Short-Term Fluctuations of Sugar Beet Damping-Off by *Pythium ultimum* in Relation to Changes in Bacterial Communities After Organic Amendments to Two Soils

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ABSTRACT

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Previously, oscillations in beet seedling damping-off by *Pythium ultimum*, measured as area under the disease progress curve (AUDPC), were demonstrated after incorporation of organic materials into organic and conventional soils. These periodic fluctuations of *P. ultimum* infections were cross-correlated with oscillations of copiotrophic CFU at lags of 2 to 4 days. For this article, we investigated whether bacterial communities and microbial activities fluctuated after a disturbance from incorporation of organic materials, and whether these fluctuations were linked to the short-term oscillations in AUDPC of beet seedling damping-off and bacterial populations (CFU) in soil. Soil microbial communities studied by polymerase chain reaction-DGGE analysis of 16S DNA after isolation of total DNA from soil and microbial activities measured as CO₂ emission rates were monitored daily for 14 days after addition of grass-clover (GC) or composted manure (CM) into organic versus conventional soils. Similar to our previous findings, AUDPC and density of copiotrophic bacteria oscillated with time. Fluctuations in species richness (S), Shannon diversity index (H), and individual amplicons on DGGE gels

were also detected. Oscillations in AUDPC were positively cross-correlated with copiotrophic CFU in all soils. Oscillations in AUDPC were also positively cross-correlated with 19 to 35% of the high-intensity DNA fragments in soils amended with GC but only 2 to 3% of these fragments in CM-amended soils. AUDPC values were negatively cross-correlated with 13 to 17% of the amplicons with low average intensities in CM-amended soils, which were not correlated with densities of copiotrophic CFU. CO₂ emission rates had remarkable variations in the initial 7 days after either of the soil amendments but were not associated with daily changes in AUDPC. The results suggest that infection by *P. ultimum* is hampered by competition from culturable copiotrophic bacteria and some high-intensity DGGE amplicons, because AUDPC is cross-correlated with these variables at lags of 1 to 4 days. However, negative cross-correlations with low-intensity DNA fragments indicate that *P. ultimum* infection could also be suppressed by antagonistic bacteria with low densities that may be nonculturable species, especially in CM amended soil. The organic soil generally had lower AUDPC values, higher bacterial diversity, and negative cross-correlations between AUDPC and low-intensity DNA fragments (after CM amendment), indicating that specific bacteria that do not attain high densities may contribute to *P. ultimum* suppression in organic soils.

Based on the temporal data of area under the disease progress curve (AUDPC) for seedling damping-off (by *Pythium ultimum*) after a nutrient impulse into soils, a new phenomenon was proposed: damping-off did not simply increase immediately after incorporation of organic materials into soils and decrease thereafter as formerly reported (9,23) but increased and decreased in a wavelike pattern with regular intervals (18). Experiments with soils which were amended with organic materials and inoculated with 0.1% (vol/vol) *P. ultimum* showed that AUDPC of sugar beet damping-off fluctuated with significant harmonics over time (18). Moreover, harmonics analysis demonstrated that AUDPC in the organically managed soil always had smaller amplitudes and lower frequencies than that in the conventionally managed soil after amendments, suggesting that the organic soil was healthier (38,39).

The fluctuations in seedling damping-off could be the consequence of microbial growth and death cycles in soils after a disturbance. Wavelike patterns in microbial populations had been observed many times (38,46). The impulse of nutrients would lead to immediate growth of microorganisms followed by death due to local nutrient limitation and, subsequently, by growth again from reuse of carbon sources supplied from decaying soil organic matter and dead cells. Given that the responses of pathogens to a nutrient pulse are similar to those of microorganisms, plant infections might also fluctuate in wavelike patterns, and even show fluctuation characteristics similar to those of microbial populations. Some species of plant pathogens have nutrient requirements similar to those of bacteria (12,25) and, indeed, AUDPC harmonics for *P. ultimum* displayed periods similar to those of CFU of copiotrophic bacteria after incorporation of organic materials into soils (18). Analogously, the periods of the wave-like distribution of *Pythium* spp. infections were similar to those of copiotrophic bacteria along wheat roots (40). However, the phase had shifted between the oscillations in pathogen infections and copiotrophic CFU in both the temporal and spatial dimensions (18,40).

