE similarities between all various hosts tested ('strong' composts) showed higher disease suppression than those that had low similarities ('weak' composts). Thus we obtained indications that the composition of bacterial rhizosphere communities is determined by a balance between the relative strengths of the influence of the compost and the influence of the plant and that the result of this determines the degree of disease suppression. The consequence for research on disease suppression induced by composts is that certain model plants used in bioassays do not necessarily predict disease suppression in crops that are of agricultural importance. Our results also indicate that suppression of *Pythium ultimum* is primarily associated with rhizosphere bacterial communities rather than with actinomycete and fungal communities. Chapters 3 and 4 collectively indicate that both bulk parameters as well as rhizosphere parameters predict disease suppression. Differential compost-induced rhizosphere communities likely develop only if bulk parameters vary also, since the great majority of rhizosphere organisms is bulk soil-derived (Costa et al., 2006).

A factor constraining compost research is that repetition of experiments with the same batch of compost is difficult since during storage further decomposition of the organic material always occurs. Also, if different composts are to be compared, storage is often necessary since they usually cannot be collected within a single day. This topic is also relevant for the predictability of disease suppressiveness of commercial composts that are stored in bags until they are used. We investigated the effects of three months of storage under different conditions (dried and stored at 20°C, frozen at -20°C or cooled at 4°C) on disease suppressive characteristics against flax wilt caused by *Fusarium oxysporum* f. sp. *lini* (Chapter 5). In 75% of the bioassays significant disease suppression was observed and there was no compost significantly stimulating disease. Storing of composts led in general to relatively small effects on their properties to suppress Fusarium flax wilt, and resulted in

several cases in a significant increase in disease suppression. Only in 12% of the cases storage resulted in considerable losses of the original bacterial bands and a significant decrease in basal respiration, resulting in a significant decrease in disease suppression. A significant storage method × compost interaction was found with respect to suppression of Fusarium wilt, indicating that the effect of storage on disease suppression is compost-dependent. Significant changes in bacterial composition and microbial activity occurred as a result of all tested ways of storage. Relatively strong changes in bacterial composition and microbial activity due to storage had a relatively strong effect on disease suppression. These changes appeared to be compost-dependent. The cooling treatment resulted in the least deviation in disease suppression from the fresh compost, although the effect of freezing gave the most reliable results with the lowest standard deviation. In general, we concluded that the effects of storage on disease suppression of Fusarium wilt were mild. Thus, disease suppressive properties of compost may be maintained for a prolonged period of time if stored properly. Wet-storage under ambient conditions (such as is the case in e.g. garden shops) most likely is not a proper way of storing compost since then maturation of compost continues and consequently a change in disease suppression can be expected (Tuitert et al., 1992).

In some of our studies (Chapters 3-5), our conclusions are partially based on the results of DGGE banding patterns as measure of microbial composition. As DGGEs mainly detect dominant rDNAs, the quality of DNA extraction depends of edaphic factors, and one band is not necessarily linked to a single species, banding patterns do not necessarily represent biodiversity (Schmalenberger and Tebbe, 2003). Still, because of the relation between banding pattern similarities between samples and disease suppression, we show here that DGGE-analysis of 16S-rDNA rhizosphere samples can yield ecologically meaningful results. In the future more work needs to be done on identifying the organisms that lead to bands related with disease suppression. However, since often multiple bands, and not a single band, varied, we are inclined to think that disease suppression was not induced by a single specific microorganism.

Section II. Phytosanitary aspects of composting

Most plant pathogens are inactivated during proper aerobic composting (Bollen, 1989, 1993; Bollen and Volker, 1996; Ryckeboer, 2001). However, the use of compost is sometimes regarded as a potential hazard because of the potential presence of pathogens in composts. Indeed, survival of a few soilborne plant pathogens during composting has been reported (Noble and Roberts, 2003). We assessed phytohygienic risks associated with compost applications in agriculture (Chapter 6). Composting temperatures and the general working conditions at the composting plant are important factors that determine the phytohygienic status of a compost. For pathogens that may survive these conditions, however, knowledge on their occurrence in the biowaste and on the crops to be grown on the sites where the compost is to be applied is necessary. So, we propose to consider not only the composting conditions but also all other factors that may or may not lead to disease. It is, for example, useless to consider pathogens that are extremely rare or absent in the Netherlands. Likewise, it is useless

to consider pathogens that do not occur in certain types of compost as significant risk factors. In Dutch green composts (made from mainly woody materials originating from gardens and parks) the likelihood of heat-surviving pathogens occurring in arable crops is highly unlikely and need not be considered. Although vegetable, fruit and garden (VFG) compost does contain organic residues originating from hobby gardens, it is quite unlikely that root systems are present that contain *Polymyxa betae*. Another example is Tobacco Rattle Virus (TRV), that can resist composting conditions, but its vectors, the nematodes *Paratrichodorus* and *Trichodorus* spp., are readily inactivated during composting and the virus is unable to infect plants without its vector. Such considerations need to be made for all pathogens considered as potential risks, preferably as quantitative as possible. The result, the expected number of propagules surviving, needs to be weighed against the actual levels occurring in soils and the damage they might inflict.

