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# Cross-Polarized Magic-Angle Spinning $^{13}C$ Nuclear Magnetic Resonance Spectroscopic Characterization of Soil Organic Matter Relative to Culturable Bacterial Species Composition and Sustained Biological Control of Pythium Root Rot

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**We report the use of a model system that examines the dynamics of biological energy availability in organic matter in a sphagnum peat potting mix critical to sustenance of microorganism-mediated biological control of pythium root rot, a soilborne plant disease caused by *Pythium ultimum*. The concentration of readily degradable carbohydrate in the peat, mostly present as cellulose, was characterized by cross-polarized magic-angle spinning $^{13}C$ nuclear magnetic resonance spectroscopy. A decrease in the carbohydrate concentration in the mix was observed during the initial 10 weeks after potting as the rate of hydrolysis of fluorescein diacetate declined below a critical threshold level required for biological control of pythium root rot. Throughout this period, total microbial biomass and activity, based on rates of [$^{14}C$]acetate incorporation into phospholipids, did not change but shifts in culturable bacterial species composition occurred. Species capable of inducing biocontrol were succeeded by pleomorphic gram-positive genera and putative oligotrophs not or less effective in control. We conclude that sustained efficacy of naturally occurring biocontrol agents was limited by energy availability to this microflora within the organic matter contained in the potting mix. We propose that this critical role of organic matter may be a key factor explaining the variability in efficacy typically encountered in the control of pythium root rot with biocontrol agents.**

Biological control of diseases caused by soilborne plant pathogens through the introduction of microorganisms has been explored intensively for decades (42, 45). Despite numerous contributions aimed at improving our understanding of the four principal mechanisms involved in biological control, competition, antibiosis, hyperparasitism, and systemic acquired resistance in the host plant (1, 14, 26, 29, 30), variability in efficacy of treatments still limits application and commercialization (45). Recent literature on the use of biocontrol agents emphasizes specific interactions between this microflora and pathogens in the rhizosphere (1, 45). It is generally assumed that rhizodeposition products hold sufficient energy reserves to sustain activity of biocontrol agents and thus disease suppression. The role of edaphic soil organic matter (OM) in disease suppression has largely been overlooked.

Knowledge of the role of OM in disease control was derived decades ago from studies utilizing specific amendments, composts, and manures and, more recently, from comparisons of organic and conventional farming systems (7, 21, 49, 50). Before disease control can be achieved, OM must be fully colonized, thus avoiding direct stimulation of plant pathogens (6, 17). Concentrations of readily available nutrients must be low enough that competition prevails and the production of lytic enzymes and antimicrobial compounds involved in hyperparasitism of the pathogen is not repressed (4, 6, 9, 12, 15). Microbiostasis prevails in these suppressive soils (4, 29). Many microorganisms contribute to this mechanism of biological control, particularly for pathogens such as *Pythium* and *Phytophthora* spp. causing root rots (3, 51). It is often referred to as general suppression sensu Gerlach (18). The basis for the length of time that an amendment remains effective (i.e., sustainability) is unknown. However, the duration of suppressiveness presumably depends in part upon the concentration of biologically available energy reserves in OM (7).

Procedures allowing nondestructive quantitative analysis of such energy reserves in OM were not available until recently (23). In the absence of this technology, microbiologists developed indirect approaches to the analysis of soil properties important to biological control. For example, dehydrogenase activity and rates of respiration are higher in suppressive than in conducive highly decomposed substrates (32, 34). The rate of hydrolysis of fluorescein diacetate (FDA activity) predicts the potential for the development of diseases caused by several soilborne plant-pathogenic fungi in both field soil and soilless potting mixes (4, 5, 22, 34, 50, 51). FDA is hydrolyzed by both free and membrane-bound esterases, lipases, and proteases in soil (38, 48). Thus, FDA activity appears to mimic OM decomposition level. FDA activity has provided meaningful insights concerning the microbial carrying capacity of soil OM relative to biological control of diseases caused by several soilborne plant pathogens sensitive to microbiostasis (2–4, 34, 50, 51).