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Microbial activity and biodiversity in soil are important factors influencing plant disease suppression (1,6,30). Therefore, another potential explanation for the wavelike patterns in AUDPC could be the fluctuation in bacterial metabolic activity and succession of competitive or antagonistic bacteria species within microbial communities. Infection could possibly take place more easily when microbial abundance and activity decline (7,30) but fluctuations in microbial activity in disturbed soil have not been widely published (4,7). The diversity of bacterial amplicons obtained by DGGE oscillated over time and along roots (10,41). The bacterial communities were similar when CFU increased or decreased in successive waves, indicating that succession took place within one waxing and waning phase of each wave rather than between waves. The succession of competitors and antagonists might result in alternating levels of competition between particular bacteria and plant pathogens, so that oscillations in disease incidence could be observed.

Various studies have been carried out on changes in soil microbial activities and community structures after implementation of agricultural practices (8,13,26,29,37,45), and several investigations have focused on the suppressive effects of soil bacterial biomass, activity, and diversity on pathogen infections (15,16,21,30,31,36). In all of these studies, microbial communities were investigated for specific groups (13), sampling times were sparse and intervals were long (8,26), or only a general description of suppressive effects of soil microbial functions was given (31). No detailed research on correlations between daily changes in plant pathogen infections and short-term variations of genetic composition of soil microbial communities or microbial activity after a disturbance has been conducted to date.

The overall goal of this research was to characterize the short-time oscillations of infections by the root pathogen *P. ultimum* in relation to bacterial communities and microbial activity after addition of organic materials to organic and conventional soils under controlled environmental conditions. Specific objectives were to investigate whether (i) short-term fluctuations in pathogen infections are correlated with changes in bacterial communities or activities and (ii) how soil bacterial communities and microbial activities fluctuate in a time series after input of organic nutrients. The experiments were carried out with two kinds of organic amendments in organic and conventional soils, which allowed us to address the additional aim of whether (iii) fluctuations in microbial activity, diversity, and plant infections differed in soils with different management strategies.

MATERIALS AND METHODS

Experimental setup. Two experiments were carried out in spring and summer 2007, respectively. Two series of PVC pots (diameter = 10.2 cm and depth = 25 cm) containing soil without amendments (control series) or soil amended with a mixture of grass and clover (GC treatment, spring experiment) or composted cattle manure (CM treatment, summer experiment) were used. The collection, addition rates, and properties of the two amendments were reported previously (18).

There were three pots for each sampling time and treatment in each experiment. The pots were randomized in blocks in a controlled environment room at 20°C. The day before the start of each experiment, moisture of the two soils was adjusted to 14.6% and both amendments were added as dry and milled materials. At the start of each experiment (day 0), inoculum of *P. ultimum* was mixed into all soils at a rate of 0.1% (vol/vol) to overwhelm the low level of *P. ultimum* naturally occurring in the soils (based on a preliminary experiment with the same soils). Fresh inoculum of *P. ultimum* was prepared in a soil-oatmeal culture (42). The soils with or without amendments were dispensed into PVC pots to a height of 20 cm. To minimize water-vapor losses while allowing air exchange, a thin plastic film was used to cover every pot.

Soils and subsampling. Experiments were carried out with two pairs of sandy soils as previously published (18).

Three pots of each series (one per block) were randomly taken from the incubator every day. The CO₂ emission rates were measured first using a closed chamber system (Innova 1412; LumaSense Technologies, Denmark). Then, subsamples were collected separately for *P. ultimum* bioassays, quantification of copiotrophic bacterial populations (CFU), and direct bacterial DNA extraction (two blocks only).

