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Short-term cover crop decomposition in organic and conventional soils: Soil microbial and nutrient cycling indicator variables associated with different levels of soil suppressiveness to *Pythium aphanidermatum*

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Abstract

Stages of oat–vetch cover crop decomposition were characterized over time in terms of carbon and nitrogen cycling, microbial activity and community dynamics in organically and conventionally managed soils in a field experiment and a laboratory incubation experiment. We subsequently determined which variables describing soil microbial community dynamics, C and N cycling could be used as predictors of *Pythium aphanidermatum* damping-off incidence and relative growth. Disease incidence and relative growth of *P. aphanidermatum* were measured in growth chamber assays and *in vitro* growth tests. No significant differences were detected between the conventional and organic farming system with respect to either relative growth or disease incidence. Stepwise discriminant analysis on three classes of disease incidence or relative growth led to selection of qualitatively similar variables. Only one soil microbial variable, total biomass of actinomycetes, was selected. Total C and N content of debris extracted from soil as well as NH_4-N content of soil were selected most consistently and show promise for assessment of potential damping-off incidence by *P. aphanidermatum* for young seedlings.

Introduction

Concerns about the sustainability of the biosphere have led to formulation of an elaborate ecological research agenda proposed by the Ecological Society of America, in which the development of indicators of ecological responses to stress constitutes one of the research priorities (Lubchenco et al., 1991). This call has been extended by the US Soil Ecology Society to explicitly include the soil as part of a sustainable biosphere initiative (Klopatek et al., 1992). At the same time, research and discussion of soil quality and soil health are becoming both more prominent and urgent [see special issues of Journal of Soil and Water Conservation 50(3) and American Journal of Alternative Agriculture 7(1–2)]. The search for indicators of soil quality, soil health or responses of soil to stress, has focused on soil invertebrates (Paoletti et al., 1991; Bongers, 1990; Stork and Eggleton, 1992), soil microorganisms (Kennedy and Papendick, 1995; Visser and Parkinson, 1992), physical and chemical aspects (Arshad and Coen, 1992; Karlen et al., 1994) and general soil and crop management practices (Reicosky et al., 1995; Karlen et al., 1992, 1994).

'Soil quality' has been defined as 'the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health' (Doran and

Parkin, 1994) and is often used synonymously with 'soil health' (Pankhurst et al., 1995). Plant health is explicitly mentioned as part of the definition and the ability of plant pathogens in soils to cause disease must be considered as part of the list of potential indicators of ecosystem health (Pankhurst et al., 1995; Van Bruggen and Grünwald, 1996). Thus, soil quality is partially determined by the risk of root infection by plant pathogens (Van Bruggen and Grünwald, 1996). Pankhurst et al. (1995) point out that among many soil biological variables measured in their experiments, root pathogens were considered to have potential as bioindicators. Unfortunately, the ability of a soil to suppress pathogens is itself a measurement that integrates many individual soil factors and, like the concept of soil quality, the concept of soil suppressiveness calls for the use of different indicator variables.

After obtaining a set of observations of different variables, the selection of a good indicator variable of soil suppressiveness to plant pathogens has to rely on different statistical procedures. Statistical methods used to select indicator variables for plant pathogens include univariate methods, such as analyses of variance or *t*-testing (Pankhurst et al., 1995; Prot and Savary, 1993), and multivariate approaches. A multivariate approach is often more promising when trying to understand which indicator variables can give an indication of the risk of infection of plants by plant pathogens. This is because single variables never explain the full range of behavior of pathogens in fluctuating environments (Wiese, 1982; Wallace, 1978). Multivariate approaches used in plant pathology include multiple regression analysis (Kincaid et al., 1970; Sallans, 1948), path coefficient analysis (Hampton, 1975), principal components analysis (Oyarzun et al., 1994), cluster analysis (Shennan, 1992), canonical and stepwise discriminant analysis (Papavizas et al., 1968), canonical correlation (Workneh et al., 1993), polar ordination (Oyarzun et al., 1994) and correspondence analysis (Savary et al., 1993, 1994, 1988; Prot and Savary, 1993; Oyarzun et al., 1994) among others. A stepwise procedure such as stepwise regression analysis or stepwise discriminant analysis is helpful in the selection of indicator variables, since the original multivariate data set is reduced to a few important variables that explain most of the pathogen or disease dynamics (Papavizas et al., 1968).

Damping-off disease caused by *Pythium* spp. has been intensively studied to understand which abiotic and biotic components of soil determine suppressiveness of soil to the pathogen (Ko and Ho, 1983; Bouhot, 1981; Martin and Hancock, 1986). Among the range of factors considered were soil moisture and temperature (Lifshitz and Hancock, 1983), soil nutrient levels (Martin and Hancock, 1986; Ko and Kao, 1989), amendments of soil (Agnihorti and Vaartaja, 1967; Schüler et al., 1989; Chen et al., 1987; Bouhot, 1981) and soil biological properties (Sugimoto et al., 1990; Boehm and Hoitink, 1992; Boehm et al., 1993; Chung and Hoitink, 1990). Despite this extensive body of work, relatively little progress has been made towards understanding, and even less so towards predicting, population establishment, growth and incidence, and severity of damping-off disease (Lumsden et al., 1990). Several experiments were conducted to study the effect of short-term cover crop decomposition on suppressiveness of soils to *Pythium aphanidermatum* using tomato plants in growth chamber assays or *in vitro* bioassays of growth (Grünwald et al., 1997, 2000). During short-term cover crop decomposition, no differences in soil suppressiveness between organic or conventionally managed soils could be established (Grünwald et al., 2000). However, the stage of decomposition of an incorporated cover crop significantly affected soil suppressiveness (Grünwald et al., 2000).

The goal of the research described in this paper was to identify indicators using stepwise discriminant analysis that predict disease suppressiveness of soil to *P. aphanidermatum* (Edson) Fitzp. in two farming systems at different stages of cover crop decomposition and to contribute to an understanding of the mechanisms underlying the natural controls observed in soils. Carbon, nitrogen and microbial dynamics during cover crop decomposition have already been described (Hu et al., 1997; Grünwald et al., 2000). The specific objectives were to identify the most important indicator variables for disease suppression, by determining which variables describing soil microbial community dynamics, C and N cycling could be used as predictors of *P. aphanidermatum* growth and damping-off incidence.

