Daily Changes of Infections by *Pythium ultimum* After a Nutrient Impulse in Organic Versus Conventional Soils

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ABSTRACT

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Bacterial populations (CFU) have been shown to oscillate in wavelike patterns after nutrient impulses in previous studies. The amplitudes and periods of oscillations could possibly be used as indicators of soil health analogous to the stability and resilience of biological populations widely accepted as indicators for ecosystem health. Limited plant and animal disease outbreaks can also be viewed as a manifestation of a healthy soil ecosystem. Two pot experiments were carried out to verify whether damping-off of beet seedlings by *Pythium ultimum*, measured as area under the disease progress curve (AUDPC), fluctuated over time after incorporation of organic materials into organic versus conventional soils, and to investigate whether daily dynamics of AUDPCs were linked to the dynamics of microbial populations and chemical parameters. AUDPCs oscillated significantly over time when *Pythium* bioassays were initiated daily after addition of ground grass and clover shoots (GC) into unplanted soils. Similar oscillations with significant harmonics of AUDPC were also observed in composted manure (CM)-amended soils but with smaller

amplitudes than in GC-amended soils. The AUDPC harmonics in amended soils had periods similar to those of CFU of copiotrophic bacteria. Cross-correlation analysis demonstrated that periodic fluctuations of *P. ultimum* infections (AUDPCs) did not coincide with those of copiotrophic CFU but were shifted in phase. It appears that competition or antagonism from some fast-growing bacteria influenced pathogen infections, because these bacterial populations were growing and dying. Soil chemical variables, including pH, dissolved organic carbon, and NO_3^- -N, and NH_4^+ -N contents, changed significantly in the initial 7 days after a nutrient impulse into soils. These changes were cross-correlated with copiotrophic CFU with time lags of ≈ 1 to 2 days but were seldom associated with daily changes in AUDPCs. Organically managed soils always had lower AUDPC ratios of amended to nonamended treatments, indicating that organic materials showed stronger suppressive abilities to *P. ultimum* in organic than in conventional soils. The oscillations in AUDPCs and copiotrophic CFU in amended organic soil also had smaller amplitudes than in amended conventional soil. These results suggested that organically managed soils had a greater resistance and resilience to the disturbance of the amendments and, therefore, could be considered healthier than conventionally managed soils.

Wavelike oscillations in microbial populations after a disturbance had been demonstrated several times (46,48). The amplitude and period of these regular oscillations were proposed to be an indicator of stability and resilience of soil ecosystems, which was suggested as a new approach for quantifying the health status of a soil (37,39). Experiments with γ -irradiated and nonirradiated soils showed that bacteria populations (CFU) oscillated in a wavelike pattern over time, and harmonics analysis demonstrated that γ -irradiated soil had larger amplitudes and periods of wavelike oscillations in microbial populations (i.e., lower stability and resilience) than nonirradiated soil after incorporation of grass and clover shoots, suggesting that nonirradiated soil is healthier (45).

Larger amplitudes of oscillations in microbial populations were also observed in a fallow soil than in a cover-cropped soil after addition of the same amount of cover crop plant material, implying that cover-cropped soils may be healthier than fallow soils (37,38).

A healthy soil ecosystem is frequently associated with limited plant and animal disease outbreaks (30). A higher microbial diversity in healthy soils can play a role in plant pathogen suppression and affect internal nutrient cycling (12,37). We hypothesize that infections by plant pathogens in perturbed soils have wavelike patterns over time with smaller amplitudes in healthier than in less healthy soils, similar to the reduced amplitudes of microbial oscillations in healthier soils. In previous experiments with wheat roots, harmonics analysis showed that infections by root pathogens fluctuated along the wheat roots (40). Furthermore, oscillations of *Pythium* spp. infections were positively correlated with oscillations in copiotrophic bacteria at a lag of 6 cm along the roots. Temporal wavelike dynamics could be deduced from ob-

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served spatial wavelike distributions (47). Indeed, in a preliminary study with sampling intervals of 7 days, temporal fluctuations of *Pythium* spp. infections on tomato seedlings followed copiotrophic bacterial populations at a lag of ≈ 6 days in fallowed and cover-cropped soils after incorporation of vetch-oat cover crop shoots (37,38). However, frequent and detailed observations of plant infections after a disturbance were not made, so that harmonics analysis could not be used to distinguish significant oscillations in those experiments.

