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Relation between soil health, wave-like fluctuations in microbial populations, and soil-borne plant disease management

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Abstract

A healthy soil is often defined as a stable soil system with high levels of biological diversity and activity, internal nutrient cycling, and resilience to disturbance. This implies that microbial fluctuations after a disturbance would dampen more quickly in a healthy than in a chronically damaged and biologically impoverished soil. Soil could be disturbed by various processes, for example addition of a nutrient source, tillage, or drying-rewetting. As a result of any disturbance, the numbers of heterotrophic bacteria and of individual species start to oscillate, both in time and space. The oscillations appear as moving waves along the path of a moving nutrient source such as a root tip. The phase and period for different trophic groups and species of bacteria may be shifted indicating that succession occurs. DGGE, Biolog and FAME analysis of subsequent populations in oscillation have confirmed that there is a cyclic succession in microbial communities. Microbial diversity oscillates in opposite direction from oscillations in microbial populations. In a healthy soil, the amplitudes of these oscillations will be small, but the background levels of microbial diversity and activity are high, so that soil-borne diseases will face more competitors and antagonists. However, soil-borne pathogens and antagonists alike will fluctuate in time and space as a result of growing plant roots and other disturbances, and the periods and phases of the oscillations may vary. As a consequence, biological control by members of a single trophic group or species may never be complete, as pathogens will encounter varying populations of the biocontrol agent on the root surface. A mixture of different trophic groups may provide more complete biological control because peaks of different trophic groups occur at subsequent locations along a root. Alternatively, regular addition of soil organic matter may increase background levels of microbial activity, increase nutrient cycling, lower the concentrations of easily available nutrient sources, increase microbial diversity, and enhance natural disease suppression.

Abbreviations: BCA – biocontrol agent; CFUs – colony forming units; DGGE – denaturing gradient gel electrophoresis; FAME – fatty acid methyl esters; GFP – green fluorescent protein

Introduction

Health is a necessary condition for the survival of individual living organisms, communities, ecosystems, and for nature in general. Ecologists have long recognized that the state of health of terrestrial, edaphic and aquatic ecosystems is important. However, it is not so easy to define, let alone measure, ecosystem health. This is also true for soil, which is considered as a living system, where many physical and chemical properties are mediated by biota, which are primarily responsible for its health (Brussaard et al., 2004). The living components of a soil possess both stable and dynamic characteristics. Recently, we proposed to use the resistance and resilience of microbial communities in response to a disturbance as quantitative indicators for soil health (van Bruggen and Semenov, 1999, 2000).

Soil as a productive system was developed and continues to develop jointly with plants that inhabit the soil. The condition of the soil, including its microbial community, reflects the condition of the (past) vegetation. In agricultural ecosystems, especially in conventional farming systems, most natural plants are considered weeds. Decreasing the vegetation diversity leads to pauperization of soil inhabitants, decreasing of interconnectedness and functional interchangeability. An extremely simplified vegetation, such as a monoculture, selects a specific microbial community, including plant pathogenic microorganisms and sometimes also their parasites or antagonists. However, such a simplified ecosystem may be very sensitive to the slightest disturbance and cannot be considered healthy.

High quality soil is the main production resource for many societies. However, this resource is disappearing at an alarming rate due to loss of organic matter as a result of erosion, oxidation, compaction, and biological impoverishment. In particular, agricultural systems with minimal biological diversity and large inputs of synthetic fertilizers and pesticides have problems with poor soil health and associated plant diseases. Action must be taken urgently to restore the balance of the soil ecosystem and its health status.

In this review we present a dynamic view of microbial populations, soil health, and disease suppression. In the following paragraph an introduction is given on soil health and its connection to disease suppression. The next two paragraphs deal with temporal and spatial oscillatory responses of bacterial communities to various disturbances. Then, we demonstrate that soil-borne pathogens respond with similar oscillations to a disturbance from a growing root. Next, agricultural management practices to control soil-borne diseases, like the use of organic amendments, tillage and biocontrol agents, will be discussed from the point of view of dynamic microbial oscillations. Finally, conclusions will be presented regarding soil health, microbial oscillations and soil-borne diseases.

Soil health and disease suppression

Rapport (1995) defined a healthy ecosystem as an ecosystem with the following characteristics: (1) integrity of nutrient cycles and energy flows, (2) biological diversity, (3) interconnectedness between functional units, (4) stability and resilience when faced with a disturbance or stress, and (5) limited plant and animal disease outbreaks. A soil ecosystem is considered healthy if it has a good balance of mineral and organic substances and living components. Such a balance is reached when an ecosystem comes to a climax condition, characterized by high biodiversity and low concentrations of easily available organic and inorganic nutrients (van Bruggen and Semenov, 1999, 2000). To maintain soil health, it is necessary to promote high primary productivity, high microbial biomass, activity and diversity, high nutrient turnover rates, and low residual nutrient pools; in other words, oligotrophic conditions. In particular, losses of mineral nitrogen and dissolved organic carbon from soil and soil biological complexity have been used to assess the functioning of soil ecosystems (Liiri et al., 2002).

Soils of natural ecosystems are generally thought of as being healthier than those of agroecosystems. Indeed, cultivated soils generally have lower microbial diversities and more severe disease problems than they had as a natural habitat (Ko, 1982; Buckley and Schmidt, 2001). Organically managed soils, where synthetic fertilizers and pesticides are not used, are closer to natural soils than conventionally managed soils even though soil fertility is maintained by regular additions of organic materials (van Bruggen, 1995; van Bruggen and Termorshuizen, 2003). Especially chlorinated pesticides have had negative impacts on microbial diversity (Mas et al., 1996). Although some authors found no differences in soil microbial diversity between organically and conventionally managed soils (Lawlor et al., 2000; Franke-Snyder et al., 2001), most researchers reported a higher biological diversity for organically than for conventionally managed soils with respect to various taxa, namely bacteria (Sivapalan et al., 1993; Drinkwater et al., 1995; Mäder et al., 2002; van Diepeningen et al., 2005), arbuscular mycorrhizal fungi (Ryan et al., 1994; Oehl et al., 2003), nematodes (Mulder et al., 2003; van Diepeningen et al., 2005), earthworms (Mäder et al., 2002), and arthropods (Drinkwater et al., 1995; Mäder et al., 2002). Also, a higher microbial activity (Workneh et al., 1993; Mäder et al., 2002) and microbial biomass (Workneh and van Bruggen, 1994; Mäder et al., 2002; Mulder et al., 2003) were found in organically managed soils.

High microbial biomass, activity, and diversity in natural or agricultural soils have been associated with suppression of soil-borne plant diseases (Nitta, 1991; Workneh and van Bruggen, 1994; Mäder et al., 2002). This kind of suppression may be due to general competition or antagonism, which may be non-specific and active against a wide range of soil-borne pathogens (Gerlagh, 1968; Whipps, 1997). However, in a few cases, no relationships were found between microbial biomass, activity or diversity and disease suppression. Boehm et al. (1993, 1997) found that the level of *Pythium* root rot suppression in peat mixes was not related to microbial biomass, activity or diversity but to the composition of the rhizosphere bacterial population. The seemingly unpredictability of disease suppression in relation to microbial community parameters may be due to a greater specificity of the relationship between pathogen and antagonist than sometimes thought, due to influences of varying soil physical and chemical characteristics (Hoper and Alabouvette, 1996), or due to variation in soil microbial communities in time and space (van Bruggen and Semenov, 2000).