Materials and methods

Soils and cover crops

Research was conducted with soils from two different comparative farming systems projects. The first of these experiments (Experiment 1) was conducted in

the field, while the second experiment (Experiment 2) was conducted in the laboratory using air-dried and rewetted field soil, to which dried cover crop residue was added. More details on soils, cover crop and experimental procedures are presented in Grünwald et al. (2000).

Experiment 1

The soils used for Experiment 1 came from a farming systems project started in 1986 (Stivers and Shennan, 1991; Shennan, 1992), from which we chose the winter-fallow plots that received 168 kg N ha^{-1} in the form of ammonium-sulfate and the green manured plots that were seeded to an oat-vetch mixture, supplying a similar amount of nitrogen. The soil is a coarse-loamy, mixed, Thermic Mollic Xerofluvent soil with a pH of 7.7, CEC of 25.4 meq 100 g^{-1} soil, and 33 ppm $NaHCO_3$-extractable P. In Fall of 1992 the experiment was terminated by planting a cover crop mixture consisting of oats (*Avena sativa* L.) and lana wooly pod vetch (*Vicia dasycarpa* cv. lana) in all plots including the previously not cover-cropped plots. Relative to time of incorporation of an oat–vetch cover crop, we took soil samples 3 days before incorporation, and 7, 20, and 35 days after incorporation of the cover crop and stored samples in plastic buckets with a lid at 5 °C until processed. See Grünwald et al. (2000) for more details.

Experiment 2

For Experiment 2, soil came from the Sustainable Agriculture Farming Systems project started in 1989 at UC Davis, where a conventional system with a 2-year rotation, and conventional, low input and organically managed systems with 4-year rotations were compared (Temple et al., 1995). Soils were sampled, air-dried on greenhouse benches and kept in buckets at air-temperature until used. Cover crops used for incorporation consisted of field grown oats (*A. sativa* L.) and lana wooly pod vetch (*V. dasycarpa* Ten.) harvested from plots adjacent to Experiment 1. Before incorporation of oat–vetch debris, 16.5 kg dry weight soil were gently brought to 10% moisture using spray-bottles, mixed and incubated for 48 h. Subsequently, we either added a cover crop mixture consisting of 76.0 g air-dried oats and 38.0 g air-dried vetch and another 3% distilled water, or just 3% water for the treatment where no cover crop was incorporated. The soil was then divided into two plastic buckets (8.0 kg each) for incubation in the dark at 22 ± 2 °C (Grünwald et al., 2000).

Effect of soil on pathogen growth and damping-off incidence

Radial growth and relative growth of *P. aphanidermatum* (Edson) Fitzp. was determined over autoclaved and natural soil using an *in vitro* method (Grünwald et al., 1997). Radial growth of a fungus was measured on a cellophane membrane overlaying either natural or autoclaved soil, inoculated with 48 h old potato dextrose agar plug cultures of *P. aphanidermatum*. Relative growth is subsequently estimated as

$$\textit{Relative growth} = \frac{\begin{array}{l}\textit{Radial growth (cm)}\\ \textit{on unsterilized soil}\end{array}}{\begin{array}{l}\textit{Radial growth (cm)}\\ \textit{on sterilized soil}\end{array}}.$$

During Experiment 1, a growth chamber bioassay was performed using *P. aphanidermatum* to determine whether the *in vitro* bioassay was a good indicator of damping-off incidence (Grünwald et al., 1997). Soils were either not inoculated, or inoculated at a low (10^3 oospores g^{-1} soil) or high level (10^4 oospores g^{-1} soil) of inoculum. Three seeds were planted per pot and damped-off seedlings were counted twice, two and three weeks after planting, to determine disease incidence as the percentage of seeds that died within 21 days after planting due to *P. aphanidermatum*.

Soil nutrient cycling

Total soil C, N, NO_3-N, NH_4-N, and hot-water soluble carbohydrates were determined (Hu et al., 1997) using a randomly obtained subsample sieved through a 4 mm mesh. Organic debris larger than 4 mm was cut and passed through the mesh. Finely ground, air-dried soil samples were measured for C and N contents by combustion using a Carlo Erba C/N analyzer (Carlo Erba, Milano, Italy) at the DANR Analytical Laboratory of the University of California. Hot-water extractable carbohydrates were determined by slightly modifying the method of Brink et al. (1960) as described in Hu et al. (1997) using glucose as the standard. Soil NO_3-N and NH_4-N were detected following 2 M KCl extraction (Keeney and Nelson, 1982). Concentrations of NO_3-N and NH_4-N in extracts were determined on an Inorganic Nitrogen Analyzer (Alltech Associates, Beerfield, IL) according to Carlson (1986). Plant debris was extracted from ca. 2000–3000 g soil using a wet-sieving method (Weinhold, 1977). Floating materials were composited

per plot, dried in an oven at 60 °C and ground for fiber analyses, and total C and N content of debris. Neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) analyses of retrieved residues were performed following a procedure modified from Robertson and Van Soest (Robertson and Van Soest, 1981). Acid-detergent lignins (ADLs) were obtained after cellulose was eliminated by 72% H_2SO_4 digestion for 3 h. Cellulose content was estimated by subtracting ADLs from ADF. All results were presented as percentages cellulose, hemicellulose, and lignin of dry matter on an ash-free basis (Hu et al., 1997).

Soil microbial biomass and activity

Microbial biomass C was determined by the chloroform fumigation–extraction method adapted from Vance et al. (1987) using a k_{ec}-factor of 0.33 according to Sparling and West (1988). Fluorescein diacetate (FDA) hydrolytic activity, generally considered as a measure of microbial activity, was determined on 5-g subsamples in three pseudoreplications per plot and three or four replications for each treatment as described previously by Workneh et al. (1993). Total bacterial numbers were estimated by direct counts after staining with fluorescein isothiocyanate (FITC) (Babiuk and Paul, 1970). Active bacteria and active or total fungal hyphal lengths were estimated by direct observation after staining with fluorescein diacetate (Ingham and Klein, 1984). Total fungi were estimated using phase contrast microscopy (Colinas et al., 1994), and hyphae which were smaller than 1 μm in width were counted as total actinomycetes. Biomass was calculated by multiplying bacterial and fungal biovolume by an average bacterial density of 0.33 g cm^{-3} and average hyphal density of 0.41 g cm^{-3} (Ingham et al., 1991). All direct counts were conducted by the Soil Microbial Biomass Service at Oregon State University (Covallis, OR, USA).