The occurrence of regular waves in bacterial populations might be due to the growth-death cycle of microorganisms and succession of different microbial species in response to an impulse of nutrients (39,42). Such a succession in bacterial communities occurred within one waxing and waning phase of an oscillation rather than between oscillations, as demonstrated by changes in microbial composition detected by denaturing gradient gel electrophoresis (8,42). The growth-death cycles of bacteria and the successions of microbial communities might result in alternating levels of competition between particular bacteria and plant pathogens, resulting in oscillations in disease incidence.

Recently, many researchers have shown effects of organic amendments on plant disease incidence in soils (3,20,25,26,29,35), and various investigations have been carried out on changes in soil chemical and microbial properties after disturbances (6,11,13,14,28,36). However, the daily dynamics of pathogen infections in soil after a disturbance have seldom been explored (17), and their correlations with soil microbial populations and chemical properties not at all.

Our research was aimed at understanding the short-term dynamics of infections by the root pathogen *Pythium ultimum* in relation to the daily changes of bacterial populations and chemical properties after incorporation of organic materials into different soils under controlled environment conditions. Specific objectives were to investigate whether (i) there are temporal wavelike patterns in infections by *P. ultimum* after a nutrient impulse into soils, (ii) such patterns correlate with the wavelike fluctuations of microbial populations after a nutrient impulse in soil, and (iii) the daily changes of chemical properties relate to short-term fluctuations in infections by *P. ultimum*. Experiments were conducted with two kinds of organic amendments in organically and conventionally managed soils, allowing us to investigate an additional aim: (iv) to compare the daily changes of damping-off, soil microbial populations, and chemical properties between two differently managed soils amended with different organic materials.

MATERIALS AND METHODS

Soil. Experiments were conducted with two pairs of sandy soils collected from organically managed fields in the Droevendaal experimental farm, which had been organic for 7 years, and neighboring conventional fields of Unifarm, Wageningen UR, The Netherlands. The organic fields had received cover-crop materials and composted cattle manure and cattle slurry to fertilize the crops, while the conventional fields had received only inorganic fertilizers. Pesticides had been used in the conventional fields only. For the experiment in spring 2007, both the organic and

conventional soils were from fields planted to maize (*Zea mays* L.) in 2006, and maize residues were still visible. For the experiment in the summer of 2007, two soils were collected from organic and conventional fields that had maize crops at the time of sampling. Soil was taken to a depth of 15 cm in each field. After collection, the soils were sieved through a screen with 4-mm mesh and stored in a controlled environment room at 20°C until the start of the experiments. The moisture contents of the soils were measured immediately. General soil properties were determined after air drying (Table 1).

Soil chemical analyses. Soil samples were dried, ground, and suspended in CaCl_2 . Soil extracts in CaCl_2 were analyzed for pH, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, total soluble N content, and dissolved organic carbon (DOC) content. The concentrations of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were determined by an automatic inorganic nitrogen analyzer (Autoanalyzer II system; Technicon Instruments, St. Dennis, France). The contents of DOC were measured by a TOC Analyzer (Shimadzu, Japan). The moisture content of the fresh soils was determined with $\approx 10\text{-g}$ samples on a Mettler LJ16 moisture content analyzer (Mettler Toledo International Inc., OH).