Plate counting of bacteria. Soil (10 g) was added to 90 ml of sterile deionized water and shaken on a rotary shaker for 15 min at 300 rpm. The suspension was sonicated for 5 min in an ultrasonic bath. Ten-fold serial dilutions were made. For amended soils, 50 µl of 10⁻⁵ and 10⁻⁶ suspensions were plated onto triplicate plates with high carbon (C at 1,000 ppm) media for copiotrophic bacteria to determine CFU per gram of dry soil as described previously (35,41). For nonamended soils, 50 µl of 10⁻⁴ and 10⁻⁵ suspensions were plated. Bacterial colonies were counted after 48 h of incubation at 25°C.

DNA isolation and polymerase chain reaction-DGGE analyses. The soil samples from days 0 to 14 in two blocks were analyzed for DNA composition. The microbial community DNA was extracted directly from soil samples (≈0.4 g). DNA extraction was done using the FastDNA SPIN Kit for soil following the manufacturer's instructions.

The polymerase chain reaction (PCR) amplification was carried out with the bacterial primers U968GC and L1401r (19) and the touchdown scheme (33) for 30 thermal cycles. The region of 450-bp-long products was checked on 1.2% agarose gels in Tris-borate-EDTA buffer and stained with ethidium bromide. Samples were randomized on DGGE gels.

DGGE was performed with the Bio-Rad Dcode system. PCR products (40 µl each) were loaded onto the denaturing gradients. A 6% polyacrylamide gel (acrylamide/bisacrylamide, 37.5:1) with 45 to 60% denaturing gradient (28) was used to separate bacterial amplicons. Electrophoresis was performed in Tris-acetate-EDTA buffer for 16 h at 100 V at a constant temperature of 60°C. Bio-Rad's Silver Stain (Bio-Rad Laboratories, Detroit) was used to stain the gels (3) according to the manufacturer's protocol.

Soil CO₂ flux measurement. The Closed Chamber Soil Trace Gas Flux Monitor (Innova 1412; LumaSense Technologies) was used to measure CO₂ emissions from the different soils. It was programmed to take seven measurements within 6 min. The gas flux $F(t)$ calculation was $F(t) = (V/A)/(dC/dt)$, where $F(t)$ = gas flux (milligrams per square meter per hour), V = chamber headspace volume (cubic meters), A = chamber surface area (square meters), and dC/dt = the slope of the linear regression of seven measurements of headspace gas concentration (milligrams per cubic meter) with measurement intervals of 1 min.

Bioassays with *P. ultimum*. The bioassays with *P. ultimum* were carried out in a separate climate chamber set at 20°C with 16 h of light. Thirty seeds of sugar beet ('Anastasia') were sown in one pot (0.6 liter) per soil sample collected daily from the main experiments. On days 14, 18, and 21 after sowing the seed, the numbers of healthy seedlings were scored and the numbers of completely damped-off or dead plants (30 minus the number of healthy seedlings) were calculated. The AUDPC (5) was calculated as described previously (18).

Statistical analysis. Untransformed CFU and AUDPC of damping-off in the soils were compared by one-sided paired *t* tests between treatments using SPSS 15.0 for Windows. Variances in CFU were small for each time period and were similar enough among treatments to warrant the use of untransformed data.

The scanned images of the DGGE gels were analyzed using Phoretix 1D (NonLinear Dynamics Ltd., Newcastle upon Tyne, UK), and the relative densities of amplified fragments were calculated. Bacterial diversity was estimated from DGGE banding patterns in two different ways: species richness and Shannon index.

Species richness was the number of DGGE fragments detected, disregarding their relative intensities. The Shannon index of bacterial diversity was calculated as $H' = -P_i \log P_i$ based on the relative band intensities (11). P_i was the relative intensity of each amplified fragment which was defined as n_i/N , where n_i is the height of a peak in intensity and N is the sum of all peak heights in the given DGGE gel profile.

Cross-correlation functions at 0.05 significance levels were calculated for all variables over time with SPSS 15.0 for Windows.