Polymyxa betae is one of the few pathogens of which the inactivation temperature has not been determined, as the available quantification technique is quite time-consuming (Tuitert, 1990): to determine the inoculum density of one sample, a bioassay needs to be done involving the microscopic inspection of the whole root system of 24 9-weeks-old seedlings of sugar beet. Our goal was not only to investigate the effect of temperature but also the effect of anoxia, as these regularly occur during composting (Veeken et al., 2002). Termorshuizen et al. (2003) showed that survival patterns under anaerobic conditions can differ from those under aerobic conditions. We investigated the survival of resting spores of *Polymyxa betae* under aerobic and anaerobic conditions at various temperatures in a water suspension and in leachate from a compost heap (Chapter 7). No survival was observed in aerated water at 60°C for incubation times of 30 min or longer. Since temperatures of 60°C are usually reached in Dutch composting plants for several days we consider the current composting circumstances safe to eradicate *P. betae*. Although the lethal temperature of microorganisms usually is determined under aerated conditions in pure water for 30 min, testing for longer periods of incubation in the presence of compost leachate is more realistic. Actual inactivation temperatures appeared to be significantly lower in the presence of compost leachate or anoxic conditions. Under these circumstances the lethal temperature was close to 40°C. Overall, our results indicate that complete eradication of *P. betae* is well guaranteed under the German norms for composting (i.e. 2 wk > 55 °C or 1 wk > 65 °C for open air composting, or 1 wk > 60°C for closed facilities; Hogg et al., 2002).

Conclusions

Disease suppression induced by compost appeared to be dependent on compost type, pathosystem, plant species, the degree of rhizosphere and peat substrate colonization by compost organisms and in many cases mutual interactions between these. Moreover, changes in microbial composition and microbial activity of composts after disturbance as a result of storage led to changes in disease suppression. Our results may be important in contributing to a general understanding of the cause of variation in disease suppression as induced by composts. Prediction of disease suppression should be focused on specific pathogens and

specific plant species, given the variation in disease suppression between different pathogens and different plant species. This will require research on specific plant-pathogen combinations that are of importance in horticulture and agriculture. In addition, we demonstrated that the type of compost and the colonization of the peat substrate and rhizospheres by compost-borne organisms are of importance in the prediction of disease suppression. We observed that colonization of the peat substrate and the rhizosphere was positively correlated with oxygen uptake rate, organic matter content, total C, total N, pH and EC and negatively correlated to total C/total N ratio. Future research should verify which factors of compost (i.e. biotic and abiotic composition) and plants (e.g. composition and quantity of root exudates) determine the colonization by compost microorganisms. Naturally good colonization of the bulk substrate and the rhizosphere by compost organisms does not guarantee high disease suppression rates, but it can be a first step in identification of disease suppressive composts.

Quantification of phytosanitary risks is still difficult since predictive data are often lacking. In general, it seems safe to use compost although the survival of some pathogens is still unknown. Our results indicate that complete eradication of *Polymyxa betae* is well guaranteed under the German norms for composting.

For the composting sector the results are interesting in various ways:

- The results indicate that there is scope for development of products, designed specifically for certain pathosystems.
- Different compost batches sampled at different times from the same composting company were to some extent comparable with respect to disease suppression (Chapter 2). This could be somewhat unexpected since the composition of the organic material varies during the year, but the results are in line with the rather mild effects of compost storage we report in Chapter 5. It would be interesting to investigate the hypothesis that a composting facility has a set of microorganisms that are 'endemic' for that facility and that is continuously recycled through the facility.
- In general, disease suppression or absence of disease suppression was the rule, and disease stimulation was the exception. So, even if no 'specialty product' specific for a pathosystem would be designed, it would be recommendable to apply compost for its disease suppressive characteristics. Better multiple regressions were obtained if biotic and/or abiotic characteristics of the compost/peat mix were used than if pure compost characterics were used. This conclusion is quite logical, since it is not the compost but the compost/peat mix where the plants, the pathogens and their antagonists are living in. However, this interferes with the wish of the composting industry to design composts that can de assigned 'disease suppressive' based on some of their inherent characteristics. For the time being it is most advisable not to focus on parameters in pure composts that predict disease suppression but first, as said above, to confirm the regressions obtained for disease suppression in relation to the characteristics measured in the compost/peat mix (Chapters 2 and 3). The difficulty in predicting disease suppression for a given pathogen based on pure compost characteristics only is likely to become even greater if the goal is to apply compost to arable soils, since then also the variability of soil characteristics has to be taken into account.
- For assessing the phytosanitary risks associated with the application of compost it is

necessary to include all risk factors and not only those occurring during composting. It seems safe to use composts produced from Vegetable, Fruit and Garden (VFG) compost although the conditions for inactivation of some pathogens are still unknown. With respect to the assessment of the phytosanitary risks associated with composting itself, the determination of the degree of inactivation as function of temperature likely overestimates the temperature needed during composting, since other factors such as temporary anoxia, including the formation of a range of compounds formed during anoxia, also contribute to inactivation of pathogens.

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Summary

In Western Europe, approximately 25% of the 200 million tons of municipal solid waste that is generated each year is of organic origin and therefore compostable. Presently 35% of this organic waste is composted, resulting in 9 million tons of compost, and used mainly in agriculture, horticulture or hobby gardens. The remainder 65% of compostable waste is either used for biogas production or is incinerated or dumped on landfill sites. Increasing the opportunities to use compost in horticulture and agriculture as a (potting) soil amendment and nutrient source for plants and the soil microbial community would contribute to the recycling of waste and reduce the use of non-renewable peat and artificial fertilizers.