Experimental design. The experiments were carried out from 23 May to 12 June and from 2 to 22 August 2007. Both experiments consisted of two series of PVC pots (diameter, 10.2 cm and depth, 25 cm) containing nonamended soil or soil mixed with 1% (wt/wt, by dry weight) of dry and milled amendments. A mixture of grass and clover (GC) was the amendment for the spring experiment. Grass and clover were cut from a field at Wageningen University and mixed in a 1:1 ratio based on fresh weight. The average carbon and nitrogen contents for the GC mixture were 41.6 and 2.6%, respectively (based on dry weights). Composted cattle manure (CM), consisting of cattle dung mixed with straw, was the amendment for the summer experiment. It was collected from a heap at the Droevendaal farm and dried and milled before use. The carbon and nitrogen contents of CM based on dry weights were 29.76 and 2.51%, respectively. The day before the start of each experiment (day 0), predetermined amounts of water were added to the soils to reach a water-filled pore space of 25% and an air-filled pore space of 20% in the pots, corresponding to 14.6% of moisture value in both experiments (assuming a soil particle density of 2.65 mg m^{-3} and depth of 20 cm). Inoculum of *P. ultimum* was mixed into all soils at a rate of 0.1% (vol/vol) on day 0 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 (44).

The final C:N ratio of amended soil ranged from 15.97 to 17.75 in the spring experiment and 13.04 to 15.77 in the summer experiment. The soils with or without amendments were filled into the PVC pots to a height of 20 cm. To prevent water losses but allow air exchange, the pots were covered with a thin plastic film. The pots with the different treatments (soil management type and with or without amendment) were randomized in three blocks in a controlled environment room at 20°C and incubated for 20 days.

Three pots of each treatment (one per block) were collected every day. All soil from one pot was mixed first; then, subsamples

TABLE 1. Characteristics of soils collected from organically and conventionally managed fields in this study^a

Soil characteristics	Spring 2007		Summer 2007	
	Organic soil	Conventional soil	Organic soil	Conventional soil
Sand content (%)	92.78 $\pm$ 1.31	93.24 $\pm$ 1.00	89.30 $\pm$ 0.06	91.17 $\pm$ 0.54
Silt content (%)	4.77 $\pm$ 0.19	4.51 $\pm$ 0.23	7.00 $\pm$ 0.62	6.13 $\pm$ 0.17
Clay content (%)	2.45 $\pm$ 0.27	2.25 $\pm$ 0.18	3.70 $\pm$ 0.09	2.70 $\pm$ 0.20
pH (CaCl_2)	5.59 $\pm$ 0.03	5.55 $\pm$ 0.04	5.37 $\pm$ 0.01	5.51 $\pm$ 0.05
Organic matter content (%)	2.85 $\pm$ 0.01	2.25 $\pm$ 0.02	3.03 $\pm$ 0.01	1.91 $\pm$ 0.01
Total N content (mg kg^{-1})	1,039 $\pm$ 26	806 $\pm$ 30	1,115 $\pm$ 41	662 $\pm$ 19

^a Values are means $\pm$ standard deviations ($n = 3$).

were taken for determination of soil moisture, pH, dissolved organic carbon (DOC), $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ contents as described above for determination of copiotrophic bacterial populations (CFU) and for *P. ultimum* bioassays. Thus, separate bioassays for *P. ultimum* disease development and determination of area under the disease progress curves (AUDPCs) were initiated daily.

Dilution plating of bacteria from soil. 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. Then, 10-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 (32,42). For unamended soils, 50 μl of 10^{-4} and 10^{-5} suspensions were plated. The agar medium contained 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g of KNO_3 , 1.3 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.06 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2.5 g of glucose, 0.2 g of enzymatic casein hydrolysate, and 15.0 g of Bacto Agar per liter, plus sterile cycloheximide at 100 ppm. The total amount of carbon available for bacterial consumption was estimated at 1,100 mg liter $^{-1}$ of medium. Bacteria colonies were counted after 48 h of incubation at 25°C.

Bioassays for root pathogen infection. The *P. ultimum* bioassays were carried out in a controlled environment chamber at 20°C (16-h day and 8-h night regime). For both main experiments, 30 sugar beet (cv. *Anastasia*) seeds were sown daily in one pot (0.6 liter) per soil sample. 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. In this disease assay, we used the AUDPC (2) to express the incidence of seedlings infected by *P. ultimum* over time. Roots of damped-off seedlings were observed for symptoms and plated onto water agar to check for the presence of *P. ultimum*.