RESULTS

***Pythium* spp. infections.** AUDPC values for sugar beet damping-off fluctuated in all treatments, with similar fluctuations for each treatment in the three blocks over a period of 14 days. Because the fluctuations were not exactly synchronous and mean values would mask any significant oscillations, the results from single blocks are presented rather than averages of the three blocks, as mentioned in a previous article (18). The AUDPC values in amended conventional soils were significantly higher than in amended organic soil ($P < 0.05$) (Fig. 1). In the spring experiment, the AUDPC values decreased immediately after input of GC into both soils but increased after 1 day, with the first peak on the second and third days in organic and conventional soils, respectively (Fig. 1A). After the first peak, the AUDPC fluctuated at intervals of 4 and 3 days in GC-treated organic and conventional soils, respectively. The AUDPC in organic soils also decreased immediately after addition of CM and increased after 2 days, with the first remarkable peak on the third day (Fig. 1B). The first peak was followed by continuous oscillations for 14 days. For the CM-amended conventional soil, the AUDPC increased immediately in the initial 2 days, reached the first peak on the

Fig. 1. Fluctuations in area under the disease progress curve (AUDPC) of damping-off of sugar beet seedlings by *Pythium ultimum* in organic (org) and conventional (conv) soils over time in days since incorporation of A, grass-clover or B, composted manure.

second day, dropped, and rose again with the second peak after 5 days (Fig. 1B). These initial two peaks were followed by two fluctuations within 14 days. The mean standard errors (three blocks) for AUDPC were 8.45 and 6.93 for GC-amended organic soil and conventional soil, respectively, and 9.72 and 11.69 for CM-amended organic soil and conventional soil, respectively. The mean errors among replications were small compared with the variations over time. The variations of AUDPC in non-amended soils were relatively small. The ranges of AUDPC were 80 to 103 and 70 to 117 in organic and conventional soil, respectively, of the spring experiment and 90 to 122 and 115 to 145 in organic and conventional soil, respectively, of the summer experiment.

Bacterial CFU. Fluctuations were also noticeable in the data of copiotrophic CFU for all three blocks but they were not exactly synchronous and, again, the results of single blocks are presented instead of averages of the three blocks. Addition of organic materials significantly enhanced the numbers of copiotrophic CFU in soils, as shown in another article (18). The average number and fluctuations of CFU in GC-amended conventional soil were significantly larger than in GC-amended organic soil ($P < 0.001$) (Fig. 2A). The CFU increased immediately after input of plant residues and reached the first peak after 1 day in both soils. These peaks appeared 1 and 2 days ahead of the first peak of AUDPC in GC-treated organic and conventional soil, respectively. The CFU continued to fluctuate, with two oscillations in both GC-amended soils, which were observed 1 to 2 days earlier than AUDPC peaks. In the summer experiment, the oscillations of CFU in CM-amended soils were much weaker than in GC-treated soils (Fig. 2B). The first peak of CFU in CM-amended organic and conventional soil occurred after an increase for 4 and 3 days, respectively, which lagged 1 day behind the first peaks in AUDPC. In the following days, the bacterial populations continued to fluctuate, with two peaks within 14 days, and these peaks

Fig. 2. Fluctuations in copiotrophic CFU in organic (org) and conventional (conv) soils over time after incorporation of A, grass-clover or B, composted manure.

appeared 2 to 4 days and 2 days behind AUDPC peaks in CM-treated organic and conventional soils, respectively. The mean standard errors for copiotrophic CFU were 15.02×10^6 and 13.30×10^6 g⁻¹ of dry soil for GC-amended organic soil and conventional soil, respectively, and 3.45×10^6 and 3.56×10^6 g⁻¹ of dry soil for CM-amended organic soil and conventional soil, respectively. Again, the mean errors for replications were negligible compared with the changes over time. In the control series, copiotrophic CFU were much lower than in amended soils, with weaker fluctuations: the ranges of CFU in organic and conventional soils were 7.82 to 19.69×10^6 and 3.61 to 12.20×10^6 g⁻¹ of dry soil, respectively, in the spring experiment and 5.41 to 9.78×10^6 and 2.19 to 6.01×10^6 g⁻¹ of dry soil, respectively, in the summer experiment.