Soil microbial populations generally fluctuate, and start to oscillate regularly in response to a disturbance, such as addition of organic material to soil (van Bruggen and Semenov, 2000). The amplitude of the waves in microbial populations (a measure of stability of the soil ecosystem), their frequency, and the time needed to return to initial conditions before organic amendment (a measure of the resilience of the system) may be used as indicators for soil health (van Bruggen and Semenov, 1999, 2000). The strongest wave-like response of microbial communities occurs in soils low in organic matter (Semenov et al., 1999). In high-organic matter soils with higher microbial biomass and activity, wave-like responses are also noticeable but the amplitudes and periods of these waves are less pronounced (Semenov et al., 1999). Stability and resilience of microbial communities after exposure to a disturbance could possibly also be related to disease suppression (van Bruggen and Semenov, 1999, 2000). Indeed, soils with a higher biological diversity and activity, such as natural or organically managed agricultural soils are frequently more suppressive to soil-borne diseases than conventionally managed agricultural soils (van Bruggen, 1995; van Bruggen and Termorshuizen, 2003).

Temporal wave-like fluctuations of microbial populations

Fluctuations in soil microbial populations have been observed many times, both in laboratory experiments and in the field with native bacterial communities (Aristovskaya, 1980; Zvyagintsev and Golimbet, 1983; Semenov, 2001). Under natural conditions, microbial fluctuations in soil appear irregular, and generally do not correlate with variations in external environmental characteristics, such as temperature and moisture content of the soil.

Irregular fluctuations can turn into regular oscillations with distinct waves after a disturbance such as addition of fresh organic matter to soil (Doebeli and Ruxton, 1997, 1998; Clarholm, 1981). Soil is generally low in easily available nutrients, especially fallow arable soil. Any disturbance providing a nutrient impulse under these conditions, such as incorporation of fresh organic matter or rewetting after drying, is likely to initiate a wave-like response of the microbial community (van Bruggen and Semenov, 1999; Caldéron et al., 2000). Hints of wave-like fluctuations were obtained in a field experiment after incorporation of cover crop debris into soil (van Bruggen and Semenov, 2000), but the observations were too sparse for time series or harmonical analysis (Shumway, 1988) to prove that regular oscillations occurred.

Only recently, we demonstrated the occurrence of regular oscillations over time using appropriate statistical techniques (Zelenev et al., 2004). Temporal oscillations of microbial populations (CFUs and microscopic cell counts) were observed for one month in soil amended with fresh plant material (grass–clover) incubated at constant temperature and moisture. Bacterial populations fluctuated with different periods and amplitudes, depending on the specific conditions of each experiment, but immediately after the disturbance they revealed remarkable oscillations with large amplitudes. The patterns of the oscillations were quite predictable, always with a small and large peak within one week after incorporation of a grass–clover mixture in soil (Zelenev et al., 2004).

Various mechanisms underlying oscillations in microbial populations could be envisaged. Ecologists would first of all think of predator–prey interactions. Total bacterial-feeding nematodes did not oscillate, but increased monotonously in the second week after grass–clover incorporation into the soil (Zelenev et al., 2004). Daily changes in active numbers of bacterial-feeding nematodes did oscillate with a frequency similar to that of bacterial oscillations due to intermittent activation of the dormant juveniles (Dauerlarvae). However, the response of bacterial-feeding nematodes was still too slow to explain the decline after the first peak in bacterial populations within two days after the incorporation of grass–clover material (Zelenev et al., 2004). Similarly, protozoa were likely to be too slow to be responsible for the first decline in bacterial populations, suggesting that bacteria initiate the oscillations, and that their predators follow suit (Zelenev et al., 2004). During the experiments with grass–clover amended soil, various chemical and physical parameters were measured, such as ammonium and nitrate concentrations, pH, and redox potential. None of these parameters oscillated over time (Zelenev et al., 2004). In a simulation model, bacterial populations started to oscillate due to a temporary shortage of easily available substrate (Zelenev, 2004). Indeed, substrate availability is a plausible explanation for initiation of the oscillations. Yet, local oxygen deprivation after intensive bacterial growth has not been excluded but remains as a potential mechanism underlying the initiation of bacterial oscillations.

Another aspect of the mechanisms underlying bacterial oscillations is whether all taxa oscillate simultaneously, or if each peak represents a different microbial community corresponding with different organic components that are decomposed subsequently, or if there are repetitive successions within each peak. This question was addressed in another time-series experiment with and without grass–clover incorporated into a sandy soil. The response of copiotrophic bacterial CFUs (de Vos and van Bruggen, 2001; Zelenev et al., 2005) to the disturbance was determined daily over a period of nine days, both for the grass–clover (GC) and the non-amended control series (CO). Copiotrophic bacteria are fast-growing bacteria, with a relatively low substrate affinity and high half saturation constant. Copiotrophic CFUs oscillated over time in a wave-like fashion after amendment of the soil, whereas in the non-amended soil the CFUs fluctuated only very slightly (Figure 1). Microbial communities were characterized daily by determining DGGE profiles using eubacterial primers, FAME composition, and physiological profiles (Biolog, Hayward, CA, USA) on mixtures of copiotrophic colonies removed from agar plates (de Vos and van Bruggen, 2001). The patterns of DGGE bands (Figure 2), fatty acid composition and Biolog profiles indicated a succession in taxonomic and functional groups over time. Discriminant analysis of the DGGE band intensities, percentages of individual fatty acids, and intensities of physiological reactions on Biolog plates (Figure 3) showed that there were repetitive cycles in the succession of bacteria over time: communities at times when CFUs increased were more similar to each other than to those when CFUs decreased and *vice-versa* (de Vos and van Bruggen, 2001).

In an attempt to relate amplitudes and periods of the oscillations (representing stability and resilience of the soil ecosystem) to soil health, grass–clover mixtures were added to γ-irradiated and non-irradiated soils, a filtered (0.8 μm) soil suspension was added to the irradiated soil, and microbial populations were enumerated daily. In the γ-irradiated soil the amplitudes and periods of the wave-like fluctuations in microbial communities in response to the disturbance by grass–clover were larger than those in the non-irradiated soil, supporting the notion that non-irradiated soil is healthier (Zelenev et al., 2004). The amplitudes of microbial populations were also generally higher in conventionally than in organically managed soils (unpublished results) and higher in a fallow soil than in a cover-cropped soil after addition of the same amount of cover crop plant material (van Bruggen and Semenov, 2000). Thus, the amplitude and period of microbial oscillations after a disturbance may indeed be good indicators for soil health (van Bruggen and Semenov, 2000; Orwin and Wardle, 2004).