Statistical analysis. CFU and AUDPC data were compared by one-sided paired *t* tests among treatments and between experiments using SPSS 15.0 for Windows. To detect the presence of significant wavelike oscillations for CFU and AUDPC of *Pythium* spp. infections, harmonics analysis (also called Fourier analysis) was conducted on individual data series (24 series for each of the experiments) according to Jenkins and Watts (22) with modifications of Gorbenko and Kryshev (15). The data were detrended by linear regression and smoothed using Hann's three-point window before harmonics analysis (33). Significant harmonics and their period, phase, and amplitude were determined at the 0.05 significance level. Cross-correlation functions at 0.05 significance levels were calculated for all variables with SPSS 15.0 for Windows.

RESULTS

Damping-off. Damping-off was caused by *P. ultimum* based on typical symptoms on the root tips and base and on isolations of the pathogen. There were fluctuations in AUDPC for all series of pots, with similar fluctuations for the three blocks. Because the fluctuations were not exactly synchronous, mean values would obscure any significant oscillations. Therefore, the results of single blocks are presented rather than averages of the three blocks per experiment. The AUDPC of damping-off of sugar beet seedlings decreased immediately after incorporation of GC into both organic and conventional soils but increased after 1 day, with the first peak on the third day and the second peak on the fifth day (Fig. 1A). The first peak in AUDPC in GC-amended organic soil was much smaller than the second one, whereas the GC-amended conventional soil had the larger peak on the third day. These two peaks were followed by three weak but significant fluctuations (*t* test, $P < 0.05$). In the summer experiment, the AUDPC in CM-treated conventional soil increased immediately and reached the

first peak after 2 days, dropped, and rose again with the second peak after 5 days (Fig. 1B). These initial two peaks were followed by continuous oscillations. Organic soil had lower AUDPC values than conventional soil after addition of manure. The first remarkable peak of AUDPC in CM-amended organic soil appeared on the third day, and AUDPC values continued to fluctuate with two gentler oscillations. The mean standard errors (three blocks) for AUDPC were 15.91 and 16.37 for GC-amended organic soil and conventional soil, respectively, and 12.41 and 9.31 for CM-amended organic soil and conventional soil, respectively. These mean errors between replications were negligible compared with the changes over time (Fig. 1).

The data of AUDPC ratio in amended soils to nonamended soils could represent suppressive abilities of organic materials to plant disease. In both experiments, the AUDPC data in control series had minor variations over time, so that the AUDPC ratio had oscillations similar to the AUDPC in amended soils and fluctuated around 1 (Fig. 1). More than 70% of the AUDPC ratio data were <1 in both amended organic soils, whereas $<50\%$ of these data in both amended conventional soils were <1 , indicating that organic materials showed stronger inhibitory abilities to plant disease in organic soils than in conventional soils, especially after 7 days. The mean standard errors for AUDPC ratios in the spring experiment were 0.14 and 0.18 for organic and conventional soil, respectively, and 0.13 and 0.11 in organic and conventional soil, respectively, for the summer experiment. Again, these mean errors for the replications were small compared with the changes over time.

Harmonics analysis of detrended and smoothed AUDPC data demonstrated that there were remarkable wavelike patterns with one and two significant harmonics in organic and conventional soils, respectively, after incorporation of GC (Fig. 2; Table 2).

Fig. 1. Daily changes in area under the disease progress curve (AUDPC) of damping-off (*Pythium ultimum*) on sugar beet seedlings in organic (org) and conventional (conv) soils after incorporation of **A**, grass-clover or **B**, composted manure; figures in the top right corners were ratio of AUDPC in amended soils to nonamended soils over time.

These harmonics contributed 33.5 and 78.99%, respectively, to the observed variation in AUDPC. In the summer experiment, the first and second harmonics were significant in both CM-amended organic and conventional soils, accounting for 65.51 and 74.99% of the variation, respectively (Table 2). The amplitudes in the GC-amended soils were much greater than in CM-amended soils, probably because GC had more readily available carbon sources than CM.