The willingness of farmers and horticulturalists to use compost is directly related to various quality aspects of the compost. Firstly, composts are commonly used in agriculture as an organic amendment to maintain or increase soil organic matter content which is essential for maintaining soil quality by improving the biological, physical and chemical soil conditions. Secondly, growers can benefit from an increased use of compost by the fact that many composts have disease suppressive properties and, therefore, use of disease suppressive compost will reduce crop losses caused by soilborne diseases. Thirdly, sanitation plays an important role in the use of compost. Risks of pathogen spread through compost have to be minimized and therefore proper sanitation measures have to be taken during the composting process.

This thesis is concerned with quality aspects of compost, varying from disease suppressive properties to phytosanitary aspects. The study aimed:

- To determine whether disease suppressive characteristics of composts are pathosystem-specific and whether these characteristics are correlated with physico-chemical, microbiological and faunal characteristics of the composts and compost-amended peat substrates.
- To determine the effects of amendment of compost to peat-based substrate on the bacterial, actinomycete and fungal communities in relation to disease suppression.
- To determine the disease suppressive characteristics of a variety of composts for *Pythium ultimum* as function of host plant species.
- To determine the effects of three months of storage under different storing conditions (dry, frozen and cool) on disease suppressive characteristics against flax wilt caused by *Fusarium oxysporum* f. sp. *lini,* and relate these effects to any possible changes in microbial composition or microbial activity.
- To assess phytohygienic risks associated with compost utilization in horticulture and agriculture.
- To determine the composting conditions required for eradication of *Polymyxa betae*.

In Section I of this thesis, disease suppressive properties of composts are investigated aiming to get more insight in which biotic factors (microbial composition of the compost and compost/peat mixes, plant species and pathogen) and abiotic factors (physico-chemical characteristics of the compost and compost/peat mixes) are decisive in disease suppression. Ultimately this may lead to a more effective use of compost as a biological control measure to

manage soilborne pathogens. In Section II of this thesis, phytosanitary aspects of composts are studied. Acknowledging that risks of pathogen spread through compost utilization should be minimized, we investigated the possibilities of phytosanitary risk assessment and determined the conditions required to eradicate the soil borne pathogen *Polymyxa betae*.

Section I. Compost-induced suppression of soilborne plant pathogens

In Chapter 2, the ability of composts to induce disease suppression was tested for 18 composts against 7 pathosystems. Compost mixed with peat (20% vol./vol.) was tested with respect to *Cylindrocladium spathiphylli* on *Spathiphyllum* sp., *Phytophthora nicotianae* on tomato, *P. cinnamomi* on lupin, *Rhizoctonia solani* on cauliflower, and *Verticillium dahliae* on eggplant; and compost mixed with loamy soil (20% vol./vol.) was tested with respect to *Fusarium oxysporum* f. sp. *lini* on flax and *Rhizoctonia solani* on *Pinus sylvestris*. From the 120 bioassays involving 18 composts and 7 pathosystems, significant disease suppression was found in 54% of the cases while only 3% of the cases showed significant disease enhancement. Pathogens were affected differently by the composts. In general, prediction of disease suppression was better when parameters derived from the compost mixes were used rather than those derived from the pure composts. Regression analyses of disease suppression of the individual pathogens with parameters of compost-amended peat-based mixes revealed the following groupings: (1) competition-sensitive: *F. oxysporum* and *R. solani* / cauliflower; (2) rhizosphere-affected: *V. dahliae*; (3) pH-related: *P. nicotianae*; and (4) specific/unknown: *R. solani* / pine, *P. cinnamomi*, and *C. spathiphylli*.

Twelve of the composts studied in Chapter 2 were further analysed. The disease suppressive properties tested in compost/peat mixes against *Cylindrocladium spathiphylli*, *Phytophthora cinnamomi*, *P. nicotianae, Rhizoctonia solani* and *Verticillium dahliae* was tested in relation to the microbial composition of the composts and compost/peat mixes as determined by DGGE. We tested the hypothesis that disease suppression is dependent on the ability of compost to change the microbial community of the substrate. Some of the compost/peat mixes showed higher similarities with the microbial composition of the pure peat than with the respective composts and were denoted as ‘peat-like’. On average, disease suppression of peat-like mixes with respect to bacteria and/or actinomycetes was significantly lower for *V. dahliae*, *P. cinnamomi*, and *C. spathipylli* but significantly higher for *R. solani* and *P. nicotianae* than disease suppression of the other mixes. We demonstrated that compost-borne microbial communities may or may not survive after mixing with peat, and that this ability may determine the extent of disease suppression. The ability of compost to change the microbial community of the substrate was significantly related to the pH of the compost/peat mix and the organic nitrogen content and microbial composition of the pure compost.

In Chapter 4, suppression of nine composts against damping-off caused by *Pythium ultimum* for five host species was determined. Analysis of variance indicated a significant ($P < 0.01$) compost × plant species interaction, indicating that disease suppression depends on the combination of type of compost and plant species. Relatively low disease suppression

rates (< 20%) were observed for plant species that showed high (> 70%) similarity between the rhizosphere bacterial DGGE banding patterns of a compost-amended mix and the non-amended peat (assigned as 'strong' plant species), while high or low disease suppression rates were observed for plant species that showed low similarities ('weak' plants). Composts that had high bacterial DGGE similarities between all various hosts tested ('strong' composts) showed higher disease suppression than those that had low similarities ('weak' composts). Our results suggest that disease suppression may be predicted by a combination of 'weak' plants and 'strong' composts. For prediction of disease suppression of Pythium damping-off the effects of both the host and the amended substrate on rhizosphere bacterial communities should be investigated.