Statistical analysis

Initial analysis consisted of descriptive statistics for each variable by experiment, farming system and decomposition stage of the cover crop. All soil nutrient cycling, microbial community, and disease variables were tested for normality by univariate analysis. Non-normally distributed variables were subjected to the Box–Cox transformation procedure (Dixon et al., 1990) to achieve normality, and then standardized to zero mean and unit variance before discriminant analysis. The final choice of whether a transformation was used or not was based on whether transformations were able to meet assumptions of subsequent statistical tests, whether a variable behaved differently in different data sets, and whether results from the discriminant analysis were affected by the transformation suggested by the Box–Cox procedure (Dixon et al., 1990). A canonical discriminant analysis using all soil microbial, C and N variables was performed to assess how well this data set discriminated between three levels of *in vitro* relative growth of *P. aphanidermatum* and disease incidence of *P. aphanidermatum* in growth chamber experiments. The three classes contained

Table 1. Definitions of abbreviations used for soil C and N cycling and microbial variables used in Experiments 1 and 2

Variable	Abbreviation	Units
Soil nutrient cycling		
Total C of soil	C soil	mg g^{-1} soil
Total N of soil	N soil	mg g^{-1} soil
C/N ratio of soil	C/N soil	—
NH_4-N of soil	NH_4-N	μg g^{-1} soil
NO_3-N of soil	NO_3-N	μg g^{-1} soil
dry weight of debris	DW debris	mg g^{-1} soil
Total C of debris	C debris	mg g^{-1} dry matter
Total N of debris	N debris	mg g^{-1} dry matter
C/N of debris	C/N debris	—
Lignin content of debris	Lignin	mg g^{-1} dry matter
Cellulose content of debris	Cellulose	mg g^{-1} dry matter
Soil microbial dynamics		
Microbial biomass carbon	MBC	μg g^{-1} soil
FDA-hydrolytic activity	FDA	μg g^{-1} soil $minute^{-1}$
Potentially mineralizable N	PMN	μg N g^{-1} soil
Total biomass of bacteria	TB	μg biomass g^{-1} soil
Active biomass of bacteria	AB	μg biomass g^{-1} soil
Total biomass of fungi	TF	μg biomass g^{-1} soil
Active biomass of fungi	AF	μg biomass g^{-1} soil
Total biomass of actinomycetes	TA	μg biomass g^{-1} soil
Active biomass of actinomycetes	AA	μg biomass g^{-1} soil

approximately equal numbers of observations. Subsequently, a stepwise discriminant analysis was conducted to select those variables best discriminating between these three groups of relative growth or disease incidence. A singularity (tolerance) of 0.1 was used to bypass multicollinearity, while significance levels regarding *F*-to-enter and *F*-to-stay values were set to 0.15 and 0.3, respectively (Afifi and Clark, 1990). The degree of differentiation between the groups obtained by entering the selected variables was interpreted in terms of the magnitude and significance level of the average squared canonical correlation (ASCC), which estimates the amount of variance that is accounted for by classifying the observations into the three groups. Discriminant analyses were performed both separately and jointly for the two experiments. Unless otherwise indicated, all statistics were performed using the procedures within the Statistical Analysis System library (1988). Abbreviations used for soil microbial, C and N variables throughout this manuscript are presented in Table 1 with the corresponding units.

Results

Disease assays

Damping-off incidence caused by *P. aphanidermatum* and relative growth of *P. aphanidermatum* were highest ten days after incorporation in Experiment 1 and 7 days after incorporation in Experiment 2 and then declined (Figure 1). Disease incidence or relative growth were