There were significant ($P < 0.05$) positive correlations between AUDPC and copiotrophic CFU at a lag of 4 and 2 days in GC-amended organic soil and conventional soil, respectively (Table 1). In the summer experiment, the AUDPC data also showed significant ($P < 0.05$) positive correlations with CFU; however, the correlations were observed when AUDPCs were moved backward 2 and 1 days in CM-treated organic and conventional soils, respectively. Indeed, the AUDPC fluctuations were shifted compared with those of copiotrophic CFU, as observed visually.

Soil CO₂ emissions. Soil CO₂ emission rate could be used as an index to represent soil microbial activity (34). Significant variations in CO₂ emissions in both experiments were observed in the initial 7 days after addition of organic materials (Fig. 3). Soil CO₂ emission rate increased immediately after GC incorporation and reached the first peak after 2 days (Fig. 3A) (i.e., 1 day behind the first peak in CFU). In the following days, CO₂ emission rate in GC-amended organic soil declined and rose appreciably but no significant oscillations appeared until the end of the experiment. CO₂ emission rate in GC-amended conventional soil reached the second small peak on the fourth day, then decreased slowly from ≈ 190 to 130 or 175 mg m⁻² h⁻¹ depending on the origin of the soil. There were significant positive correlations between CO₂ emission and CFU at a lag of 1 day (Table 1). This meant that soil microbial activity for all bacteria took place 1 day later than copiotrophic bacteria reproduction after the addition of GC to soil. In the summer experiment, the CO₂ emission rate also went up immediately after input of CM, with the first peak on the first day (Fig. 3B), which was much faster than copiotrophic

CFU. Thus, soil CO₂ emission rates were significantly positively correlated with CFU, and changes in CO₂ emission took place 1 day earlier than that in copiotrophic CFU (Table 1). After the initial peak, soil CO₂ emission declined sharply in the following 5 days and changed with negligible fluctuations to the end of the experiment. Final emission rates were ≈ 17 to 20 mg m⁻² h⁻¹. There were less significant fluctuations in CO₂ emission after the first peak, so that AUDPC fluctuations could not be attributed to soil CO₂ release. Indeed, no significant correlations were found

Fig. 3. Daily changes in soil CO₂ emission rate in organic (org) and conventional (conv) soils after incorporation of A, grass-clover or B, composted manure.

TABLE 1. Cross-correlations (CC) (significant at $P = 0.05$) among area under the disease progress curve (AUDPC), CFU, CO₂ emission rates, bacterial richness (S), and Shannon diversity index (H) in organic (Org) and conventional (Conv) soils after incorporation of grass-clover (GC) or composted manure (CM)^a

Variable, treatment	AUDPC		CFU		CO ₂		S		H	
	CC	Lag (days)	CC	Lag (days)	CC	Lag (days)	CC	Lag (days)	CC	Lag (days)
CFU										
Org-GC	0.627	-4	...	...	...	...	...	...	...	...
Conv-GC	0.509	-2	...	...	...	...	...	...	...	...
Org-CM	0.724	2	...	...	...	...	...	...	...	...
Conv-CM	0.494	1	...	...	...	...	...	...	...	...
CO ₂										
Org-GC	ns	...	0.611	1	...	...	...	...	...	...
Conv-GC	ns	...	0.570	1	...	...	...	...	...	...
Org-CM	ns	...	0.555	-1	...	...	...	...	...	...
Conv-CM	ns	...	0.693	-1	...	...	...	...	...	...
S										
Org-GC	ns	...	ns	...	ns	...	...	...	...	...
Conv-GC	ns	...	ns	...	ns	...	...	...	...	...
Org-CM	ns	...	ns	...	0.591	1	...	...	...	...
Conv-CM	ns	...	-0.570	2	ns	...	...	...	...	...
H										
Org-GC	ns	...	ns	...	0.554	1	0.811	0	...	...
Conv-GC	ns	...	ns	...	ns	...	0.952	0	...	...
Org-CM	ns	...	ns	...	0.746	1	0.897	0	...	...
Conv-CM	ns	...	-0.598	2	ns	...	0.686	0	...	...