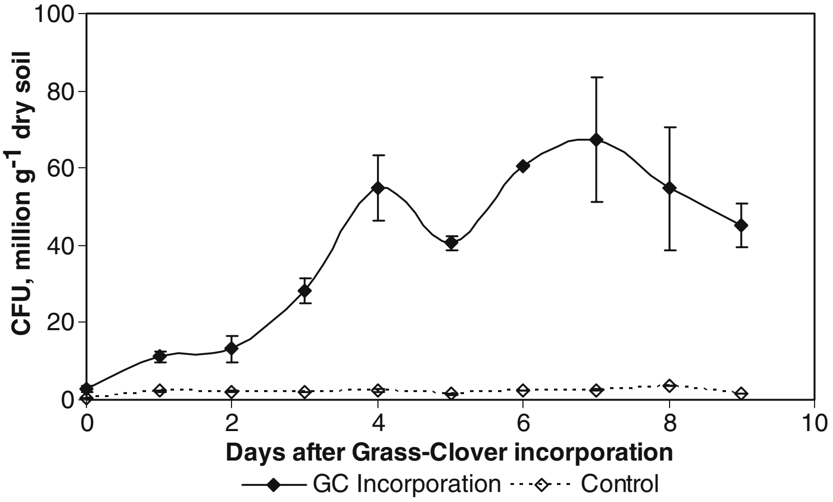

Figure 1. Wavelike fluctuations in numbers of copiotrophic bacteria isolated from sandy soil zero to nine days after incorporation of a grass–clover (GC) mixture and the relative stable numbers in the non-amended control soil.

Soil health is also frequently associated with limited disease outbreaks (Rapport, 1995; van Bruggen and Semenov, 2000), and indeed, root disease suppression is generally greater in non-irradiated than in γ-irradiated soil (Workneh and van Bruggen, 1994), in natural than in agricultural soil (Ko, 1982), and in organic than in conventional agricultural soil (van Bruggen, 1995; van Bruggen and Termorshuizen, 2003). This leads to the following questions: do plant pathogens also fluctuate in soil after a disturbance, and are the amplitudes greater in less healthy soils? Incorporation of vetch/oats cover crop debris in fallowed *versus* cover-cropped soil resulted in temporal fluctuations in copiotrophic bacterial CFUs over the next five weeks (van Bruggen and Semenov, 1999, 2000), and in similar fluctuations in damping-off incidence of tomato seedlings (caused by naturally occurring *Pythium ultimum* and *Pythium aphanidermatum*) in soil samples taken daily from the same experiment (Figure 4). The oscillations showed similar periods but were shifted in time: disease incidence increased when copiotrophic – and possibly antagonistic - populations decreased. It would be interesting to

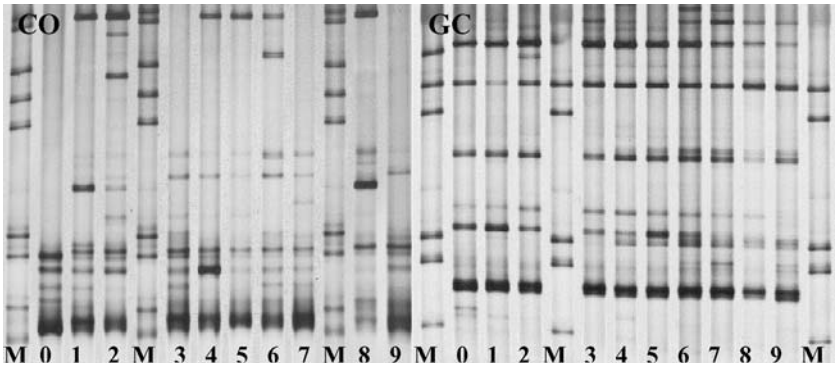

Figure 2. DGGE patterns of PCR products derived from DNA from copiotrophic bacterial colonies. The numbers represent the number of days after the incorporation of a grass/clover mixture in soil (GC) and in a non-amended control soil (CO). M represents a set of eubacterial marker strains. The urea/formamide denaturing gradient was between 40% and 48%. Note that the bacterial composition of CO does not change in time, while the composition of GC changes over time, the composition being similar after 0, 5 and 9 days and different on the other days.

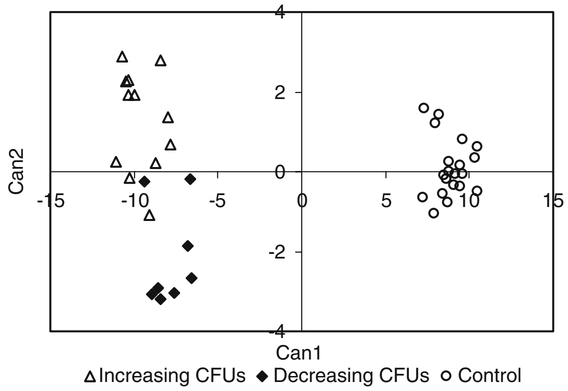

Figure 3. Discriminant analysis of the Biolog data of the copiotrophic bacterial population zero to nine days after grass–clover incorporation into a sandy soil and in the control. All samplings were done in duplicate; daily data were compared to the data on the next sampling day to determine whether CFUs were increasing or decreasing. The clustering of increasing and decreasing data shows that there are different, repetitive, stages in the succession of bacteria in time.

investigate if the decline in bacterial CFUs and the increase in *Pythium* infections were associated with a decrease in oxygen availability.

Spatial wave-like fluctuations of microbial populations in the rhizosphere

Distribution patterns of microbial populations within root systems have been investigated extensively (Schippers and van Vuurde, 1978; van Vuurde and Schippers, 1980; Scott et al., 1995; Kim et al., 1997; Semenov et al., 1999). High microbial densities have generally been observed close to the root tip and in middle and upper sections of the roots, and patterns in microbial density along roots have been thought to be a direct reflection of patterns of exudation and sloughing off of cortex cells (Rovira, 1973; Schippers and van Vuurde 1978; van Vuurde and Schippers, 1980; McCully and Canny, 1985). This is a rather static viewpoint in which growth and death of microbial populations is not explicitly considered. After a series of experiments on the distribution of microbial populations along roots, we arrived at a very different and dynamic concept of microbial community development in the rhizosphere, namely that bacterial communities respond to the influx of nutrients from a root tip with growth and death cycles at any location where the root tip passes, resulting in wave-like patterns along each root (Semenov et al., 1999; van Bruggen et al., 2000; Zelenev et al., 2000).