Another approach used to monitor the impact of OM on biological soil properties has been to analyze the microflora itself. Bacterial species diversity among suppressive substrates high in biologically available carbon versus more decomposed conducive substrates does not differ (3). On the other hand, the composition of bacterial species and other microorganisms in the rhizosphere and edaphic soil is affected dramatically by

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OM decomposition level in both field soil and soilless potting mixes (3, 21, 49). This also has been shown through analysis of phospholipid fatty acids extracted directly from the rhizosphere and edaphic substrates (43). Pseudomonads, *Pantoea* spp. (formally *Enterobacter* spp.), and other copiotrophic microorganisms such as *Trichoderma* spp. predominate in suppressive potting mixes and soils high in lignocellulosic substances (3, 20, 27). Isolates and strains of these genera rank among the most efficacious biocontrol agents (1, 45). In conducive highly stabilized OM, pleomorphic gram-positive bacteria and putative oligotrophic microorganisms are abundant (3). The latter microflora is not effective in biological control of *Pythium* spp., for example, when applied as seed treatments (40, 41) and has been associated with highly mineralized niches in soils (24). What remains uncharacterized is a direct relationship between OM decomposition level and the dynamics of shifts in microbial populations and functional activities relative to biological control. Answers to these questions should begin to more critically illustrate the role of OM in sustenance of biological control.

In this paper we report on the role of OM in biological control in a light peat potting mix that changes from suppressive to conducive to pythium root rot over a relatively short time during the production of poinsettia. We apply cross-polarized magic-angle spinning $^{13}C$ nuclear magnetic resonance spectroscopy (CPMAS $^{13}C$-NMR) to the nondestructive analysis of the fate of the carbohydrate fraction (mostly cellulosic substances) in OM in a slightly decomposed light sphagnum peat potting mix. We reveal the temporal changes in FDA activity, microbial activity, and biomass, based on the rate of incorporation of [$^{14}C$]acetate into phospholipids, as well as culturable bacterial species composition over time. It has been shown previously that (i) trends in disease suppressiveness and FDA activity in this potting mix are negatively correlated with pathogen (*Pythium ultimum*) population and disease (poinsettia root rot) development during the production of a crop (2), (ii) the composition of bacterial species varied significantly within peats of various decomposition levels (3), and (iii) peat decomposition level significantly affected the efficacy of biological control agents (3). While others have shown the correlation of OM to biological control, this is the first analysis of the dynamics of the changes in biological energy availability in OM relative to these other factors affecting biological control of root rot.

## MATERIALS AND METHODS

**Peat potting mixes.** Three different sources of sphagnum peat, $H_2$, $H_3$, and $H_4$ on the von Post decomposition scale (37), stored in bales at −15°C until use to prevent further decomposition, were from the same batches as those used by Boehm and Hoitink (2). Peats were prepared into potting mixes, treated with fertilizer, planted with poinsettia, and incubated in a greenhouse as described previously (2). The suppressiveness of these peat mixes to pythium root rot has been characterized previously (2).

**CPMAS $^{13}C$-NMR characterization of peat decomposition level.** The decomposition level of $H_2$, $H_3$, and $H_4$ sphagnum peat samples was characterized by CPMAS $^{13}C$-NMR spectroscopy (11, 19, 36). Changes in the decomposition level of the $H_3$ peat mix as suppressiveness declined were determined with the NMR spectral subtraction procedure described previously by Hammond et al. (19). At various times after potting, $H_3$ peat mix samples incubated as described previously (2) were fractionated by washing through a series of mesh sieves (4.00, 2.00, 1.00, 0.500, 0.250, 0.105, and 0.053 mm). Fractionated samples (three replicates per treatment) were lyophilized and stored under vacuum. Two fractions, one containing coarse particles (1- to 2-mm diameter) and the other containing fines (105- to 205-μm diameter), were analyzed by CPMAS $^{13}C$-NMR spectroscopy. Spectra were acquired on a home-built 200-MHz spectrometer equipped with a Chemagnetics multinuclear probe utilizing a Tecmag pulse generator (Technology for Magnetic Resonance, Houston, Tex.). Samples were spun at the magic angle at −3 kHz in a zirconium rotor. A contact time of 2 ms for all samples was used to assume full polarization of all carbon atoms and accurate integrations. A 1.0-s pulse delay was used. Chemical shifts were referenced to the methyl of hexamethylbenzene at 17.0 ppm. The number of scans acquired for each sample was 10,000. The number of datum points was 512 per scan. All peaks in the samples exhibited a single relaxation time, as determined by variation of contact time. Postprocessing of data was performed with the software programs NMRI (New Methods Research, Inc., Syracuse, N.Y.) and Tecmag MACNMR (Technology for Magnetic Resonance).