^a Lag < 0 means that variable indicated in the leftmost cell (i.e., first variable) leads the variable indicated in the top cell; Lag > 0 means that variable indicated in the far left column follows the variable indicated in the top cell with |Lag| number of days; ns = not significant.

between AUDPC and soil CO₂ emission rate (Table 1). Non-amended soils had comparatively small changes in CO₂ emission rate over time: the ranges of CO₂ emission in organic and conventional soils were 12 to 28 and 11 to 59 mg m⁻² h⁻¹, respectively, in the spring experiment and 5 to 14 and 2 to 5 mg m⁻² h⁻¹, respectively, in the summer experiment.

Bacterial community composition. More than 100 fragments could be distinguished on DGGE gels of bacterial DNA from all amended soils. On average, 123 and 114 dominant eubacterial

amplicons could be distinguished on gels of DNA extracted from GC-amended organic and conventional soils, respectively, and 138 and 98 bacterial fragments in CM-treated organic and conventional soils, respectively, supporting the idea that higher diversity existed in the soils with lower density of bacterial CFU. Both indicators of bacterial diversity in amended soils, species richness and Shannon index, fluctuated over time (Fig. 4). During the first week after GC incorporation, the diversity indices were relatively low, but they climbed again in the second week in an oscillatory fashion. Oscillations in microbial diversity were also seen after CM amendment but without a specific trend during the 2-week observational period. Species richness had significant positive cross-correlations with soil CO₂ emission rates in CM-amended organic soil (Table 1). The Shannon index was positively correlated with CO₂ emission in the two amended organic soils when CO₂ emission rates were shifted backward 1 day. There were only significant negative correlations between bio-

Fig. 4. Species richness (S) and Shannon diversity index (H) of DGGE fragments detected in samples from **A**, grass-clover-amended organic soil; **B**, grass-clover-amended conventional soil; **C**, composted manure-amended organic soil; **D**, and composted manure-amended conventional soil.

Fig. 5. Relative band intensities of single amplicons of 16S rDNA directly extracted from organic soil after incorporation of grass-clover. **A**, Amplicon at relative band position 111, which went up first and down subsequently, had similar oscillations as copiotrophic CFU, and cross-correlated to area under the disease progress curve (AUDPC); **B**, amplicon at relative band position 69, which went down first and up subsequently, cross-correlated to AUDPC but was not significantly related to CFU; **C**, amplicon at relative band position 56, which was absent in the beginning of the experiment and was negatively correlated to AUDPC later.

diversity measures and copiotrophic CFU in CM-amended conventional soil. However, no significant correlations were found between fluctuations in AUDPC and biodiversity measures.

The intensities of individual bacterial DNA fragments also fluctuated over time after the nutrient inputs (Fig. 5). We divided amplified fragments into bands with high intensities and bands with low intensities. The cut-off points for high-intensity versus low-intensity bands on DGGE gels are shown in Table 2. Amplified fragments with a higher overall intensity often went up first and then declined (Fig. 5A). These fragments always had oscillations similar to those of copiotrophic CFU, and correlated with AUDPC at short lag periods. The intensities of weak bands sometimes declined first, then increased, and fluctuated in tandem with the AUDPC data at lags of several days but were not related to CFU (Fig. 5B). Some weak bands were absent in the beginning and negatively correlated to AUDPC later (Fig. 5C). Cross-correlations analysis demonstrated that the amplicons with significant (mostly positive) correlations to AUDPC formed 38.37 and 29.15% of all bands in GC-amended organic and conventional soils, respectively (Table 3). In the CM-treated organic and conventional soils, the amplified fragments significantly related to AUDPC represented 20.30 and 16.53% of all bands, respectively, but the correlations were mainly negative (Table 3).