A factor constraining compost research is that repetition of experiments with a similar batch of compost is impossible since storage affects the organic material including the microbial communities. This was investigated in Chapter 5. The objective was to study the effects of 3 types of storage (dry at +20°C; freezing at -20°C and cooling at +4°C) of 8 composts for 12 wk on the suppression of *Fusarium oxysporum* f. sp. *lini* induced by mixes of composts with peat substrate (20/80%, vol./vol.). A significant ($P < 0.0001$) storage method × compost interaction was found with respect to suppression of fusarium wilt of flax, indicating that the effect of storage type on disease suppression is compost-dependent. For 7 composts storage had no (13 cases) or a significantly positive effect (8 cases) on disease suppression and for 1 compost there was a significant negative effect of storage on disease suppression. Significant changes in microbial activity and 16S-rDNA DGGE banding patterns of the composts were observed as a result of all tested ways of storage and these changes could be related to changes in disease suppression: relatively strong changes in microbial activity and bacterial composition due to storage had a relatively strong effect on disease suppression. The cool storage treatment (4°C) resulted in the least deviation in disease suppression from the fresh compost, although the effect of freezing gave the most reliable results with the lowest standard deviation.

Section II. Phytosanitary aspects of composting

Composting is an efficient process for treatment of biowastes in order to obtain stable organic matter that contributes to soil microbial activity. Proper composting guarantees the eradication of the majority of plant pathogens residing in biowastes due to heat generated during the thermophilic phase of the composting process. However, survival at peak compost temperatures > 62°C for 21 days has been reported for few soilborne plant pathogens. Phytosanitary risk assessment associated with compost utilization is therefore desired and should not only focus on the survival of plant pathogens during the composting process but should also pay attention to the composition of the original waste and several aspects related to compost utilization. In Chapter 6 the idea is put forward that in order to assess the phytosanitary risks associated with application of composts in agriculture the focus should not only be on survival aspects during composting. Although a few plant pathogens are heat resistant and they may survive a properly monitored and controlled composting process, the

phytosanitary risks associated with these pathogens may be limited. For example, if it is highly unlikely that the organic material that is to be composted contains such pathogens, their survival during composting is a non-issue. The following parameters are considered to be key-factors: (1) the proportion of host biomass relative to the total quantity of biowaste, (2) the proportion of host infected with a pathogen, (3) the density of infected host material, (4) the proportion of propagules of a pathogen that survived the process, and (5) the threshold density of a pathogen in soil above which disease of the host is expected to develop. While the first two parameters may be rather easy to estimate and information on survival of many plant pathogens can be obtained from the literature, little knowledge exists on the density of the pathogens in host materials or on threshold values. This applies particularly to virus diseases. The phytosanitary risk of several types of plant pathogens is discussed in some detail and recommendations are given for testing of a composting process for phytohygienic safety.

Temperature is considered the major factor determining the inactivation of plant pathogens during composting under aerobic conditions while toxic agents are considered to be responsible for pathogen inactivation during decomposition under anaerobic conditions. Composting conditions to eradicate pathogens have been defined on the basis of standard experimental conditions of 30 minutes of incubation in aerated water. The conditions prevailing during the thermophilic phase of composting are different from the aerated water suspensions used in standard test conditions. During the thermophilic phase of composting, which may last several days, temporary and local anaerobic conditions widely occur, leading to decomposition products that may be toxic to multiple organisms. Thus, in a composting heap, combined effects of temperature and anaerobic circumstances may become responsible for pathogen eradication. One of the test organisms to determine the phytosanitary status of a composting process that has been used extensively in the Netherlands is *Polymyxa betae*, the vector of beet necrotic yellow vein virus (BNYVV) in sugar beet. Remarkably, *P. betae* is one of the few pathogens of which the inactivation temperature has not been determined. In Chapter 7, the survival of resting spores of *P. betae* was determined under aerobic (30 min, 4 and 21 days) and anaerobic (4 days) conditions under several temperature regimes in a water suspension and in leachate extracted from an aerobic compost heap. In water under aerobic conditions the lethal temperature was 60, 55, and 40°C for exposure times of 30 min, 4, and 21 days respectively. The effect of compost leachate and/or anaerobic conditions on survival of *P. betae* depended on temperature. After incubation for 4 days at 20°C, no significant effects of anaerobic conditions or leachate on the survival of *P. betae* were found. However, at 40°C for 4 days under anaerobic conditions, survival of *P. betae* was 0.3% compared to survival under aerobic conditions in water at the same temperature. In leachate taken from an aerobic compost heap, aerobically incubated at 40°C for 4 days, survival of *P. betae* was 6% compared to survival in water at the same temperature. Since anaerobic spots are prevalent in aerobic compost heaps, especially during the thermophilic phase, actual inactivation temperatures under composting conditions are likely to be lower than the temperatures we found for eradication in water under aerobic conditions.

For the composting sector the results are interesting in various ways:

- There is scope for development of products, designed specifically for certain

pathosystems.