In the above-mentioned experiments, wheat plants (*Triticum aestivum* L.) were grown in 60 or 90 cm long root observation boxes with soil high or low in fresh organic matter. After two, three, and five weeks, 2 cm root sections were cut at 4 cm intervals. Copiotrophic and oligotrophic bacteria were isolated from the rhizosphere and corre-

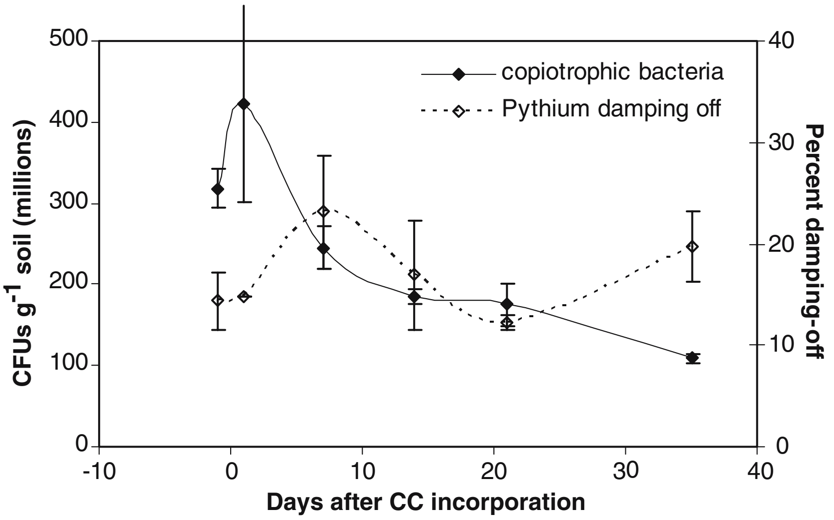

Figure 4. The CFUs of copiotrophic bacteria and the percentage damping-off of tomato seedlings caused by *Pythium ultimum* and *Pythium aphanidermatum* naturally occurring in soils collected one day before, one day after and one, two, three and five weeks after incorporation of a vetch/oats cover crop (after van Bruggen and Semenov, 2000).

sponding bulk soil on carbon-rich and carbon-poor media, respectively (Semenov et al., 1999). For the first time, wave-like distributions of bacterial populations were demonstrated along plant roots using harmonics analysis (Semenov et al., 1999). Peaks in oligotrophic populations were slightly shifted upwards on the root compared to those of copiotrophic populations, indicative of the possibility that oligotrophs would follow copiotrophs in a succession starting from the tip. There were no (cross) correlations of either bacterial group with number of mature lateral roots per section, or with concentrations of soluble total organic carbon (TOC) in the rhizosphere (Semenov et al., 1999). The oscillations shifted from week to week, and were justifiably called ‘moving waves’.

To ascertain that the spatial pattern in microbial populations was not related to lateral root formation, we did an experiment with an artificial nutrient source moving through soil. A tube, through which a solution with sugars and amino acids was pumped, was pulled at a speed of 1 or 4 cm per day through a dialysis sleeve buried in soil. This experimental setup gave the expected wave-like patterns in bacterial populations along the path of the moving nutrient source similar to the patterns in real rhizospheres along wheat roots (van Bruggen et al., 2000). Oscillations in space were transformed to oscillations in time, taking the moving rate of the tube into account. This resulted in oscillations with similar periods, regardless of the moving rate of the tube, indicating that the periods are dictated by growth and death rates of the bacteria, not by the growth rate of a root.

These experiments led to the so-called moving-wave hypothesis for bacterial populations in the rhizosphere: ‘Waves originate from bacterial growth on nutrients from the root tip, followed by death when nutrients become exhausted and re-growth from recycled carbon sources plus substrate from soil organic matter’. This hypothesis was visualized by means of the results of a simulation model (Zelenev et al., 2000). We envisage the following scenario. As the root tip moves into bulk soil, releasing nutrients, dormant bacteria (and probably fungi) are activated, grow, and then die as nutrients become exhausted; dead bacteria lyse and a new generation grows on recycled nutrients (plus additional substrate from soil and roots). Thus, there are growth and death cycles at any point where the nutrient source passes resulting in waves in space (Figure 5).

Not only total bacterial communities, but also individual bacterial strains exhibit wave-like fluctuations along roots. The biocontrol agent *Pseudomonas fluorescens* 32-*gfp*, marked with the green fluorescent protein gene, was added to soil samples from neighbouring conventional and organic farms at Heelsum, the Netherlands, and re-isolated from the rhizosphere along the total length of wheat roots after three weeks of growth. Both CFUs on selective media and fluorescent microscopic counts oscillated significantly and similarly along the length of the roots (Semenov et al., 2004). The oscillations had a much greater amplitude and period in the conventionally than in the organically managed soil (Figure 6). In the last soil, *P. fluorescens* 32-*gfp* populations were zero towards the root tip. The reason was the lower survival of *P. fluorescens* 32-*gfp* in the organically than in the conventionally managed soil, presumably due to more intense competition in the organic soil. Apparently, when root tips reached a depth of 10–35 cm below soil level, the majority of the intro-

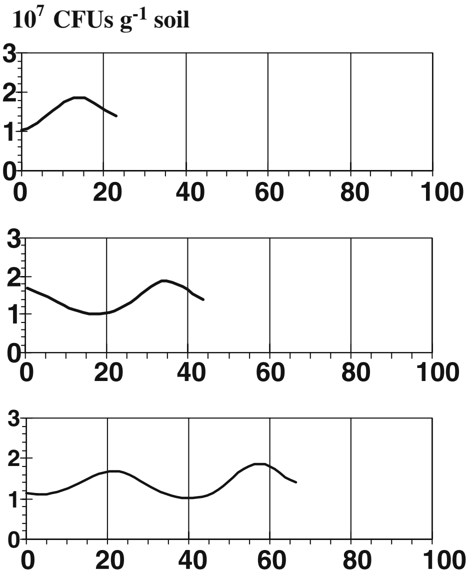

Figure 5. Moving waves: Oscillations in numbers of copiotrophic bacteria along the root in distance from the root base after two, three and four weeks of growth of wheat. With the growing root tip (to the right of each graph) the population moves to a maximum, followed by a harmonic iteration of minima and maxima.

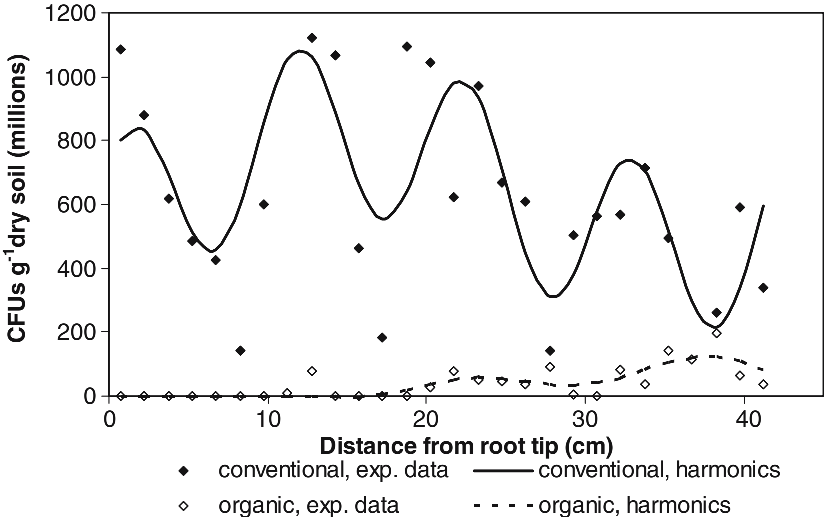

Figure 6. Experimental data and harmonic fluctuations of a GFP-labelled strain of biocontrol agent *P. fluorescens* (CFUs/g^{-1}dry - soil) along the root of wheat in a conventionally and an organically managed sandy soil plotted against distance (cm) from the root tip. The conventional soil had a higher available N and K content and a higher pH than the organic soil. Organic C contents were similar. The contribution of the harmonics to the total variance was 75.6% for the conventional soil and 82.5% for the organic soil. The amplitudes of *P. fluorescens* in the organic soil were lower than in the conventional soil and *P. fluorescens* could not be detected any more around the organic root tip, probably due to a reduced survival in the organic soil compared to the conventional soil.

duced cells had died, so that no cells or CFUs were detected in this region at the time of sampling.