DISCUSSION

We used 16S DNA amplification combined with DGGE to study the daily changes in bacterial communities after a nutrient impulse and relations between fluctuations in measurable bacterial communities and those in pathogen infection during 14 days in two different soils. Harmonics analyses were not carried out for this relatively short period (due to limitations of the number of lanes on a DGGE gel) but significant oscillations were shown previously for longer series (21 days) of AUDPC for *Pythium* damping-off and CFU over time (18). In the 14-day experiments, AUDPC values were significantly positively cross-correlated to copiotrophic populations when AUDPC were moved backward or forward several days in experiments with GC and CM, respectively. This result was similar to those of the 21-day experiments (18) but values of cross-correlation functions were higher in the 14-day data sets, confirming that the strongest oscillations in AUDPC and CFU took place during the first 2 weeks after nutrient inputs (18).

In this article, we showed that short-term changes of individual bands in DGGE gels with DNA extracted directly from soil showed oscillatory patterns after incorporation of organic ma-

terials into soil. This result was similar to the wavelike pattern in individual bacterial DNA fragments from DGGE analysis observed along wheat roots (41). For the first time, we demonstrated that the variations of a large proportion of individual DGGE bands correlated or cross-correlated with the extent of *P. ultimum* infection expressed as AUDPC. High-intensity fragments in the DGGE gels had oscillations similar to the AUDPC values but with short, mostly positive time lags, meaning that changes in the amplicons preceded similar changes in AUDPC. The fluctuations in the high-intensity amplicons were matched by oscillations in copiotrophic CFU. These DGGE fragments may have represented competitive species and antagonists among fast-growing bacteria. Other, less-intense fragments had mostly negative cross-correlations with AUDPC but no significant relations with copiotrophic bacteria. The species with lower population densities (and band intensities) seemed to dominate the microbial communities in organic- and CM-amended soils. Moreover, the DGGE fragments which were absent in the beginning also showed relations with AUDPC, suggesting that organic amendments could increase the activity and biomass of antagonistic bacteria over time or bring new species into the soil. In previous studies, single species of antagonistic bacteria were shown to suppress root pathogen infections (14,17) but reports of relationships between groups of soil microbial communities based on DGGE analysis are relatively rare (22,30,44). Workneh et al. (43), Hiddink et al. (20), and van Bruggen et al. (39) argued that general root disease suppression was not associated with one particular species but with bacterial communities and microbial diversity.

The short-term fluctuations of AUDPC mostly showed positive correlations or cross-correlations with changes of amplicons detected by DGGE in the spring experiment whereas, in CM-amended soils, AUDPC was mainly negatively correlated or cross-correlated to relative intensity of individual bands, suggesting that input of different organic materials to soil might represent diverse pathogen-suppressive principles. CM likely had much more native antagonistic bacteria to pathogens (24,32) and steadier nutrient levels than GC. Consequently, CM-amended soils seemed to be more stable, with smaller fluctuations in AUDPC than GC-amended soils. GC had higher concentrations of available carbon and nitrogen (18), which could be utilized by both bacteria and pathogen immediately after addition into soils. Presumably, competition between fast-growing bacteria and the pathogen was the major principle of temporary disease suppression in GC-amended soils, indicated by alternating peaks in copiotrophic CFU and AUDPC (at 2- to 4-day lags) as well as high-intensity individual bands.

TABLE 2. Cut-off points for high-intensity (HI) versus low-intensity (LI) bands on DGGE gels of 16S rDNA directly extracted from grass-clover (GC) or composted manure (CM) amended organic (Org) and conventional (Conv) soil

Parameters	Org-GC		Conv-GC		Org-CM		Conv-CM	
	HI	LI	HI	LI	HI	LI	HI	LI
Range of intensities	2.48–10.99	0–2.48	2.03–9.39	0–2.03	1.82–6.91	0–1.82	2.49–9.61	0–2.49
Total intensities (%)	51–56	44–49	58–64	36–42	59–64	36–41	59–61	39–41
Total bands (%)	23–28	72–77	29–33	67–71	33–38	62–67	24–26	74–76