**Microbial activity and biomass.** Microbial activity was measured as the rate of hydrolysis of FDA as described previously (2) and by a slightly modified version of the procedure described by van Veldhoven and Mannaerts (44). Microbial activity and total microbial biomass, based on the rate of incorporation of [$^{14}C$]acetate into phospholipids, were determined as follows. At various times after potting, 5-g peat samples (three replicates per treatment) were collected, suspended in distilled water (95 ml), and shaken. Next, 2.85-ml aliquots of the resulting suspensions were transferred in duplicate to 20-ml glass scintillation vials and amended with 150 μl of [1,2-$^{14}C$]acetate. Controls consisted of vials containing peat suspensions without [$^{14}C$]acetate and vials containing [$^{14}C$]acetate alone. Metabolic activity was stopped after a 2-h incubation in the dark on a rotary shaker (175 rpm) by the addition of 7.5 ml of methanol and 3.75 ml of $CHCl_3$ to establish a methanol-$CHCl_3$-water ratio of 2:1:0.8 (vol/vol/vol) (46). The mixture was shaken vigorously for 5 h on a rotary shaker (175 rpm, 24°C). After 18 h, equal volumes of $CHCl_3$ and water were added to yield a final methanol-$CHCl_3$-water ratio of 1:1:0.9 which partitioned the lipid-containing $CHCl_3$ fraction from the aqueous phase. The $CHCl_3$ phase (7.0 ml) was then transferred to a new vial and dried under a stream of $N_2$ (55°C). Finally, the dried lipid was redissolved in 4 ml of $CHCl_3$ and fractionated for microbial activity and biomass determinations. Specific activity was calculated on the basis of microbial activity ($^{14}C$ incorporation) per unit of biomass. This entire experiment was performed twice.

**Bacterial species composition.** Shifts in culturable rhizosphere bacterial populations in the $H_3$ peat mix were assessed by baiting the $H_3$ peat mix with cucumber seedlings (*Cucumis sativus* L. 'Straight eight', 90% germination) at 8, 38, 57, and 172 days after potting. Rhizosphere bacteria were isolated as described by Boehm et al. (3). Shifts in culturable bacterial populations in the $H_3$ peat mix were assessed directly by dilution plating of the mix at 6, 100, and 172 days after potting. Bacteria were enumerated, purified, and identified by gas chromatographic-fatty acid methyl ester (GC-FAME) analysis as described previously (3). Strains with a similarity index of ≥0.5 were assigned to a taxon, strains with values <0.5 and ≥0.1 were assigned to a genus, and strains with values <0.1 were assigned to a group (GC similarity group) based on the similarity of their fatty acid profiles. Pure cultures were stored at −70°C in a 15% (vol/vol) sterile glycerol-water solution (39). The relative population density of bacterial taxa for each sample was determined as $100n_i/N$, in which $n_i$ is the number of strains assigned to the $i$th taxon and $N$ is the total number of strains for a given root tip segment. This experiment was repeated three times.

**Efficacy of rhizosphere bacteria as biocontrol agents.** The ability of bacteria isolated from cucumber root tips 172 days after potting in the $H_3$ peat mix to induce biocontrol of damping-off and root rot caused by *P. ultimum* was determined in cucumber bioassays, as described previously (4). The frequency of rhizosphere bacteria with biocontrol activity against *Pythium* recovered from cucumber roots in the $H_3$ and $H_4$ peat mixes 10 days after potting was described previously by Boehm et al. (3).