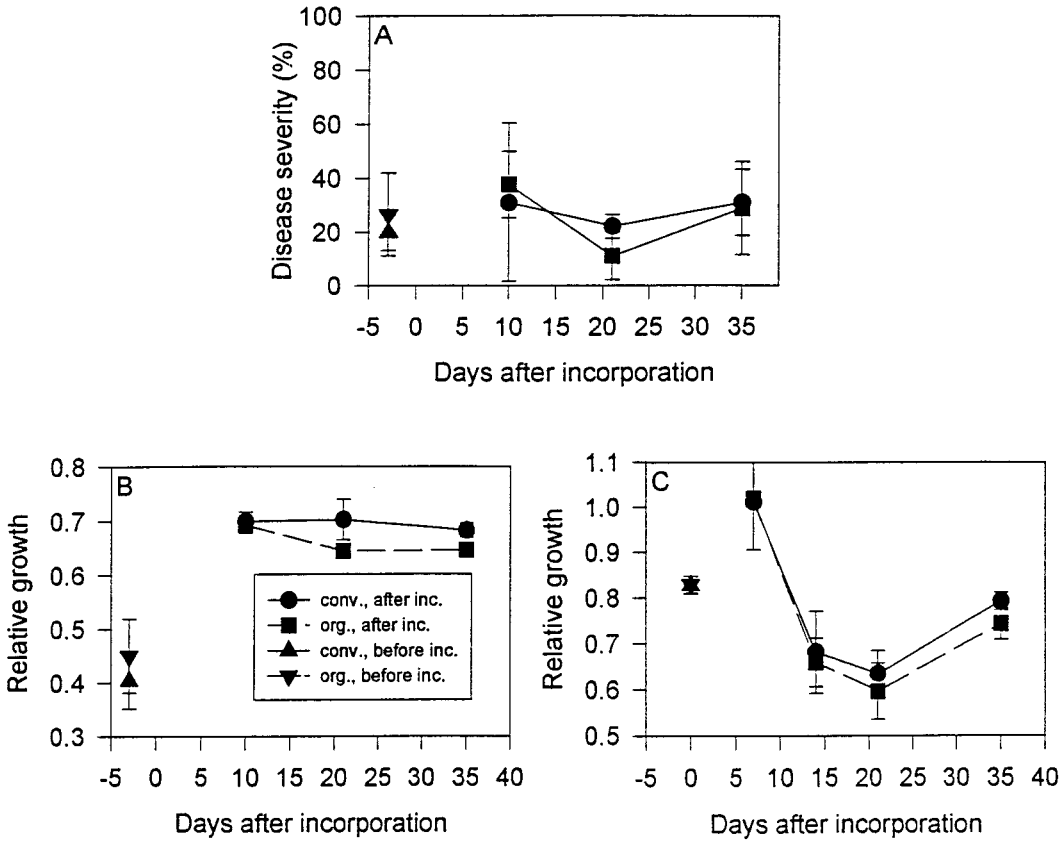

Figure 1. (A) Disease incidence in growth chamber assays in Experiment 1 at low level of inoculation with *P. aphanidermatum* (% of total plants damped off due to *P. aphanidermatum* after subtraction of plants damped off in control due to native microflora) and relative growth of *P. aphanidermatum* at different stages of cover crop decomposition in organically and conventionally managed soils from (B) Experiment 1 and (C) Experiment 2. The abscissa reflects samples taken 3 days before, and 7, 20, and 35 days after incorporation of the oat–vetch cover crop. Data presented in (B) and (C) have been previously presented in Grünwald et al. (1997). Shown are mean ± standard errors of the means.

lowest 20–21 days after incorporation (Figure 1). No significant differences were detected between the conventional and organic farming systems with respect to damping-off incidence or relative growth ($P \geq 0.05$).

Factors affecting distinctions among classes of relative growth

Ordination resulting from a canonical discriminant analysis on all soil nutrient cycling and microbial variables distinguished well between three classes of relative growth in Experiments 1 and 2 (Figure 2). Canonical function 1 explained 69% and 67% of the variance in Experiments 1 and 2, respectively. Separation of low, medium, and high levels of relative growth were better in Experiment 2 (Figure 2B vs D), but the behavior of the three classes was qualitatively the same for both canonical axes 1 and 2. Results from the stepwise discriminant analysis using three classes of relative growth of *P. aphanidermatum* for Experiment 1 resulted in selection of N debris and lignin content of extracted debris, when only cover crop treatments were included and N of debris, when all treatments were included (Table 2). In Experiment 2, the same kind of analysis selected NH_4-N, C of debris, and total biomass of actinomycetes with only cover-cropped treatments and NH_4-N, C of debris with inclusion of all treatments (Table 2). When both data sets from Experiments 1 and 2 were combined, cellulose and C content of debris were selected, when only cover cropped treatments were included, while N content of debris, soil NH_4-N, and microbial biomass carbon were selected, when cover-cropped and non-cover- cropped treatments were included (Table 2). Generally, the ASCCs were higher in Experiment 2 or the combination of Experiments 1 and 2 (Table 2). As indicated

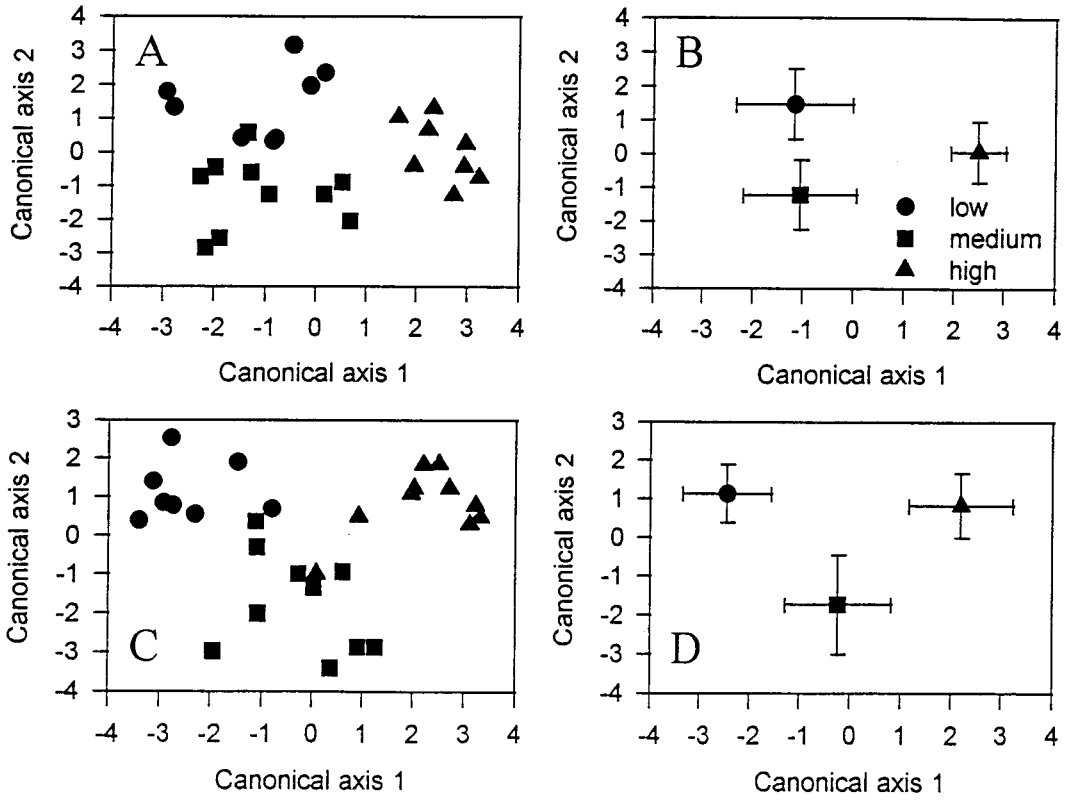

Figure 2. (A) Grouping of samples from Experiment 1 and (C) Experiment 2 resulting from a canonical discriminant analysis performed on three classes of relative growth (i.e., low, medium, and high) with all soil microbial, C and N variables. Classes were designed to have distinct breaks between classes, yet approximately equal numbers of observations. (B) and (D) show the means and standard deviations of the canonical variable scores of Experiments 1 and 2, respectively.