Furthermore, we investigated if the bacterial communities fluctuated as a whole along the wheat root or whether there is a succession in bacterial composition from peak to peak or within peaks (van Diepeningen, pers. comm.). Therefore, rhizosphere microbial communities along roots of wheat were studied in detail (20–25 rhizosphere and bulk soil samples along the total root length) by colony enumeration and DGGE analysis of extracted DNA in the same organic and conventional soils used for the experiments with *P. fluorescens* 32-*gfp*. Similar to our previous findings, the numbers of copiotrophic and oligotrophic bacteria oscillated with significant harmonics along each root, independent of soil moisture or lateral roots. The oscillations and rhizosphere effects were more pronounced in the conventionally managed soil. For amplified eubacterial 16S rDNA fragments from DGGE analysis three different groups could be distinguished: those fluctuating in intensity in phase with CFU oscillations (19.2% of the total number of bands, representing 37.4% of the total band intensity); those fluctuating in intensity in opposite phase with CFU oscillations (26% of the total number of bands, representing only 25.0% of the total band intensity), and remaining bands whose intensity showed no relationship with CFU oscillations or that were restricted to certain root zones (54.8% of the total number of bands, representing only 37.5% of the total band intensity). Discriminant analysis of the bacterial populations in root sections with increasing and decreasing phases in the oscillations showed that the community compositions of waxing populations are more similar to each other than to those of waning populations, especially in conventionally managed soil (Figure 7). Again the succession appeared to be cyclic, in space as well as over time.

Two measures of bacterial biodiversity in soil, species richness *S* and the Shannon index *H*, were calculated based on the DGGE data. Both biodiversity measures oscillated with significant harmonics along the root in opposite phase to total bacterial CFUs. The bacterial diversity along the root was negatively correlated with the numbers of oligotrophic and copiotrophic bacterial CFUs in the conventional soil and with oligotrophic bacterial CFUs in the organic soil

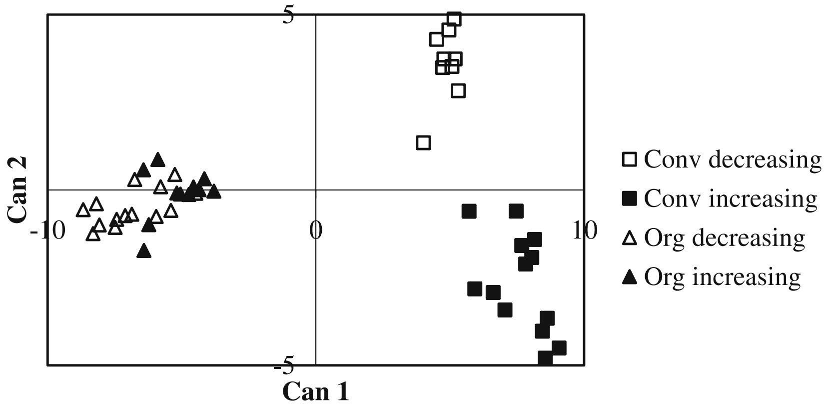

Figure 7. Discriminant analysis of populations in the increasing and decreasing parts of the wave-like oscillations in the rhizosphere of wheat, in an organic and conventional sandy soil. Soil samples originated from neighbouring organic and conventional farms. The conventional soil had a higher available N and K content and a higher pH than the organic soil. Organic C contents were similar.

(Table 1). This indicates that a limited number of fast growing taxa were growing and dying over time and in space.

Besides fluctuations in the vertical direction along the root, running waves of bacterial populations have also been observed in the horizontal direction away from the root surface. Kozhevin (1989) observed fluctuations in cells of introduced *Bradyrhizobium japonicum* 1021 perpendicular to the length of soybean roots (*Glycine max*) at a microscopic scale (up to 1 mm from the root surface), using immunofluorescence. The pattern of the oscillations shifted over time, and these spatial–temporal distributions were described as 'running waves' of bacteria literally moving towards the root surface (Kozhevin, 1989). In seawater, chemotactic bacteria were shown to occur in concentric spheres with alternating higher and lower bacterial densities around point sources of diffusing nutrients, forming wave-like patterns both in space and over time at scales of a few μm and seconds. These patterns were attributed to the combined effects of molecular diffusion of the attractant, congregation and subsequent dispersal of the motile bacteria (Blackburn et al., 1998), and were simulated by nonlinear diffusion-reaction models. The running waves observed by Kozhevin (1989) may also be the result of diffusion-reaction mechanisms.

Table 1. Cross-correlation coefficients (CCF) between oligotrophic and copiotrophic bacterial CFUs at various depths in the rhizosphere of wheat grown in conventional and organic soil, and band intensity in DGGE gels of amplified 16S rDNA fragments from DNA isolated from corresponding rhizophere samples

		CCF	lag[a]
Conventional soil	Oligotrophic CFUs	−0.552[b]	2
	Copiotrophic CFUs	−0.635	2
Organic soil	Oligotrophic CFUs	−0.466	−1
	Copiotrophic CFUs	n.s.[c]	

Soil samples originated from neighbouring organic and conventional farms. The conventional soil had a higher available N and K content and a higher pH than the organic soil. Organic C contents were similar.

[a] One lag corresponds to 1.5 cm.

[b] Significant at $P = 0.05$.

[c] Not significant.

As mentioned by Kozhevin (1989), there must be a connection between bacterial oscillations in space and time. Indeed, we demonstrated that spatial wave-like fluctuations of microbial populations along the path of a moving nutrient source could be transformed to spatial moving waves by taking the rate of root growth into account (van Bruggen et al., 2000). The connection between spatial and temporal oscillations was used to create a simulation model to describe and predict microbial dynamics in the rhizosphere (Zelenev et al., 2000). This model could also be used to predict the distribution of infections by pathogens in a root system, since infection could possibly take place more easily when microbial abundance and activity decline, at the waning phases of microbial waves.