**Statistical analyses.** Bacterial species (or taxon) diversity was determined for each root tip or peat mix sample (i.e., sampling unit [SU]) at each harvest date as described previously (3). Similarity in bacterial species composition among SUs was measured by using Dice (3, 31) similarity coefficients (resemblance functions) and polar ordination (3, 31). Dice similarity coefficients were calculated as $2a/(2a + b + c)$, in which $a$ is the number of common taxa between two SUs ($X$ and $Y$), $b$ is the number of unique taxa which occur in SU $X$ but not in SU $Y$, and $c$ is the number of unique taxa which occur in SU $Y$ but not in SU $X$. This resemblance function is equal to 0 at "no similarity" and approaches 1 when the SUs are identical in bacterial species composition (31). The polar ordination method of Bray and Curtis (31) was used to position SUs within a coordinate system such that the distances between SUs reflected their similarity in bacterial species composition as well as their relation to underlying environmental gradients such as OM decomposition level. In this study, SUs were arranged on the basis of percent dissimilarity. Percent dissimilarity between SUs was calculated as 100(1 − Dice similarity index). The computer programs used for these analyses were SPASSOC.BAS, SUDIST.BAS, and PO.BAS (31).

Chi-square tests were used to determine significant differences in the relative population densities over time (first to last assessment) of (i) gram-positive and gram-negative bacteria (among identified taxa), (ii) GC groups (unknown taxa), and (iii) strains lost upon subculturing during purification and identification (10).

## RESULTS

**CPMAS $^{13}C$-NMR characterization of peat decomposition level.** Subtraction spectra of potting mix samples prepared from fine (105- to 250-μm-diameter)-particle fractions of the $H_2$ and $H_4$ peats were distinguished from each other by CPMAS $^{13}C$-NMR spectroscopy (Fig. 1). Distinct resonances cen-

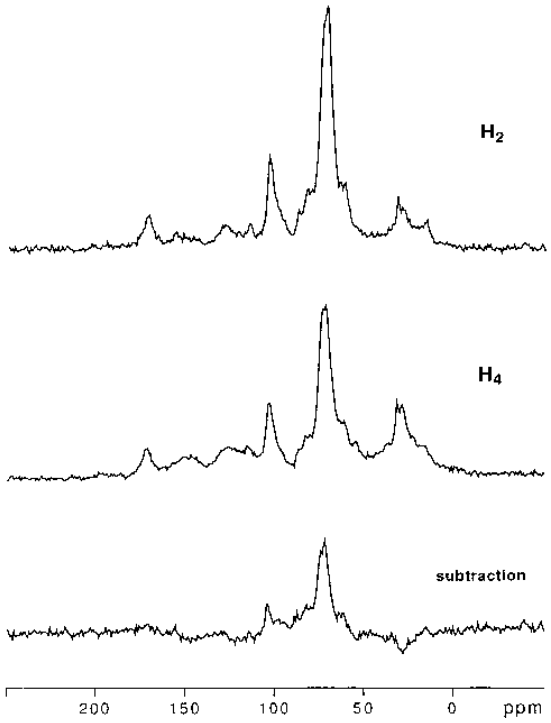


FIG. 1. Solid-state CPMAS $^{13}$C-NMR spectra revealing differences in carbohydrate content within the fine (105- to 250-μm-diameter)-particle fraction of a slightly decomposed light ($H_2$) and a decomposed dark ($H_4$) Canadian sphagnum peat. The subtraction spectrum is the $H_2$ spectrum minus the $H_4$ spectrum.