TABLE 3. Relationships between area under the disease progress curve (AUDPC) and amplicons in DGGE gels of 16S rDNA directly extracted from grass-clover (GC) or composted manure (CM) amended organic (Org) and conventional (Conv) soil^a

Correlation	Org-GC bands (%)			Conv-GC bands (%)			Org-CM bands (%)			Conv-CM bands (%)		
	Lag = 0	Lag < 0	Lag > 0	Lag = 0	Lag < 0	Lag > 0	Lag = 0	Lag < 0	Lag > 0	Lag = 0	Lag < 0	Lag > 0
Positive	5.04	13.95	16.28	2.78	6.25	10.40	0	1.49	0.99	0.9	1.42	1.42
Negative	0.78	1.16	1.16	2.08	3.47	4.17	2.97	4.95	9.90	2.84	4.74	5.21
Total (%) ^b	38.37			29.15			20.30			16.53		

^a Lag < 0 means that variation of AUDPC leads the changes of amplicons; Lag > 0 means that variation of AUDPC follows the changes of amplicon; the lag unit is 1 day.

^b Total numbers of bands were 123, 114, 138, and 98 for Org-GC, Conv-GC, Org-CM, and Conv-CM, respectively.

Another potential reason for different relations between damping-off intensity and measurable bacterial composition in the two experiments could be oxygen concentration. Although we did not determine O₂ content, much higher concentrations of NH₄⁺ (18) and larger peaks of N₂O (unpublished data) in the GC-amended than CM-amended soil indicated that temporary anaerobic conditions occurred to a greater extent in the GC-amended soils. The microbial community structures were likely different in anaerobic and aerobic microniches, leading to differences in bacterial abilities to compete with *P. ultimum*.

The biodiversity detected by DGGE analysis in both amended soils of two experiments exhibited short-term fluctuations after a disturbance by nutrient incorporation. The oscillations in bacterial diversity dampened with time, especially after CM incorporation, but the diversity remained relatively high. In contrast, oscillations in bacterial diversity continued while the average diversity level increased in GC-amended soils, associated with a gradual decline in AUDPC oscillations. Thus, the effect of GC amendment on bacterial communities and pathogen infection was of shorter duration than that of CM amendment, which was also observed before (2,27) but not in a time series.

No correlations were found between copiotrophic CFU and biodiversity in soils. This result was different from the results of van Diepeningen et al. (41), who reported that an increase of fast-growing bacterial populations might cause local loss in biodiversity after a disturbance in soil. These different findings could be attributed to the higher biodiversity in our study (≈70 to 80 DGGE bands were detected in the study of van Diepeningen and her colleagues whereas >100 bands were found in our study). The more biodiversity exists in soil, the less fast-responding species influence soil community structure (39).

Microbial activity, assessed as CO₂ respiration, increased significantly in organic and conventional soils after nutrient impulses from fresh plant material or CM. Fluctuations in CO₂ emission rates occurred within the first 7 days and were less pronounced than those in copiotrophic CFU. Chen et al. (7) also found that microbial activity, measured by the rate of hydrolysis of fluorescein diacetate, fluctuated during a 7-day experiment after amending container media with composted hardwood bark. Significant correlations between microbial activity and disease severity were observed (7). In the research reported here, however, CO₂ respiration from total soil microorganisms did not seem to influence the fluctuations in *P. ultimum* infection after nutrient impulses in 14-day experiments. The background levels of available nutrients might be the reason for the different results.

In conclusion, soil measurable bacterial communities and microbial activities fluctuated after nutrient input into two different soils. The oscillations in damping-off intensities by *P. ultimum* could be explained by increases and decreases of groups of microbial species in amended soils but could not be directly related to variations of bacterial activities. Although it is difficult to detect specific bacteria for plant disease control among hundreds of species in soil, our observations lead to an approach to identify antagonists or competitors of plant pathogens by searching at particular moments in time relative to a disturbance, cutting selected DNA fragments from the DGGE gels, and sequencing these fragments. Finally, the higher biodiversity and much steadier fluctuations in microbial community composition after a disturbance by CM than by GC indicated a greater stability in CM-amended soils.

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