Table 2. Soil C, N and microbial variables selected by stepwise discriminant analysis for differentiating between three levels of relative growth of *P. aphanidermatum* based on Experiments 1 (field experiment) and 2 (controlled incubation experiment)

Obs.[1]	Experiment 1				Experiment 2				Experiment 1 and 2 combined			
	Variable[2]	$P > F$	Cumulative ASCC[3]	$P >$ ASCC	Variable[2]	$P > F$	Cumulative ASCC[3]	$P >$ ASCC	Variable[2]	$P > F$	Cumulative ASCC[3]	$P >$ ASCC
CC[4]	N debris	0.0643	0.1150	0.0643	NH_4-N	0.0001	0.3360	0.0001	Cellulose	0.0001	0.1815	0.0001
	Lignin	0.0859	0.2204	0.0302	C debris	0.0181	0.4957	0.0001	C debris	0.0456	0.2454	0.0001
					TA	0.0333	0.5901	0.0001				
All[5]	N debris	0.0034	0.1951	0.0034	NH_4-N	0.0001	0.2616	0.0001	N debris	0.0001	0.1674	0.0001
					C debris	0.0964	0.3200	0.0003	NH_4-N	0.0062	0.2484	0.0001
									MBC	0.0290	0.2885	0.0001

[1]Observations from treatments with and without cover crop (CC) included in discriminant analysis.
[2]See Table 1 for explanation of variable names.
[3]Average squared canonical correlation.
[4]Only cover cropped (CC) soils included.
[5]All soils before and after cover crop incorporation included.

by the total-sample correlations between the canonical variable 1 and the original variables, nitrogen and carbon content of debris were negatively correlated with increases in relative growth classes (data not shown). Total biomass of actinomycetes, and microbial biomass carbon of soil and lignin and cellulose content of debris were positively correlated with increases in relative growth class. However, NH_4-N was an exception in that it was positively correlated with increase in relative growth class in Experiment 2 and negatively correlated in the analysis where both experiments were combined. Since NH_4-N was positively correlated to increasing relative growth in both Experiments 1 and 2 (within group) and negatively correlated in the combined data set (between groups), the correlation in the combination of the two experiments may be an artifact.

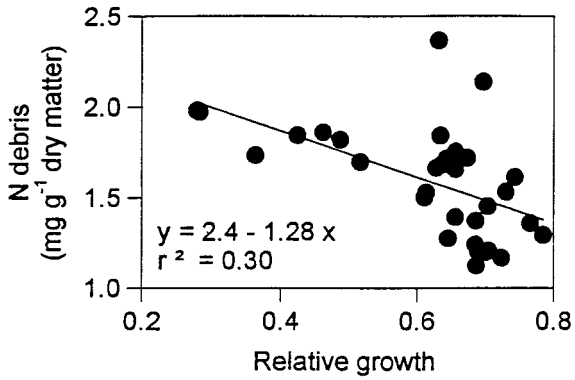

Figure 3. Relationship between relative growth and N debris in Experiment 1. N debris is significantly negatively correlated with relative growth ($P < 0.05$).

Figures 3 and 4 present linear regressions between variables selected in the stepwise discriminant analysis including all observations for Experiments 1 and 2, respectively (Table 2). N debris was significantly negatively correlated with relative growth ($P < 0.05$) (Figure 3). C debris was significantly negatively correlated and NH_4-N was significantly positively correlated with relative growth in Experiment 2 ($P < 0.05$) (Figure 4).

Although the sets of variables selected in the stepwise discriminant analyses were somewhat different, they can be exchanged with each other to a certain extent by looking at correlation coefficients between a selected variable and other variables. Thus, variables which are highly correlated with one another are expected to be interchangeable to a certain degree. In Experiment 1, C of debris could replace N of debris (Table 3), while in Experiment 2, N debris could replace C debris (Table 4). Similarly, in the combined analysis (Experiments 1 and 2) cellulose content of debris could be replaced with N debris, and lignin (Table 5). To further explain the selection of different variables, we carried out additional discriminant analyses using subsets of variables previously selected in one experiment in a stepwise analysis using the data set from the other experiment (Table 6). Except for one case, significant ASCCs were obtained in all cross validations, although in most cases they were lower and consisted of fewer variables. N debris, C debris, and NH_4-N most consistently emerged as indicator variables from the stepwise discriminant analysis (Tables 2 and 6) and, except for NH_4-N, were negatively correlated with relative growth (Figures 3 and 4).

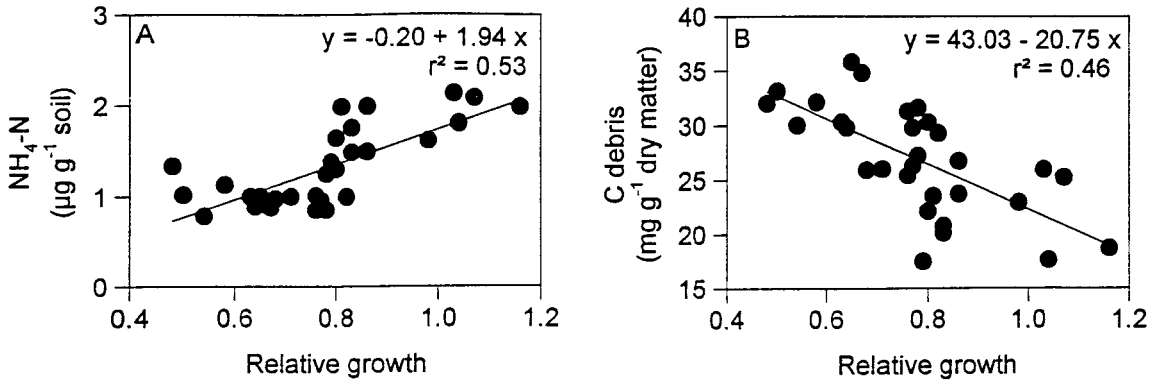

Figure 4. Relationships between relative growth and (A) NH_4-N or (B) C debris in Experiment 2. C debris is significantly negatively correlated and NH_4-N is significantly positively correlated with relative growth in Experiment 2 ($P < 0.05$).