- Different compost batches sampled at different times from the same composting facility were to some extent comparable in terms of disease suppression.
- Significant disease suppression or no effect on disease is the rule, disease stimulation the rare exception.
- Phytosanitary risk assessment of compost needs to include all risk factors and not only those occurring during composting. It seems safe to use composts produced from Vegetable, Fruit and Garden (VFG) compost even though the conditions for inactivation of some pathogens are still unknown.
- The determination of the degree of inactivation as function of temperature likely overestimates the temperature needed during composting.

Samenvatting

In West-Europa bestaat zo'n 25% van de 200 miljoen ton huishoudelijk afval uit organische resten, waarvan ca. 35% gecomposteerd wordt, wat resulteert in 9 miljoen ton compost. Deze compost wordt toegepast in de land- en tuinbouw en in hobbytuinen. De rest van het organisch afval wordt vergast of gestort. Een verdere toename in de afzet van compost draagt bij aan het hergebruik van grondstoffen en beperkt de noodzaak tot gebruik van onvervangbare grondstoffen, zoals veen voor potgrond en kunstmest.

Compost wordt aangewend om het organisch-stofgehalte van de bodem te verhogen en om de grond of het substraat meer ziektewerend te maken, met name tegen bodemgebonden plantenpathogenen. De bereidheid van agrarische ondernemers om compost toe te passen hangt samen met de kwaliteit van compost. Het verschil tussen compost en niet-gecomposteerde organische gewasresten is dat tijdens compostering de in de gewasresten aanwezige plantenpathogenen afgedood worden, door de hitte die tijdens het composteringsproces ontstaat.

Dit proefschrift behandelt twee kwaliteitsaspecten van compost: ziektewerendheid en hygiëne. Het doel van het onderzoek was:

- Te bepalen of de ziektewerende eigenschappen van compost pathosysteem-specifiek zijn en of ziektewering gecorreleerd is met fysisch-chemische of biotische eigenschappen van de composten en van de compost/veen-mengsels.
- Het effect vast te stellen van menging van veen met compost op de bacteriële, actinomyceten- en schimmelgemeenschappen en hun relatie met ziektewerende eigenschappen.
- Te onderzoeken of de ziektewerende eigenschappen van composten tegen omvalziekte veroorzaakt door *Pythium ultimum* afhankelijk is van de plantensoort.
- Het effect van bewaring van compost op de ziektewerendheid tegen verwelking veroorzaakt door *Fusarium oxysporum* f. sp. *lini* in vlas vast te stellen.
- De fytosanitaire risico's in te schatten van het gebruik van compost in de land- en tuinbouw.
- De dodingscondities vast te stellen die tijdens compostering nodig zijn voor *Polymyxa betae*, de veroorzaker van rhizomanie in suikerbiet.

In Sectie I van dit proefschrift worden de aspecten met betrekking tot de ziektewerende eigenschappen van compost behandeld. Aandacht wordt vooral besteed aan welke biotische factoren (microbiële samenstelling van de composten en compost/veen-mengsels) en abiotische factoren (fysisch-chemische samenstelling van de composten en compost-veenmengsels) gerelateerd zijn aan ziektewering. Uiteindelijk doel is te komen tot een meer effectief gebruik van compost als doelgericht middel om bodemgebonden ziekten te beheersen. In Sectie II van dit proefschrift worden de fytosanitaire aspecten van compost behandeld. Terwijl risico's ten aanzien van aanwezigheid van ongewenste micro-organismen nooit uitgesloten kunnen worden, is een realistische risico-inschatting dringend gewenst.

Sectie I. Compost-geïnduceerde ziektewering van bodemgebonden plantenpathogenen

In hoofdstuk 2 werd voor 18 composten de ziektewerende werking vastgesteld tegen 7 bodemgebonden plantenpathogenen. Compost gemengd met veen (20% compost, 80% veen) werd getoetst met betrekking tot de plantenpathogenen *Verticillium dahliae* (veroorzaker van verwelking in vele planten, hier getoetst op aubergine), *Rhizoctonia solani* (wortelpathogeen van zeer vele plantensoorten, hier getoetst op kiemplanten van bloemkool, die na aantasting omvallen), *Phytophthora nicotianae* (veroorzaker van wortelrot bij veelal jonge planten, getoetst op tomaat), *Phytophthora cinnamomi* (veroorzaker van wortelrot bij vele plantensoorten, hier getoetst op lupine), en *Cylindrocladium spathiphylli* (veroorzaker van wortelrot bij Spathiphyllum); compost gemengd met leemgrond (20% compost, 80% leemgrond) werd getoetst met betrekking tot de plantenpathogenen *Rhizoctonia solani* (getoetst op kiemplanten van Grove den) and *Fusarium oxysporum* f. sp. *lini* (veroorzaker van verwelking bij vlas). Van de 120 biotoetsen met 18 composten en 7 pathosystemen (voor één van de pathogenen werden 6 composten niet getoetst) werd statistisch significante ziektewering gevonden in 54% van de gevallen. Slechts in 3% van de toetsen werd significante stimulering van de ziekte door compost waargenomen. De pathogenen bleken verschillend op de composten te reageren. Voorspelling van ziektewering was beter op basis van eigenschappen van het compostmengsel (compost/veen of compost/leemgrond) dan op basis van eigenschappen van de ongemengde compost. Regressie-analyse van ziektewering op de individuele pathosystemen met compost/veen-mengsels leidde tot de volgende groepen: (1) competitie-gevoelig: *F. oxysporum* en *R. solani* / bloemkool; (2) rhizosfeer-beïnvloed: *V. dahliae*; (3) pH-gerelateerd: *P. nicotianae*; en (4) specifiek/onbekend: *R. solani* / Grove den, *P. cinnamomi*, en *C. spathiphylli*.