Bacterial CFU. Similar to the fluctuations in AUDPC, there were fluctuations in CFU for all three blocks, which were not exactly synchronous. Therefore, the results of single blocks are presented rather than averages of the three blocks. The numbers of copiotrophic CFU in amended soils were significantly ($P < 0.001$) higher than in nonamended soils (Fig. 3). The conventional soils always had the greater fluctuations after incorporation of organic materials. In the spring experiment, CFU increased immediately after incorporation of plant residues with the first peak after 1 day in both amended soils, which was observed 2 days earlier than the first peak of AUDPC (Fig. 3A). In the

following days, the bacterial populations continued to fluctuate with weak oscillations in GC-amended organic soil, whereas CFU in GC-amended conventional soil fluctuated at similar intervals of 4 to 5 days and reached high levels again after 9 days. These peaks appeared 1 to 2 days ahead of the AUDPC peaks. Copiotrophic CFU in CM-treated soils increased more slowly than in GC-amended soils and fluctuated with weaker oscillations (Fig. 3B). The first peak of CFU in both CM-amended soils occurred after a monotonous increase for 3 days. After the first peak, the CFU fluctuated with several peaks, and these peaks were observed 1 to 2 days and 2 to 3 days behind AUDPC peaks in CM-amended conventional and organic soils, respectively. The mean standard errors for copiotrophic CFU were 10.73×10^6 and 7.47×10^6 g⁻¹ of dry soil for GC-amended organic soil and conventional soil, respectively, and 3.64×10^6 and 5.36×10^6 g⁻¹ of dry soil for CM-amended organic soil and conventional soil, respectively.

There were significant wavelike oscillations for daily changes of copiotrophic CFU (Fig. 2; Table 2). The harmonics for CFU data accounted for 42.4 and 56.14% of the variation in GC-

Fig. 2. Harmonic oscillations in area under the disease progress curve (AUDPC) of damping-off on sugar beet seedlings infected by *Pythium ultimum* and copiotrophic CFU/g of dry soil over time in **A**, grass-clover-amended organic soil; **B**, grass-clover-amended conventional soil; **C**, composted-manure-amended organic soil; and **D**, composted manure amended conventional soil.

TABLE 2. Characteristics of significant harmonics detected by harmonics analysis for daily dynamics of area under disease progress curve (AUDPC) of damping-off of seedling infected by *Pythium ultimum* and CFU/g of dry soil in organic (org) and conventional (conv) soils after incorporations of grass-clover (GC) or composted manure (CM)

Variable	Treatment	Harmonic no. ^a	Amplitude ^b	Phase (days)	Period (days)	Frequency (days ⁻¹)	Contribution to variance (%)	F estimated ^c	n
AUDPC	Org-GC	2	28.35	4.69	10.5	0.0952	33.5	3.35*	21
		1	30.73	10.88	21	0.0476	49.7	4.97**	21
		3	23.60	2.86	7	0.1429	29.29	2.929*	21
CFU	Org-GC	2	43.9×10^6	-0.554	10.5	0.0952	42.4	4.24**	21
		2	67.8×10^6	-1.044	10.5	0.0952	29.91	2.991*	21
		3	63.5×10^6	2.140	7	0.1429	26.23	2.623*	21
AUDPC	Org-CM	1	16.1	-1.7	21	0.0476	30.01	3.001*	21
		2	17.5	-0.08	10.5	0.0952	35.5	3.55**	21
		1	17.4	-1.59	21	0.0476	26.09	2.609*	21
CFU	Org-CM	1	8.52×10^6	1.66	10.5	0.0952	48.9	4.89**	21
		2	8.93×10^6	10.3	21	0.0476	32.3	3.23*	21
		2	8.93×10^6	2.95	10.5	0.0952	35.4	3.54**	21
AUDPC	Conv-CM	1	8.39×10^6	4.45	10.5	0.0952	36	3.6**	21
		2							
		2							

^a Number of oscillations.

^b Half of the distance between maximum and minimum in an oscillation.

^c Significant levels: * and ** = 0.10 and 0.05, respectively.

amended organic and conventional soils, respectively, and 67.7 and 36% of the variation in CM-treated organic and conventional soils, respectively (Table 2). The periods and frequencies of oscillations in CFU were similar to the harmonics of AUDPC in each amended soil but the phases were shifted (Fig. 2).