Table 3. Simple correlations among variables selected by stepwise discriminant analysis in Table 2 for Experiment 1 ($n = 26$). Numbers in bold typeface reflect correlations that are significant at $P \leq 0.05$

	N debris[1]	C debris	Lignin	Cellulose	NH_4-N	TA	MBC	RG
N debris	1.00 0.0000							
C debris	**0.84** **0.0001**	1.00 0.0000						
Lignin	−0.35 0.0776	−0.13 0.5219	1.00 0.0000					
Cellulose	**−0.45** **0.0224**	−0.19 0.3439	**0.71** **0.0001**	1.00 0.0000				
NH_4-N	−0.28 0.1625	**−0.63** **0.0006**	−0.21 0.2981	−0.001 0.9944	1.00 0.0000			
TA	0.09 0.6639	0.02 0.9320	−0.08 0.7064	−0.03 0.8849	0.13 0.5189	1.00 0.0000		
MBC	**−0.48** **0.0130**	**−0.42** **0.0337**	0.25 0.2102	**0.49** **0.0105**	0.24 0.2434	0.30 0.1407	1.00 0.0000	
RG	**−0.50** **0.0096**	−0.18 0.3829	**0.83** **0.0001**	**0.76** **0.0001**	−0.11 0.5778	−0.04 0.8504	**0.44** **0.0265**	1.00 0.0000

[1]See Table 1 for explanation of variable names.

Table 4. Simple correlations among variables selected by stepwise discriminant analysis in Table 2 for Experiment 2 ($n = 30$). Numbers in bold typeface reflect correlations that are significant at $P \leq 0.05$

	N debris[1]	C debris	Lignin	Cellulose	NH_4-N	TA	MBC	RG
N debris	1.00 0.0000							
C debris	**0.80** **0.0001**	1.00 0.0000						
Lignin	**0.39** **0.0308**	0.07 0.7164	1.00 0.0000					
Cellulose	**−0.44** **0.0144**	−0.09 0.6360	**0.39** **0.0308**	1.00 0.0000				
NH_4-N	**−0.74** **0.0001**	**−0.64** **0.0001**	−0.10 0.6024	0.13 0.4994	1.00 0.0000			
TA	**−0.39** **0.0318**	−0.18 0.3542	**−0.37** **0.0419**	**0.56** **0.0014**	0.31 0.0902	1.00 0.0000		
MBC	−0.10 0.5858	0.33 0.0767	**−0.61** **0.0003**	**0.43** **0.0169**	−0.07 0.7047	**0.39** **0.0327**	1.00 0.0000	
RG	**−0.74** **0.0001**	**−0.68** **0.0001**	**−0.39** **0.0316**	**0.44** **0.0139**	**0.73** **0.0001**	0.34 0.0635	−0.05 0.7980	1.00 0.0000

[1]See Table 1 for explanation of variable names.

Factors affecting distinctions among classes of disease incidence

The grouping resulting from the canonical discriminant analysis on all soil nutrient cycling variables distinguished well between three classes of disease incidence from the growth chamber assay conducted for Experiment 1 (Figure 5). Canonical variable 1 accounted for 74% and 81% of the variance in the low and high inoculum treatments, respectively. Using classes of disease incidence from the growth chamber experiment conducted in Experiment 1, the stepwise discriminant analysis selected N content of debris, C/N ratio of debris, C content of debris, C/N of soil,

Table 5. Simple correlations among variables selected by stepwise discriminant analysis in Table 2 for Experiments 1 and 2 combined ($n = 56$). Numbers in bold typeface reflect correlations that are significant at $P \leq 0.05$

	N debris[1]	C debris	Lignin	Cellulose	NH_4-N	TA	MBC	RG
N debris	1.00							
	0.0000							
C debris	**0.74**	1.00						
	0.0001	0.0000						
Lignin	**−0.45**	−0.04	1.00					
	0.0006	0.7496	0.0000					
Cellulose	**−0.54**	−0.07	**0.93**	1.00				
	0.0001	0.6265	**0.0001**	0.0000				
NH_4-N	**0.35**	−0.17	**−0.88**	**−0.88**	1.00			
	0.0077	0.2047	**0.0001**	**0.0001**	0.0000			
TA	**−0.52**	−0.11	**0.73**	**0.85**	**−0.70**	1.00		
	0.0001	0.4163	**0.0001**	**0.0001**	**0.0001**	0.0000		
MBC	−0.39	0.01	**0.29**	**0.47**	**−0.32**	**0.52**	1.00	
	0.0026	0.9371	**0.0320**	**0.0003**	**0.0169**	**0.0001**	0.0000	
RG	**−0.68**	**−0.43**	**0.34**	**0.48**	**−0.37**	**0.48**	0.24	1.00
	0.0001	**0.0009**	**0.0104**	**0.0002**	**0.0051**	**0.0002**	0.0794	0.0000

[1]See Table 1 for explanation of variable names.

Table 6. Soil C, N and microbial variables previously selected by stepwise discriminant analysis for differentiating between three levels of relative growth of *P. aphanidermatum* based on Experiments 1 (field experiment) and 2 (controlled incubation experiment) (Table 2) were used for cross-validation by subjecting a set of selected variables to stepwise discriminant analysis in one of the other two data sets

Variable set[1]	Experiment 1			Experiment 2			Experiments 1 and 2		
	Variable entered	ASCC	$P >$ ASCC	Variable entered	ASCC	$P >$ ASCC	Variable entered	ASCC	$P >$ ASCC
N debris				N debris	0.19	0.0013	N debris	0.17	0.0001
NH_4-N	NH_4-N	—	—				NH_4-N	0.13	0.0001
C debris	C debris	0.096	0.0866				C debris	0.24	0.0001
N debris	N debris	0.195	0.0034	N debris	—	—			
NH_4-N	NH_4-N	—	—	NH_4-N	0.26	0.0001			
MBC	MBC	—	—	MBC	0.31	0.0004			

[1]Variables selected in previous stepwise discriminant analyses (Table 2). See Table 1 for explanation of variable names.

and NH_4-N as variables best discriminating between three classes of disease incidence (Table 7). Figure 6 presents linear regressions between variables selected for Experiment 1 for low and high levels of inoculation of soil with *P. aphanidermatum* in the stepwise discriminant analysis including all observations (Table 7). N debris, and C/N debris were negatively correlated with disease incidence, while NH_4-N was positively correlated with disease incidence (Figure 6).