Wave-like fluctuations of plant pathogens in the rhizosphere

The occurrence of microbial growth and death cycles at any point along a root could have important consequences for infection by plant pathogens. Infections by plant pathogenic fungi are rarely uniformly distributed in the root system. Some fungi preferentially infect in the vicinity of the root tip, while others infect primarily older sections of the roots. Given that there are wave-like patterns of saprotrophic microbial populations in space and time in the rhizosphere along roots, and that pathogens often have a saprotrophic phase before infecting a host, it is likely that there are also wave-like patterns in root infections. Indeed, when sclerotia of *Rhizoctonia solani* were placed uniformly along the total length of wheat roots growing in root observation boxes, the proportions of root sections (of eight roots) from which *R. solani* were isolated showed wave-like fluctuations when detrended data were plotted *versus* distance from the root tip (van Bruggen et al., 2002). Similarly, the proportions of root sections from which naturally occurring *Pythium ultimum* was isolated were distributed in a wave-like fashion along the root (Figure 8). The first peak in *Pythium* infections was closer to the root tip than that of *R. solani* (van Bruggen et al., 2002). In the same experiments, copiotrophic bacteria were enumerated at the time of inoculation with *R. solani*, one week before isolation of the pathogens from root sections. For comparison of peaks in infection with those in bacterial populations, the bacterial curves were shifted 14 cm to the right since the root tip moved down 2 cm day^{-1} during the week since the bacterial populations had been assessed. Both pathogens oscillated in a different phase relative to the bacterial oscillations. There were negative correlations between densities of copiotrophic bacteria and *R. solani* infections at 0 cm lag, while there were positive correlations between copiotrophic bacteria and *Pythium* infections at a lag of 6 cm (Figure 8). Infection by *R. solani* was probably inhibited when large bacterial populations were encountered on the root surface at the time of inoculation. It is not known when *P. ultimum* infection took place, but possibly a few days after passing of the root tip, when the first wave of copiotrophic bacteria was already declining. This shift in *Pythium* infection relative to the first peak in copiotrophic bacteria after passing of the root tip is similar to the shift in the *Pythium* damping-off peak relative to the first peak in bacterial CFUs after grass–clover incorporation in soil, as discussed in a previous section of this paper.

In addition to the oscillations in root infections along the length of a root, there is the probability of root infection by plant pathogens located at increasing distances from the root surface that can fluctuate in space. The probability of infection generally declines with perpendicular

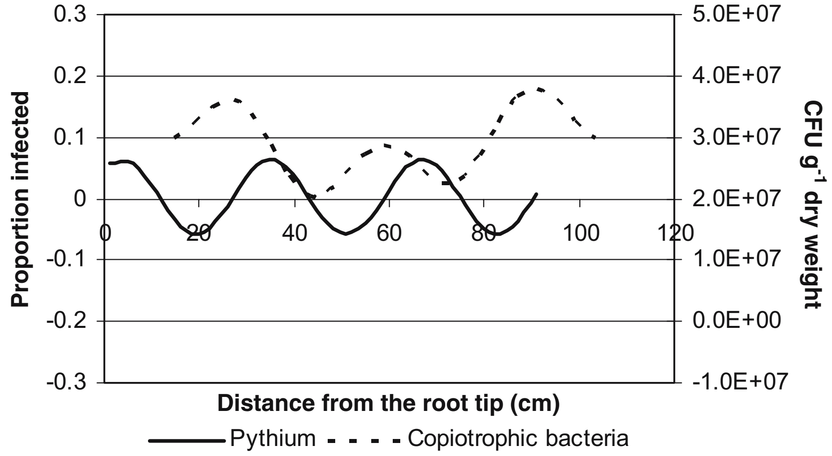

Figure 8. Harmonic fluctuations of the proportion of natural *Pythium* infection and the numbers of copiotrophic bacteria along the wheat root.

distance of propagules from the root, but the decline is generally not monotonous (Mol and van Riessen, 1995; Gilligan and Bailey, 1997). Gilligan and Bailey (1997) placed propagules of *R. solani* at 1–4 mm intervals for a maximum distance of 15 mm horizontally from the host (radish seed) and calculated probability of infection with distance from the host. Close to the host, there was frequently a small peak in the probability of infection, followed by a decline in this probability with distance from the host. After detrending and estimating some missing data by linear interpolation, we re-analyzed their data with harmonics analysis. Most of the data presented by Gilligan and Bailey (1997) actually declined in a wave-like fashion with distance from the host (Figure 9). Harmonics analysis showed that there were significant waves in the horizontal direction, similar to the waves found for *B. japonicum* by Kozhevin (1989). Similarly, the data of Mol and van Riessen (1995) on the probability of infection by *Verticillium dahliae* seemed to decrease in a wave-like fashion with increasing distance from the root surface of various crop species and potato cultivars; unfortunately, the published data were not suitable for harmonics analysis.

The realization that wave-like patterns of saprotrophic and pathogenic microorganisms occur both in the horizontal and vertical direction in the rhizosphere, led to a new view of root infection in relation to microbial population dynamics. Although not demonstrated, it is likely that wave-like distribution patterns of the microbial community are generated along any roots, including lateral roots, initiated from the growing root tips. Exudation from root tips leads to creation of waves both in the vertical direction (macro-waves along the root) and in the horizontal direction (micro-waves perpendicular to the root).

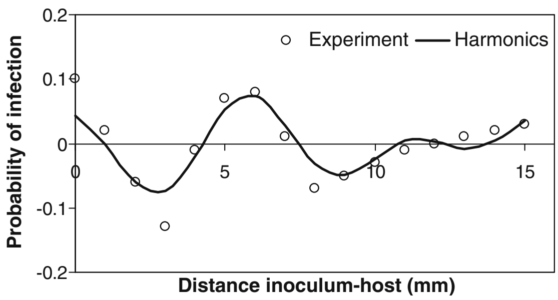

Figure 9. The probability of infection by *Rhizoctonia solani* in relation to distance from the host. Detrended values are shown (derived form Gilligan and Bailey, 1997).

The reasons for fluctuating probabilities of infection with distance of propagules from the root are not clear. If propagules of a pathogen are randomly or regularly distributed in space and a root passes releasing exudates, then hyphae growing towards the roots arrive at different distances from the tip depending on their original distance from the host. Upon arrival at the root surface they encounter more or less bacteria, depending on the phase of bacterial waves, so that they have a lower or higher chance of infecting the host. This would result in fluctuating probabilities of infection with distance from the root, and waves of infection along the length of the root.

Alternatively, if there are waves of nutrients moving into the rhizosphere as a result of waves in substrate utilization and release at the root surface or due to a day–night rhythm in exudation, the probability of infection may also fluctuate. If zoospores, for example, are pulled three steps forward and one backward as the waves pass, synchronization of zoospores would take place, so that they arrive in waves at the root surface just behind the root tip. These horizontal dynamics of pathogens would result in waves of infection along the root as the root tip moves on (provided that the root tip does not die from *Pythium* infection). Thus, the occurrence of microbial growth and death cycles at any point along a root does indeed seem to have important consequences for infection by plant pathogens along the root.

Management of soil-borne disease taking microbial oscillations into account

Based on the premise that microbial communities in healthy soils have strong resistance and resilience against disturbances and suppress disease outbreaks (Rapport, 1995), we would need to manage microbial communities so that the amplitudes of the oscillations (resistance) and the time to return to quasi-stationary conditions (resilience) are minimized. To accomplish this, we would need to enhance the biological buffering capacity of a soil by enhancing the background level of microbial activity and food web complexity, for example by regular additions of relatively stable organic matter to soil. On the other hand, we may want to stimulate a specific part of the microbial community to antagonize certain plant pathogens by applying appropriate external disturbances to stimulate oscillations and succession, for example by tillage. In this paragraph, we will only discuss those management strategies that influence short-term oscillations of microbial populations, and saprotrophic growth and infection by plant pathogens. Management practices that affect long-term population dynamics of microbial communities, such as crop rotation, will not be discussed here.