Twaalf van de composten uit hoofdstuk 2 werden verder onderzocht in hoofdstuk 3. De microbiële samenstelling van de compost/veen-mengsels werd nader geanalyseerd met een moleculaire techniek (DGGE) en gerelateerd aan ziektewering tegen bovenvermelde *Cylindrocladium spathiphylli*, *Phytophthora cinnamomi*, *P. nicotianae, Rhizoctonia solani* and *Verticillium dahliae*. We testten de hypothese of ziektewering voorspeld kan worden aan de hand van door de compost geïnduceerde microbiële veranderingen in de rhizosfeer. De compost/veen-mengsels die wat betreft bacteriën en actinomyceten een relatief grote overeenkomst vertoonden met die van de veen hadden ook een relatief geringe ziektewerende werking voor wat betreft *Verticillium dahliae*, *Phytophthora cinnamomi* en *Cylindrocladium spathiphylli*, terwijl juist het omgekeerde het geval was voor wat betreft *Rhizoctonia solani* en *Phytophthora nicotianae*. We concluderen dat het overgaan van microorganismen uit compost naar het compost/veen-mengsel een determinant kan zijn voor ziektewering, althans voor sommige plantenpathogenen. De zuurgraad (pH) en het organisch-stikstofgehalte kan hierbij een rol spelen.

In hoofdstuk 4 werd onderzocht of ziektewering geïnduceerd door compost tegen *Pythium ultimum*, de veroorzaker van omvalziekte bij kiemplanten van veel plantensoorten, afhangt van de plantensoort. Hiertoe werd de ziektewerende werking van 9 composten bepaald tegen *Pythium ultimum* bij 5 plantensoorten: erwt, komkommer, peen, suikerbiet en tomaat. Verschillende plantensoorten bleken inderdaad verschillend te reageren op de

composten; met andere woorden, een statistisch significante interactie tussen compost en plantensoort werd gevonden. Relatief geringe ziektewering (<20%) werd waargenomen voor die plantensoorten waarvoor een hoge (>70%) gelijkenis werd gevonden in de bacteriële samenstelling van de rhizosfeer van planten in de compost/veen-mengsels en die in het pure veen. Deze plantensoorten werden verder aangeduid als 'sterke' plantensoorten, omdat relatief weinig compostorganismen teruggevonden werden in de rhizosfeer van de planten die in het compost/veen-mengsel stonden. Daarentegen werden zowel hoge als lage waarden voor ziektewering gevonden voor de andere plantensoorten ('zwakke' soorten). De resultaten suggereren dat de hoogste ziektewering verwacht kan worden door een combinatie van 'zwakke' planten en 'sterke' composten. Meer in het algemeen impliceren de resultaten dat ziektewering tegen een pathogeen bij een bepaalde plantensoort niet zomaar mag worden geëxtrapoleerd naar die van een andere plantensoort.

Een probleem bij onderzoek aan compost is dat bij herhaling van een experiment de originele compost verandert, of deze nu bewaard wordt onder bepaalde omstandigheden, of dat deze opnieuw wordt verzameld bij een composteerder. Dit was het onderwerp van onderzoek in hoofdstuk 5. Het doel was om het effect van 3 typen van bewaring (droog bij +20°C; bevroren bij -20°C en gekoeld bij +4°C) gedurende 12 weken van 8 composten te onderzoeken op de ziektewerende werking tegen *Fusarium oxysporum* f. sp. *lini* in vlas. De bewaarde composten werden gemengd met veen (20% compost, 80% veen) en de ziektewerendheid werd onderzocht in een biotoets. Een statistisch significante interactie tussen type bewaring en compost werd gevonden. Voor 7 composten had bewaring geen of een positief effect op ziektewering en voor één compost was er een duidelijk negatief effect. Er kon een verband worden gelegd met microbiële activiteit en met bacteriële samenstelling op basis van DGGE-patronen: relatief sterke veranderingen in microbiële activiteit en bacteriële samenstelling ten gevolge van bewaring hadden ook relatief sterke effecten op de ziektewering. De gekoelde bewaringsmethode (4°C) had de geringste afwijking in ziektewering ten opzichte van de verse compost tot gevolg, terwijl de gevolgen van bevriezen de geringste standaardafwijking te zien gaf.