N debris, C debris, and NH_4-N had been selected previously in the analysis conducted with relative growth, while C/N debris, and C/N soil were not selected previously (Tables 2 and 7). Thus, N debris, C debris, and NH_4-N were the variables most consistently chosen by the stepwise discriminant analysis with different levels of either relative growth or disease incidence.

Discussion

Two kinds of assays were conducted to test soils for suppressiveness to *P. aphanidermatum*, namely an *in vitro* assay and a growth chamber bioassay. Both assays yielded comparable results, and in both cases conventionally or organically farmed soils were not significantly different. The two assays were significantly

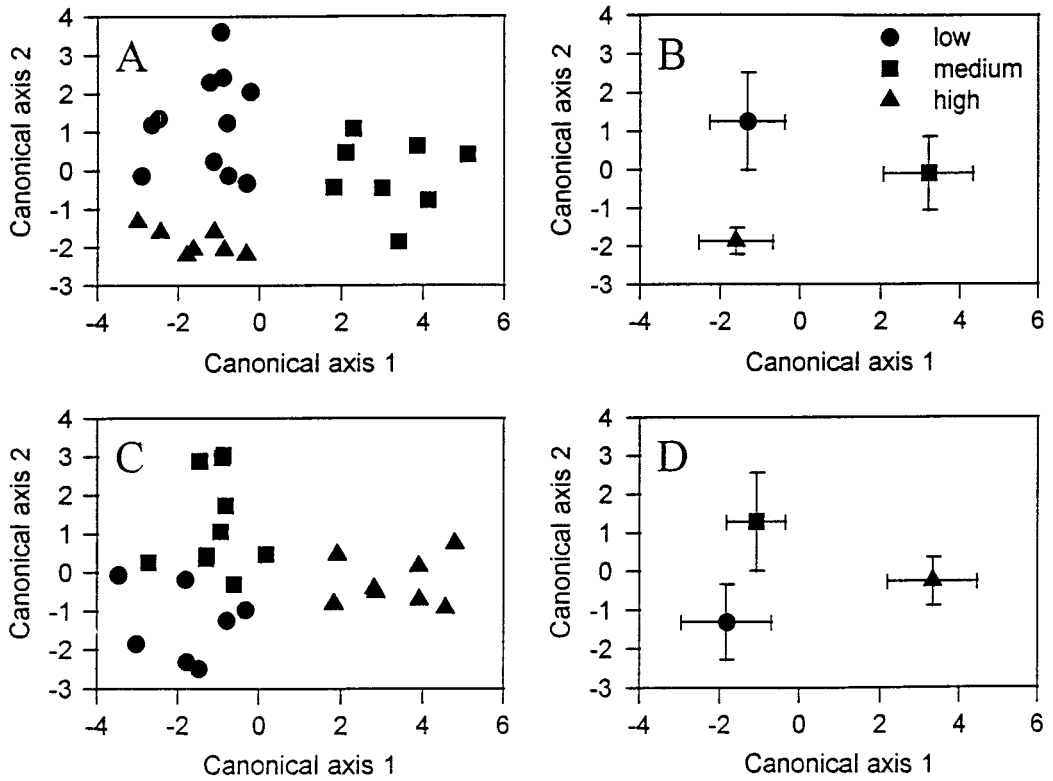

Figure 5. (A) Grouping of samples from low inoculum and (C) high inoculum of *P. aphanidermatum* treatments in Experiment 1 resulting from a canonical discriminant analysis performed on three classes of damping-off incidence (i.e., low, medium, and high) with all soil microbial, C and N variables. Classes were designed to have distinct breaks between classes, yet approximately equal numbers of observations. (B) and (D) show the mean and standard deviations of the canonical variable scores of the low and high inoculum treatment, respectively.

Table 7. Soil C, N and microbial variables selected by stepwise discriminant analysis for differentiating between three levels of disease incidence of tomato due to *P. aphanidermatum* based on growth chamber assays in Experiment 1

	Low inoculum of *P. aphanidermatum*				High inoculum of *P. aphanidermatum*			
Obs.[1]	Variable[2]	$P > F$	Cumulative ASCC[3]	$P >$ ASCC	Variable[2]	$P > F$	Cumulative ASCC[3]	$P >$ ASCC
CC[4]	N debris	0.0175	0.1598	0.0175	C debris	0.0124	0.1709	0.0124
	C/N debris	0.1220	0.2543	0.0134	C/N soil	0.1079	0.2633	0.0107
All[5]	N debris	0.0044	0.1881	0.0044	N debris	0.0029	0.1992	0.0029
	C/N debris	0.0757	0.2926	0.0027	NH_4-N	0.0667	0.3079	0.0017

[1]Observations from treatments with and without cover crop (CC) included in discriminant analysis.
[2]See Table 1 for explanation of variable names.
[3]Average squared canonical correlation.
[4]Only cover cropped (CC) soils included.
[5]All soils before and after cover crop incorporation included.

correlated, however, indicating that the *in vitro* assay can be used to estimate disease incidence (Grünwald et al., 1997).