Cross-correlation analysis (Table 3) demonstrated that the AUDPC data were positively correlated with copiotrophic CFU at a lag of 4 and 2 days in GC-amended organic and conventional soils, respectively. Similarly, AUDPC data in the summer experiment showed significant ($P < 0.05$) positive correlations with CFU; however, the correlations were observed when the AUDPC data were moved forward 3 and 2 days in CM-amended organic and conventional soils, respectively. Thus, AUDPC fluctuations were indeed shifted compared with those of copiotrophic CFU, as observed visually.

Soil chemical properties. The soil chemical properties in two experiments had similar variations after incorporation of organic

materials, and significant changes were always observed in the initial 7 days (Figs. 4 and 5). Additions of organic materials increased pH levels and reached the first peak after 2 days (Fig. 4). In the following 5 days, pH dropped continuously to the initial levels and no significant fluctuations were observed after the first

Fig. 3. Daily changes in copiotrophic CFU in organic (org) and conventional (conv) soils. **A**, soils with or without grass clover (GC) and **B**, soils with or without composted manure (CM).

TABLE 3. Cross-correlations (0.05 significance level) between area under disease progress curve (AUDPC) and CFU in organic (org) and conventional (conv) soils after the incorporation of grass-clover (GC) or composted manure (CM)

Variable	Treatment	AUDPC	
		CCF	Lag ^a
CFU	Org-GC	0.484	-4
	Conv-GC	0.492	-2
	Org-CM	0.464	3
	Conv-CM	0.484	2

^a Lag is the number of days one series is shifted compared with another series. Lag < 0 means that the variable indicated in the leftmost column (i.e., first variable) leads the variable indicated in the top row; Lag > 0 means that the variable indicated in the leftmost column follows the variable indicated in the top row with [Lag] number of days.

Fig. 4. Daily changes in pH value and concentrations of dissolved organic carbon (DOC) in organic (org) and conventional (conv) soils after incorporation of **A**, grass-clover or **B**, composted manure.

Fig. 5. Daily changes in concentrations of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in organic (org) and conventional (conv) soils after incorporation of **A**, grass-clover or **B**, composted manure.

peak. The DOC contents increased 10 and 2 times after incorporation of GC and CM, respectively (Fig. 4) into soils. DOC concentrations increased immediately after organic matter incorporation, then declined sharply in the following 5 days and continued to decrease with very weak fluctuations until the end of the experiments. The decline of DOC in GC-amended soils was much more precipitous than in CM-amended soils, probably because more stable organic matter existed in CM. Nonamended soils had considerably smaller changes in pH and DOC over time: pH in control series changed within 5.5 to 5.6 and 5.2 to 5.4 in the spring and summer experiment, respectively; the ranges of DOC contents in organic and conventional soils were 80 to 88 and 63 to 69 mg kg⁻¹, respectively, in the spring experiment, and 66 to 73 and 44 to 49 mg kg⁻¹, respectively, in the summer experiment.

The NH₄-N concentrations increased immediately after addition of organic materials and reached a maximum on the second day (Fig. 5). The NH₄-N contents declined precipitously in the following 5 days, and no fluctuations were observed after the first peak. There were no significant variations for NO₃-N concentrations in amended soils during the first 2 days but NO₃-N started to increase when NH₄-N concentrations started to drop because of nitrification (Fig. 5). There were comparatively lower changes in NH₄-N and NO₃-N in control treatments over time: NH₄-N changed within the range of 1.6 to 4.0 mg kg⁻¹ for control treatments in the spring experiment and 1.0 to 2.5 mg kg⁻¹ in the summer experiment; in nonamended soils, changes in NO₃-N ranged between 5 to 15 and 10 to 20 mg kg⁻¹ in the spring and summer experiment, respectively.

There were no significant wavelike oscillations in soil chemical properties after the initial peak, so that the fluctuations in AUDPC of pathogen infection and bacterial populations could not be attributed to changes of these chemical properties. Indeed, none of the pH, DOC, and NH₄-N contents had significant correlations with AUDPC data. Daily changes of AUDPC were only correlated with NO₃-N when AUDPC data were shifted forward 5 days in GC-amended organic soil and 6 days in CM-amended conventional soil (Table 4). However, the fluctuations in CFU were always cross-correlated with changes in chemical variables in amended soils with a time differences of 1 to 2 days (Table 4).