Sectie II. Fytosanitaire aspecten van compost

Compostering is een proces waarbij organisch afval omgezet wordt naar een stabiele organische stof die bij kan dragen tot verhoogde bodemmicrobiële activiteit en een verhoogd organisch-stofgehalte van de bodem. Een juiste wijze van composteren garandeert de doding van in het organisch afval aanwezige plantenpathogenen, wat met name veroorzaakt wordt door de hoge temperaturen die bereikt worden. Er zijn echter enkele pathogenen waarbij de doding tijdens composteren enigszins problematisch is. Overleving tijdens temperaturen van meer dan 62°C gedurende 21 dagen zijn gerapporteerd voor enkele bodempathogenen. De risico's die hierdoor ontstaan bij de toepassing van compost zijn nog maar nauwelijks in kaart gebracht: normaliter wordt alleen in beschouwing genomen of het composteringsproces kan leiden tot doding van deze pathogenen, maar aspecten of deze pathogenen aanwezig kunnen zijn in het organisch afval en of eventueel overleefde pathogenen daadwerkelijk voor schade

kunnen zorgen dienen bij een risico-analyse ook meegenomen te worden. Dit wordt verder uitgewerkt in hoofdstuk 6. In ieder geval zijn de volgende factoren van belang: (1) het aandeel in organisch afval van de waardplant met mogelijk een risicopathogeen, (2) het aandeel van de waardplant dat daadwerkelijk geïnfecteerd is met het risicopathogeen, (3) de dichtheid van het risicopathogeen, (4) de mate van overleving van het risicopathogeen gedurende de compostering, en (5) de hoeveelheid risicopathogeen per oppervlakte-eenheid (op de plaats waar de compost toegepast gaat worden) waarbij daadwerkelijk schade is te verwachten. Als deze factoren worden meegenomen dan neemt in het algemeen het fytosanitaire risico van toepassing van gecomposteerd organisch afval in de land- en tuinbouw af. Dit wordt aan de hand van een aantal voorbeelden verder uitgewerkt. Een aantal risicopathogenen is bijvoorbeeld uiterst zeldzaam.

De temperatuur tijdens de thermofiele fase van het composteringsproces wordt beschouwd als de belangrijkste factor die doding van plantenpathogenen bewerkstelligt tijdens aërobe compostering, terwijl toxische fermentatieproducten als oorzaak worden beschouwd voor de afdoding bij mesofiele anaërobe vergisting. De condities van afdoding voor pathogenen zijn veelal bepaald onder standaardomstandigheden van 30 minuten incubatie in geaëreerd water bij verschillende temperaturen. Deze condities verschillen echter met die van het aërobe composteringsproces: de thermofiele fase duurt veelal verscheidene dagen en tijdelijke en lokale anaërobe omstandigheden ontstaan in grote delen van de composthoop. Daarom werd in hoofdstuk 7 onderzocht of gecombineerde condities van verhoogde temperaturen en anaërobe omstandigheden leiden tot meer doding dan bij alleen verhoogde temperaturen. Dit werd onderzocht aan *Polymyxa betae*, de vector van het Bietenrhizomanievirus (BNYVV) in suikerbiet, waarvan de dodingstemperatuur nog niet bekend was. Rustsporen van *P. betae* werden aëroob (gedurende 30 minuten en 4 en 21 dagen) en anaëroob (gedurende 4 dagen) bij verschillende temperaturen geïncubeerd in water en in lekwater van een aërobe composteringsinstallatie. Onder aërobe omstandigheden was de dodingstemperatuur 60, 55 en 40°C bij een incubatieduur van respectievelijk 30 minuten en 4 en 21 dagen. Het effect van incubatie in lekwater en van incubatie onder anaërobe omstandigheden hing af van de temperatuur. Na incubatie gedurende 4 dagen bij 20°C werd geen effect van lekwater of anaërobe omstandigheden op de overleving van *P. betae* waargenomen, maar bij incubatie bij 40°C gedurende 4 dagen onder anaërobe omstandigheden bedroeg de overleving van *P. betae* slechts 0.3% vergeleken met de overleving onder aërobe omstandigheden in water. Ook was er een sterk effect van incubatie in lekwater onder aërobe omstandigheden: hier was de overleving bij 40°C 6% ten opzichte van incubatie in water. De conclusie is dat toetsing van de overleving van pathogenen in water de omstandigheden in een composthoop niet juist weerspiegelen.

Voor de compostsector zijn de resultaten van dit proefschrift in verschillende opzichten interessant:

- Er is ruimte voor de ontwikkeling van *special products* voor bepaalde gewassen.
- Verschillende compostmonsters van dezelfde composteringsinstallatie, genomen op verschillende tijdstippen, waren tot op zekere hoogte vergelijkbaar met elkaar wat betreft ziektewering.
- Compost heeft óf een statistisch significante ziektewering, óf geen effect op ziektewering

(afhankelijk van plantensoort, pathogeen en compostmonster); ziektestimulering is komt slechts bij uitzondering voor.

- Bij inschatting van de fytosanitaire risico's van toepassing van compost dienen alle risicofactoren in ogenschouw genomen te worden en niet uitsluitend factoren die te maken hebben met het composteringsproces zelf. Het gebruik van compost geproduceerd van groente-, fruit- en tuinafval (GFT) kan uit fytosanitair oogpunt als veilig worden beschouwd, hoewel de omstandigheden voor overleving van enkele plantenpathogenen nog niet bekend zijn.
- Bepaling van de dodingstemperatuur van organismen in water geeft een overschatting van de dodingstemperatuur in een composthoop.

Curriculum Vitae

Ik werd geboren op 15 augustus 1973 te Zoeterwoude-Rijndijk (Zuid-Holland) en groeide op in mijn geboorteplaats. In 1991 behaalde ik mijn VWO-diploma met exacte vakken en biologie aan het Bonaventura College te Leiden. In datzelfde jaar begon ik aan de studie Agrosysteemkunde aan de toenmalige Landbouwuniversiteit te Wageningen. Naast mijn studie besteedde ik veel tijd aan mijn studentenvereniging (SSR-W) en reizen (naar onder andere Zuid-Oost Europa en de Filippijnen). Hierdoor geïnspireerd koos ik voor de studie Bodem, Water en Atmosfeer aan Wageningen Universiteit. Ik verrichtte modelmatig onderzoek naar de correcte parameterisatie van een model ter beschrijving van de afbraak van organische stof in landbouwgronden bij de vakgroep Bodemkunde en Plantenvoeding en deed onderzoek naar verschralingsbeheer van natte, soortenrijke graslanden bij de vakgroep Terrestrische Oecologie en Natuurbeheer.