Organic matter management

Soil organic matter has generally not been managed explicitly by conventional farmers. Addition of synthetic fertilizers over many years has resulted in loss of organic matter by stimulating decomposition of native soil organic matter and enhancing microbial respiration. Combined with the negative effects of synthetic pesticides on components of the microbial communities and associated food webs, conventionally managed soils are generally biologically impoverished. Any addition of substrate for microbial growth such as crop residues, dead organisms after tillage, or root exudates, will result in large oscillations in microbial populations, including fast-growing plant pathogens with saprotrophic abilities such as *Pythium* spp. (van Bruggen and Semenov, 2000).

Large pools of mineral nitrogen in soil may even exacerbate the fluctuations. Soil and plant nitrogen concentrations can have a profound effect on both ecosystem health and disease severity: high levels of nitrogen in the soil, particularly in the form of nitrate, may enhance several fungal diseases (Workneh et al., 1993; van Bruggen, 1995; Tamis and van den Brink, 1998, 1999; Clark et al., 1999).

Several things can be done to restore the biological buffering capacity and enhance internal nutrient cycling in soil. Regular addition of fairly stable organic matter, including solid animal manure, composts of plant and animal origin, and lignified roots of deep-rooted plants such as alfalfa, rye or grass–clover would enhance microbial biomass, activity, and diversity, and food web complexity in soil (Sivapalan et al., 1993; Ryan et al., 1994; Workneh and van Bruggen 1994; Mäder et al., 2002; Schjønning et al., 2002; van Diepeningen et al., 2005). It would also enhance suppression of many soil-borne pathogens (van Bruggen, 1995; van Bruggen and Termorshuizen, 2003). For example, densities of *Phytophthora* and *Pythium* propagules in soil were lower and those of the antagonist *Trichoderma* higher in soils amended with various organic materials (composted cotton-gin trash, composted yard waste, or cattle manure) than with synthetic fertilizer (Bulluck et al., 2002). Wave-like responses of these pathogens to introduction of the organic materials were not investigated.

Considering the reaction to a disturbance by incorporation of a winter cover crop or weeds into soil, sowing of a subsequent crop needs to be timed so that the inoculum of a facultative saprotrophic pathogen is not at its peak at that time. The quality of the organic matter in terms of easily available substrate and the C:N ratio, and the activity of the microbial community will determine if facultative saprotrophic pathogens can multiply in this material. Pathogens such as *Pythium* and *Rhizoctonia* species multiply easily in fresh substrate, and may cause serious damping-off problems when a crop is sown within three to four weeks after incorporation of fresh plant material (van Bruggen and Semenov, 2000). For example, when a mixture of vetch and oats was incorporated in soil that had been fallow or had been cover-cropped, damping-off of tomatoes by *Pythium* species was most severe seven days after incorporation of the plant material, and five days after the first peak in copiotrophic bacteria (Figure 4). The peaks in bacterial CFUs and damping-off incidence were higher in the previously fallowed soil than in the cover-cropped soil, indicating that the cover-cropped soil was more stable (van Bruggen and Semenov 1999, 2000). In another study where a vetch–oats cover crop was incorporated in organically and conventionally managed soils, *in vitro* growth of *P. aphanidermatum* peaked after 7–10 days while that of *R. solani* peaked after 21–35 days (Grünwald et al., 1997, 2000). Microbial measurements were generally lower and *in vitro* growth of the pathogens higher in the conventionally compared to the organically managed soils, but these differences were temporarily nullified after cover crop incorporation (Grünwald et al., 2000).

Different from the effects of cover crop incorporation, high-nitrogen-containing organic amendments such as cattle and poultry manure or soy meal had an immediate suppressive effect on several root pathogens and nematodes, as a result of ammonia release immediately after initiation of microbial decomposition (Lazarovits et al., 2001). Bulluck et al. (2002) also documented immediate suppressive effects of various types of compost and cattle manure applied at moderate to high rates on southern blight of tomatoes. However, these materials may not be suppressive to *Pythium*, which thrives well under high ammonium concentrations (van Bruggen and Semenov, 1999).

After repeated applications of organic materials, higher organic matter and microbial activity in bulk soil would result in a 'masking of the rhizosphere effect' (Gilbert et al., 1994), reduce the microbial oscillations along roots, and limit substrate concentrations seeping into soil, thereby reducing the attraction of root pathogens to the root surface and decreasing the chance of infection by many pathogens. Reducing the amount of easily available mineral nutrients and soluble carbon compounds by reducing fertilizer applications and the addition of stable carbon sources, would lead to oligotrophication. This promotes mycorrhizal infections, which can also suppress various root diseases (Sharma et al., 1992; Ryan et al., 1994). Whether mycorrhizal infections also occur in wave-like patterns along the roots is not known.

Tillage

No-till or reduced tillage has been promoted in recent years primarily to reduce soil erosion. However, tillage practices also have pronounced effects on survival of fungi and micro- and macrofauna in soil. Deep tillage can enhance the bacteria to fungi ratio and eliminate predatory nematodes, affecting especially the *k*-strategists (Berkelmans et al., 2003). No-till or reduced tillage is often associated with higher microbial biomass and activity and a more complex food web in the upper soil layers compared to regular tillage, i.e. plowing (van Diepeningen et al., 2005).

Tillage is a form of disturbance resulting in clear fluctuations in microbial activity and biomass. Caldéron et al. (2000) showed clear fluctuations in microbial biomass during the first eight days after simulated tillage in the laboratory, namely mixing of soil samples collected from a grassland and a vegetable field. Such a disturbance may also give facultative saprotrophic pathogens a chance to grow, but wave-like fluctuations may be dampened sooner than in the case of a disturbance by fresh plant materials. A report on a lower incidence of *Pythium* damping-off of sugar beet in a farm with reduced tillage than in a conventional farm with regular tillage (El Titi and Richter, 1987) is in agreement with the notion that reduced tillage decreases the chance that *Pythium* would grow explosively in fresh substrate after tillage. On the other hand, pathogens that survive in stubble could become problematic in no-till fields. Roget (1995) demonstrated that after conversion from regularly tilled to no-till wheat production *Rhizoctonia* root rot increased in the first few years. However, this increase was followed by a decline in *Rhizoctonia* root rot after about five years of no-till.

(Partial) soil sterilization

It is well known that soil-borne plant pathogens can wreak havoc when introduced into steamed greenhouse soil or fumigated field soil due to the existence of a biological vacuum (Bollen, 1974; Kreutzer, 1965). Any disturbance of a recently sterilized (or γ-irradiated) and re-colonized soil leads to wild fluctuations in microbial populations (Zelenev et al., 2004), and may lead to similar fluctuations in facultative saprotrophic plant pathogens. A good alternative to soil sterilization may be biological soil disinfestation (Blok et al., 2000), which does not result in enhanced disease pressure when pathogens are re-introduced, and provides long-lasting disease control (Goud et al., 2004).