DISCUSSION

For the first time, we demonstrated significant wavelike oscillations in the extent of infection of sugar beet by a plant pathogen (*P. ultimum*) over time after a disturbance of the soil by incorporation of fresh plant material or composted manure. The extent of infection was expressed as AUDPC in successive bioassays initiated daily after the disturbance was applied to unplanted soil, and it was assumed that AUDPC reflected the inoculum or infection potential of *P. ultimum* at the time of

planting of the beet seed. Previously, wavelike distributions of infections by *P. ultimum* and *Rhizoctonia solani* were observed along roots of wheat (40). In that case, exudation from the root tip and sloughed-off root cap cells were considered the disturbing factor in bulk soil when the root tip arrived. Oscillations in time were inferred from the observed oscillations in space but, in this article, we showed that temporal oscillations in infections do indeed occur.

For the first time, we also demonstrated that the periods of the oscillations in AUDPC in amended soils were similar to those of bacterial populations over time but that the phase had shifted. This result was similar to the phase shift between bacterial populations and *Pythium* spp. infections observed along wheat roots (40). This indicates that the responses of *P. ultimum* and copiotrophic bacteria to a nutrient pulse were similar but their reaction speeds may have been different. Ellis et al. (10) found similar nutrient requirements for *P. ultimum* and *Pseudomonas fluorescens* 54/96, which is a well-known copiotrophic organism (11). Indeed, *Pythium* spp. and copiotrophic bacteria are both r-strategists, which have a faster response to disturbances than k-strategists (23,38). The shift in phase indicates that there was competition between *Pythium ultimum* and copiotrophic bacteria. In the organic soil amended with GC residues and the conventional soil amended with CM, the shift was approximately half as long as the period (Table 2), so that the oscillations were in opposite direction (Fig. 2A and D), indicating that *P. ultimum* could infect only after copiotrophic populations had subsided. In the conventional soil amended with GC and the organic soil amended with CM, the shift was one-quarter to one-third as long as the period, so that the oscillations in bacterial populations and AUDPC followed each other more closely (Fig. 2B and C). The reason for this difference in shift time is not clear.

The maxima in bacterial populations appeared earlier than those in AUDPCs in GC-amended soils whereas, in CM-amended soils, oscillations of CFU followed AUDPC fluctuations, suggesting that concentrations of available carbon and nitrogen in organic amendments might influence the pioneer group of competition between pathogens and bacteria. The GC-amended soil had higher concentrations of available carbon and nitrogen than the CM-amended soils immediately after incorporation of the amendments, and copiotrophic bacteria proliferated faster than *P. ultimum* in GC-amended soils. In the CM-amended soils, the lower concentrations of readily available carbon and nitrogen might not have supported the rapid reproduction of bacteria immediately and, consequently, copiotrophic bacteria were out competed by *P. ultimum*. Alternatively, the rapid growth of bacteria in GC-amended soil resulted initially in such high ammonium levels that these might have been toxic to *P. ultimum*, so that infection was initially reduced in both organic and conventional GC-amended soils. Ammonia was shown earlier to reduce survival of *Pythium* spp. so that Pythium disease could be controlled (21).

TABLE 4. Values of cross-correlation functions (0.05 significance levels) among chemical variables, area under disease progress curve (AUDPC), and CFU after incorporation of grass-clover (GC) or composted manure (CM) into organic (org) and conventional (conv) soils

Variable	Treatment	NO ₃ -N		NH ₄ -N		pH		Dissolved organic carbon	
		CCF	Lag ^a	CCF	Lag	CCF	Lag	CCF	Lag
AUDPC	Org-GC	0.646	-5	ns	...	ns	...	ns	...
	Conv-GC	ns	...	ns	...	ns	...	ns	...
	Org-CM	ns	...	ns	...	ns	...	ns	...
	Conv-CM	0.512	-6	ns	...	ns	...	ns	...
CFU	Org-GC	0.716	-1	-0.713	0	-0.552	-1	0.649	-1
	Conv-GC	0.464	-1	-0.514	-2	-0.651	-1	0.610	-1
	Org-CM	ns	...	-0.541	2	ns	...	-0.526	1
	Conv-CM	-0.658	-2	0.630	-1	0.721	-1	0.628	-2