In juli 2000 betrok ik een functie als applicatie-ontwikkelaar bij IQUIP Informatica B.V. te Diemen en zette ik me in voor de correcte ontsluiting en update van databases van verzekeringen en de belastingdienst. Omdat ik de ‘groene’ aspecten van de landbouw miste trok ik in augustus 2001 naar Zuid-Frankrijk alwaar ik met mijn gezin tegen kost en inwoning op een biologisch melkvee bedrijf / kaasmakerij werkte. In maart 2002 begon ik aan mijn promotieonderzoek bij de leerstoelgroep Biologische Bedrijfssystemen aan Wageningen Universiteit, onder leiding van prof. dr. ir. Ariena van Bruggen en dr. ir. Aad Termorshuizen. Mijn belangrijkste expertise en wetenschappelijke interesses liggen op het gebied van bodemgezondheid en bodemvruchtbaarheid in het algemeen en in het bijzonder in relatie tot het gebruik van organisch materiaal. Vaardigheden als statistisch analyseren, modelleren en programmeren voor wetenschappelijke analyses mag ik daarbij graag gebruiken.

List of publications

Gaag, D.J. van der, Noort, F.R. van, Stapel-Cuijpers, L.H.M., Kreij, C. de, Termorshuizen, A.J., Rijn, E. van, Chen, Y., Zmora-Nahum, S., in preparation. The use of Dutch composted green waste in peat-based substrates: suppressiveness against soil-borne diseases and fertilization.

Rijn, E. van, Termorshuizen, A.J., Overbeek, L. van, Speksnijder, A., 2007. The effect of amending peat-based substrate with 12 composts on the microbial communities in bulk compost/peat mixes in relation to disease suppression against 5 pathosystems. Soil Biology and Biochemistry, submitted.

Rijn, E. van, Termorshuizen, A.J., Blok, W.J., Spyropoulos, V., 2007. Compost-induced suppression of damping-off caused by *Pythium ultimum* is host and compost dependent. Soil Biology and Biochemistry, submitted.

Rijn, E. van, Termorshuizen, A.J., Bruggen, A.H.C. van, 2007. Storage method affects disease suppression of flax wilt induced by composts. Soil Biology and Biochemistry, submitted.

Rijn, E. van, Termorshuizen, A.J., 2007. Conditions required for eradication of *Polymyxa betae* during composting. Journal of Phytopathology, accepted.

Termorshuizen, A.J., Rijn, E. van, Gaag, D.J. van der, Alabouvette, C., Chen, Y., Lagerlöf, J., Malandrakis, A.A., Paplomatas, E.J., Rämert, B., Ryckeboer, J., Steinberg, C., Zmora-Nahum S., 2006. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. Soil Biology and Biochemistry 38: 2461-2477.

Termorshuizen, A.J., Rijn, E. van, Blok, W. J., 2005. Phytosanitary aspects of composts. Compost Science & Utilization 13: 108-115.

Blok, W.J., Rijn, E. van, Termorshuizen, A.J., Volker, D., 2005. Compostonderzoek aan de leerstoelgroep Biologische Bedrijfssystemen, Wageningen Universiteit; thema bodemweerbaarheid. Gewasbescherming 36: 212-215.

Termorshuizen, A.J.; Blok, W.J.; Lozano, J.; Rijn, E. van; Volker, D., 2005. Ziektewerendheid van compostonderzoek. Gewasbescherming 36: 178-179.

Gaag, D.J. van der, Rijn, E. van, Termorshuizen, A.J., 2004. Disease suppression in potting mixes amended with Dutch yard waste composts. Multitrophic Interactions in Soil and Integrated Control, IOBC wprs Bulletin 27: 291-295.

PE&RC PhD Education Statement Form

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 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (2 credits)

- Biological farming systems (2002)

Writing of Project Proposal (2 credits)

- Management of soil health in horticulture using compost/compost management (2002)

Post-Graduate Courses (6 credits)

- Soil ecology: linking theory to practice (PE&RC) (2003)
- Basic and advanced statistics (PE&RC) (2005)
- Science of organic production: from ecology to socio-economics (BFS Wageningen) (2005)

Deficiency, Refresh, Brush-up and General Courses (4 credits)

- Molecular ecology (GEN-20304) (Phytopathology) (2002)
- Biology of fungi (G200-205) (Biological farming systems) (2005)

PhD Discussion Groups (5 credits)

- Agricultural production systems (2002-2005)

PE&RC Annual Meetings, Seminars and Introduction Days (2 credit)

- Introduction weekend (2003)
- Annual meeting (2004)

International Symposia, Workshops and Conferences (4 credits)

- Compost management EU: NAGREF (Greece) (2003)
- Compost management EU: INRA (France) (2004)
- Compost management EU: HUJI (Israël) (2005)
- First international conference on soil and compost eco-biology (SoilAce) (2004)