The ordinations using all soil microbial, C and N variables distinguished well between different classes of relative growth and disease incidence of *P. aphanidermatum* (Figures 2 and 5). In general, the first canonical variable accounted for 70–80% of the variance. A stepwise discriminant analysis was conducted on three classes of either relative growth or disease

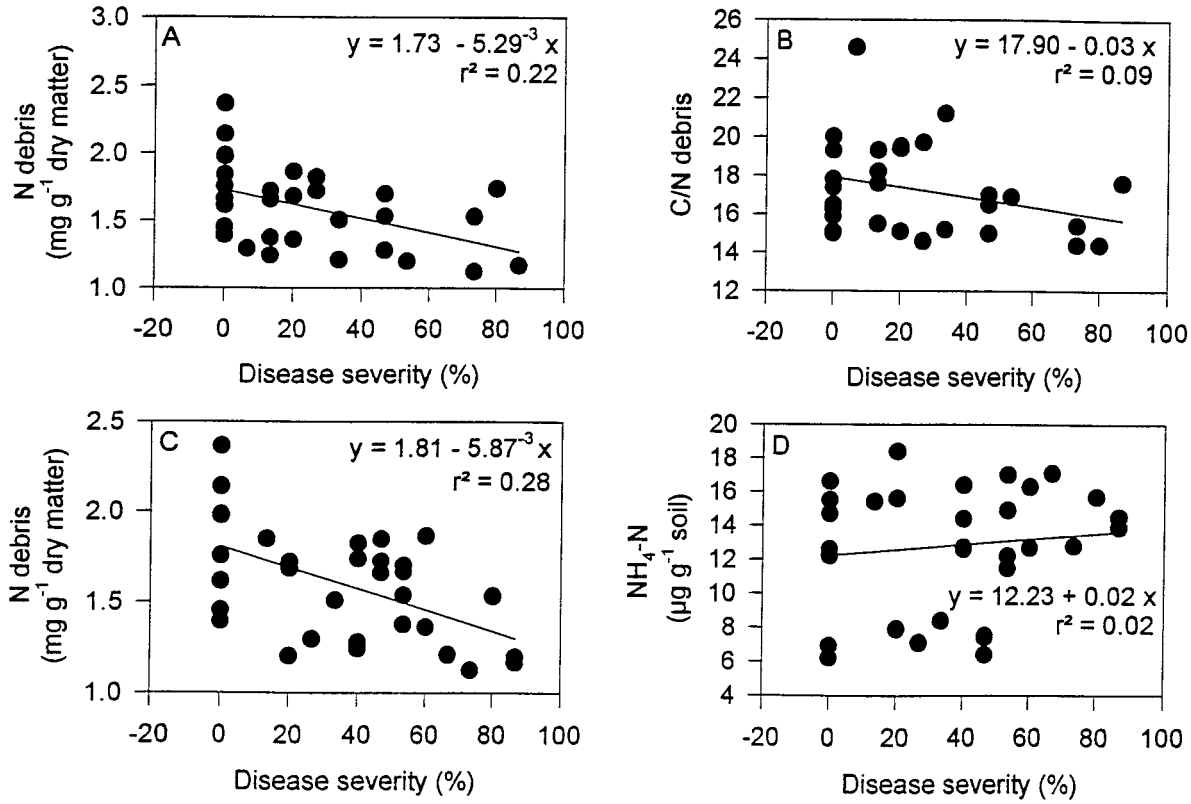

Figure 6. Relationships between disease severity and (A) N debris ($P < 0.01$), (B) C/N ratio ($P = 0.11$) of debris in low inoculum treatment and (C) N debris ($P < 0.01$), (D) NH_4-N ($P = 0.5075$) in high inoculum treatment in growth chamber assay in Experiment 1.

incidence. Sets of variables were obtained that were significant in terms of classifying observations into three classes of disease incidence/relative growth that accounted for most of the variance (Tables 2 and 7). Average squared canonical correlations were higher in Experiment 2, presumably because this experiment was more controlled with cover crop decomposition measured in small batches under uniform conditions. In all cases, one to three variables selected by the stepwise discriminant analysis gave the best relationships (Tables 2 and 7). C and N content of debris, and NH_4-N of soil were the variables that consistently and significantly appeared in the original stepwise discriminant analyses (Tables 2 and 7) as well as in subsequent cross-validations (Table 6). C and N contents of debris were negatively correlated with disease incidence or relative growth, and thus more damping-off would be expected at lower levels of total C or N of debris. As presented in Grünwald et al. (2000), C and N debris are highly correlated and follow similar trends over time being highest 20–21 days after incorporation and dropping thereafter. The pattern over time of C and N debris is inverse to that for relative growth (Figure 1). These results seemingly conflict with the general ideas that sugar-fungi like *Pythium* readily colonize any fresh plant-tissue substrates (Garrett, 1970), and would be expected to be positively correlated with C of debris. Our results might not follow traditional expectations, in that debris was wet-sieved and air-dried before analyzing it for total C and N content. We effectively only looked at the C and N content of rough debris that floats and is larger than 250 µm, thus excluding soluble carbohydrates and finer debris. The negative correlation might be further explained by competition between the soil microbial community and the C and N containing residue. Watson (1971) reported the effect of nutrient competition as accounting for reductions in pathogenic activities of *P. ultimum*. After amendment of soil with lettuce debris, he found that inoculum density of *P. ultimum* increased, but inoculum potential of propagules eventually decreased with time. Accordingly, Watson (1971) attributed the reduction

in inoculum potentials to enhanced competition from other soil microbes stimulated by the amendment with lettuce. Similarly, the negative correlation between disease incidence/relative growth and C and N of debris could reflect the enhanced competition between soil microorganisms and *P. aphanidermatum* at increasing levels of C and N content of debris. *Pythium* species are known to be poor saprophytes on organic matter that has been previously colonized by other soil microorganisms (Barton, 1961; Hine and Trujillo, 1966).

To our surprise, few microbial variables were selected by the stepwise discriminant analysis method. A similar result was obtained by Mandelbaum and Hadar (1990), who found that total microbial activity (FDA hydrolysis) or microbial densities (bacterial and fungal counts) alone were not reliable predictors of suppression of *Pythium*. Ratios of available or soluble C or N to biomass might prove to be more fruitful as indicator variables. These kinds of indicator variables could indirectly take competition for nutrients into account by scaling the availability of substrates by the biomass present in soil.

To the best of our knowledge this is the first time an integrated approach has been applied to the study of a damping-off disease, in which soil N and C cycling, as well as soil microbial community dynamics, were monitored and related to disease incidence or relative growth. A set of indicator variables was identified. These consist of total C and N content of debris extracted from soil, as well as the NH_4-N content of soil. These variables show promise for assessment of potential damping-off incidence by *P. aphanidermatum* for young seedlings.

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