^a Lag is the number of days one series is shifted compared with another series. Lag < 0 means that the variable indicated in the leftmost column (i.e., first variable) leads the variable indicated in the top row; Lag > 0 means that the variable indicated in the leftmost column follows the variable indicated in the top row with [Lag] number of days; ns = not significant.

However, taking the whole time series into account, the changes in $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations, as well as pH and DOC, did not seem to influence the fluctuation in *P. ultimum* AUDPCs because there were no significant cross correlations. In previous studies, chemical variables associated with organic matter decomposition were considered to affect growth and death of *Pythium* spp. directly (1,3,27). However, the chemical variables in those studies were measured only occasionally, so that cross correlations over time could not be calculated. Although the daily soil chemical measurements in this study were not significantly cross-correlated with AUDPCs, they were cross-correlated with bacterial populations, and were likely the result of increases and decreases in bacterial metabolic activities and growth rather than the cause of the oscillations in bacterial populations.

Soilborne plant pathogens have frequently been reported to be suppressed after addition of organic amendments (14,29), and this suppression was often associated with competition or antagonism by bacteria (4,19,35). In the research reported here, *P. ultimum* damping-off did not simply decrease when daily observations were made after incorporation of organic materials into soils; however, the infection rates declined and increased with significant harmonic oscillations. Variations in pathogen infections with time had been published before (7,24,34,37) but temporal wave-like patterns of pathogen infections had not been proven statistically or noted by the authors, partially because observations were not made frequently enough. Considering the occurrence of oscillations in bacterial populations and infections by soilborne pathogens (40; this article), it can now be understood why amendments with organic materials, including compost, sometimes resulted in increased rather than decreased damping-off depending on the maturity level of the compost used and the time elapsed between incorporation of the material and planting of a crop (17,24). More research is needed to determine how long farmers need to wait after incorporation of compost or a cover crop before planting the next crop but the results presented here suggest that this time interval may need to be several weeks, depending on the kind of amendment and the temperature (which affects the decomposition rate and amplitudes and extinction time of the oscillations).

In addition to these practical implications of our research, the discovery of oscillations in inoculum or infection potential also has broader implications. Higher incidences of diseases in a soil system are indicative of an unhealthy ecosystem (9). Ecosystem health could be measured by resistance and resilience of the system after a disturbance, expressed by the amplitudes and dampening period of fluctuations in biological populations (16,38). In our experiments, the amplitudes and frequencies in fluctuations of pathogen infections and copiotrophic CFU were always smaller in amended organic soil than in amended conventional soil. Soil in organically managed farms has been considered to be a stable ecosystem with resistance to a perturbation and more resilience (41,42). Long-term use of chemical fertilizer and pesticides and frequent cultivation reduces bacterial diversities and competition in conventional soil (41), allowing fast-growing bacteria to reach a higher biomass after a disturbance and return to a steady state more slowly. Furthermore, higher microbial activity and larger bacterial diversities in organic soils could result in stronger suppressive abilities to *Pythium* damping-off (4,5,18,43). In our study, the amended organic soils had higher microbial activity and microbial diversity and more limited microbial oscillations than amended conventional soils (*unpublished data*), resulting in lower AUDPC ratios in the organically managed soils. These results supported the notion that organic soils are healthier than soils from conventional farms (37,38).

In conclusion, disease incidence by *P. ultimum*, as measured by AUDPC, oscillated after nutrient impulses into soils, similar to copiotrophic bacteria. Our observations create an opportunity to detect competitive or antagonistic bacteria of plant pathogens in

soil at particular times relative to a disturbance, which is useful for the development of biological control of plant disease. Moreover, indicators for the health status of a soil, including disease suppressiveness, could be calculated based on the harmonic oscillations of plant pathogens and bacteria after a disturbance (31).

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