Mixed cropping

Mixed cropping – a system where two or more crops are grown in the same field – can enhance food web diversity and decrease severity of foliar plant diseases (Finckh and Wolfe, 1997). Although positive correlations between above-ground and below-ground biodiversity have seldom been demonstrated (De Deyn et al., 2004), suppression of root disease (Burdon and Chilvers, 1976; Villich, 1993) and enhanced soil microbial diversity in mixed cropping systems have sometimes been found (G.A. Hiddink, pers. comm.).

Microbial composition in the rhizosphere is strongly dependent on plant species (Smith et al., 1999). Thus in a mixture of roots of different species it may be more difficult for a pathogen to find its host and the saprotrophic phase of pathogens may be limited by a greater variety of antagonists.

Cultivar selection

Choice of crops and cultivars will influence the microbial communities that are selectively enhanced or suppressed in the rhizosphere by the quality of root exudates (Grayston et al., 1998; Kowalchuk et al., 2002; Garbeva et al., 2004). Differential interactions between plant genotypes and beneficial microorganisms have been demonstrated for species of mycorrhizal fungi, rhizobia, and general plant growth-promoting rhizobacteria (PGPR) (Smith et al., 1999). PGPR have been used as biofertilizers and biological control agents (Germida, 1996). They can be directly antagonistic towards plant pathogens or can stimulate systemic induced resistance in the plant (Kloepper et al., 1997).

Besides exudate quality, exudation rates can also vary per cultivar, and these rates determine to a large extent the amplitude of the ensuing microbial oscillations. However, plant breeders have generally not taken exudation rates and exudate quality into account. There would be a great opportunity to select cultivars for their ability to stimulate specific microbial communities that can contribute to disease suppression.

Biological control

Many microorganisms have been found with biological control potential against various plant pathogens. Biological control agents may use a variety of inhibitory and suppressive mechanisms: (1) competition for resources and space, (2) antibiotic production, (3) removal of pathogenicity factors produced by the pathogen, (4) production of degrading enzymes that target the pathogen and (5) the induction of resistance in the host plant (Whipps, 2001). However, many biological control agents perform poorly under field conditions (Fravel, 1999) and only few biocontrol species have been registered for field use (Copping, 2001). Biocontrol of soil-borne pathogens has been more successful under controlled environmental conditions using simplified potting mixes presumably low in microbial diversity (Fravel, 1999). Moreover, inoculation of soil with a single strain of a biocontrol agent rarely leads to a high level of protection and often the positive effect is inconsistent (Weller, 1988; Koch, 1999). Better results have been obtained with combinations of strains or species (e.g. Pierson and Weller, 1994; Guetsky et al., 2001, 2002; Szczech and Shoda, 2004).

These results with biocontrol agents can now be interpreted in view of the general occurrence of microbial oscillations in time and space in the rhizosphere. Introduced biocontrol agents are likely to oscillate similarly to the native soil microbial communities. Densities of *Pseudomonas fluorescens* introduced on wheat seed seemed to form wave-like patterns along the length of the root, the amplitudes tapering off towards the root tip (Scott et al., 1995). In an experiment in our laboratory with GFP labelled, phloroglucinol-producing *P. fluorescens* mixed into soil we conclusively proved a wave-like distribution of this bacterium along growing wheat roots (Figure 6), similar to the oscillations of native bacterial populations (Semenov et al., 1999). We also showed that there is a succession in microbial communities within each wave, repeating from wave to wave. Thus, microorganisms that may be good antagonists *in vitro*, may take a different position in the succession compared to the target pathogen, and may therefore not be effective as biocontrol agents. This might mean that biocontrol can only be accomplished if waves in populations of the biocontrol agent coincide more or less with potential waves in pathogen populations (unless there is systemic induced resistance). Potential biocontrol agents may need to be selected so that their populations are maximal in the region along the root where the target pathogen invades the root. Differences in succession and position along the root may also explain the greater success of biocontrol mixtures than of single biocontrol agents (Guetsky et al., 2001, 2002; Szczech and Shoda, 2004).

In organically managed soil with high microbial diversity and activity (and therefore low concentrations of easily available nutrients) introduced biocontrol agents may survive less compared to biologically impoverished conventional soil (van Bruggen et al., 2000). The difference between the effectiveness of biocontrol agents in biologically impoverished soil *versus* biologically diverse and active soils, may be explained by reduced availability of easily decomposable substrate in these latter soils. The rhizosphere effect would be masked and microbial oscillations subdued in such soils (van Diepeningen, pers. comm.). In biologically impoverished soils, high substrate concentrations can be expected where wildly oscillating microbial communities are at a minimum; biocontrol agents with a slightly different niche compared to the majority of these oscillating communities may have a chance to survive and even grow. However, they may not grow in a rhizosphere with very diverse microbial communities. An introduced phloroglucinol-producing, gfp marked *P. fluorescens* strain declined faster in three organically managed soils than in three neighbouring conventionally managed soils (van Bruggen et al., 2004). The same strain showed only mild wave-like oscillations along a wheat root in an organically managed soil compared to a conventionally managed soil (Figure 6), and had less effect on take-all disease of wheat in soils from three organic farms than in soils from three neighbouring conventional farms with a lower microbial diversity (van Bruggen et al., 2004). Thus, it is questionable if inundative biological control can be effective in soils with a high microbial biomass, activity and diversity, and low levels of easily available substrate.

Conclusions

In this review we showed that populations of different trophic groups of bacteria develop in a wave-like fashion with repetitive growth and death cycles, both in time and space after an impulse of readily utilizable substrate. Oscillatory development of bacterial populations may be a universal phenomenon after a disturbance, which could possibly be used to compare soils in terms of stability and resilience, and consequently soil health. Indeed, the amplitudes of the oscillations are smaller and decline more quickly in soils with a high microbial biomass, activity and diversity, and low levels of easily available substrate. These are characteristics of soil health. It is argued that healthy soils are more suppressive to soil-borne plant pathogens than biologically impoverished soils.

Single species of saprotrophic bacteria, biocontrol agents and phytopathogenic fungi also show wave-like fluctuations in bulk soil and along plant roots. Different trophic groups and species may fluctuate with different periods and phases. A cyclic succession occurs in response to nutrient input; in the waxing phases of successive oscillations, microbial communities are taxonomically and physiologically more similar to one another than to the communities in the waning phases. This has consequences for the selection of biocontrol agents and cultivars. Introduction of a single biocontrol agent to a soil may not lead to the expected results due to wave-like fluctuations in the rhizosphere of the biocontrol agent and the target pathogen, if they are out of phase. A mixture of biocontrol agents of different trophic groups may be more successful.

The main strategies to control soil-borne diseases can be classified into three categories: (1) enhancement of general microbial biomass and diversity resulting in a masking of the rhizosphere effect, a reduction of the amplitude of wave-like oscillations and an increase in natural disease suppression, (2) removal of dormant propagules or pathogens in their saprotrophic phase from their food base by stimulating wave-like fluctuations in populations of potentially competitive microorganisms, for example by soil tillage, and (3) augmentation of microbial communities by biocontrol agents, which must be able to survive and grow in the rhizosphere. The first strategy is the main strategy used by organic farmers, while the second and third strategies are typical for conventional farms. In all cases, dynamic oscillations of microbial communities and individual species must be taken into account. This constitutes a new view of plant disease control.

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