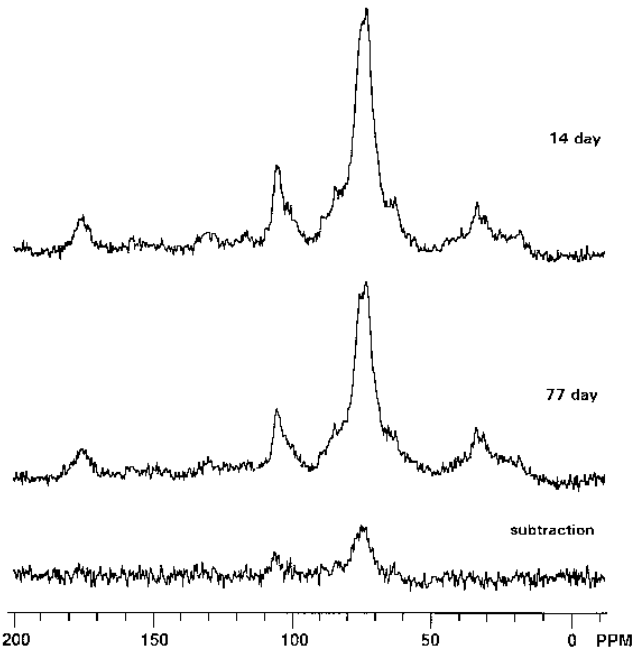


FIG. 2. CPMAS $^{13}$C-NMR spectra revealing the carbohydrate content within the fine (105- to 250-μm-diameter)-particle fractions of $H_3$ peat samples collected 14 and 77 days after planting. The subtraction spectrum is the 14-day spectrum minus the 77-day spectrum.

tered at ~73 and ~107 ppm were due to carbohydrates (11, 13, 16, 47). Such resonances in the subtraction spectra ($H_2$ minus $H_4$) revealed that the carbohydrate concentration decreased as the degree of peat decomposition increases. Similar results were obtained for the coarse (1- to 2-mm-diameter) particles for these peat mixes (data not shown).

The subtraction spectra of the fine particles (105- to 205-μm diameter) recovered by wet sieving from the $H_3$ peat mix at 14 (suppressive to root rot) and 77 (conducive) days after planting (2) reveal a decrease in carbohydrate content with time as evidenced by the distinct resonances centered at ~73 and ~107 ppm (Fig. 2). The equivalent subtraction spectra of the coarse particles (1- to 2-mm diameter) recovered from the same potting mix samples did not reveal this difference (data not shown). NMR spectra of the coarse and fine particles recovered on day 14 were indistinguishable from one another (data not shown). A slight although not significant difference in carbohydrate content was detected in the subtraction spectra of the coarse minus the fine fractions on day 77. In summary, the magnitude of the decrease in carbohydrate content appears directly related to the ratio of surface area to volume for these particles, being most pronounced for the fine particles.

**Microbial activity and biomass.** FDA activity at the time of planting in the light $H_3$ peat mix was 4.2 μg of FDA $min^{-1}$ g (dry weight) of $mix^{-1}$ (Fig. 3). A steady and significant ($P$ = 0.05) decline was observed thereafter until after 50 days, where it was below the critical threshold level of 3.2 μg of FDA $min^{-1}$ g (dry weight) of $mix^{-1}$ required for sustained suppression of pythium root rot (2). FDA activity in the conducive $H_4$ peat mix was low at all times, thus verifying our previous report for these peat mixes (2).

Microbial biomass increased significantly ($P$ = 0.05) after planting (Fig. 4A). It stabilized at ~0.25 μmol of $P_i \cdot$ g (dry weight) of $substrate^{-1}$ between days 10 and 50. Microbial biomass in the conducive $H_4$ peat mix (Fig. 4A) followed similar trends. It increased significantly ($P$ = 0.05) immediately after planting and then stabilized within the range of 0.11 to 0.19 μmol of $P_i \cdot$ g (dry weight) of $substrate^{-1}$. Although similar in trends, microbial biomass in the conducive $H_4$ peat mix was significantly ($P$ = 0.01) below that in the $H_3$ peat mix at all times (Fig. 4A).

Uptake of [$^{14}$C]acetate revealed a burst in microbial activity within days after potting in the $H_3$ and $H_4$ peat mixes (Fig. 4B).

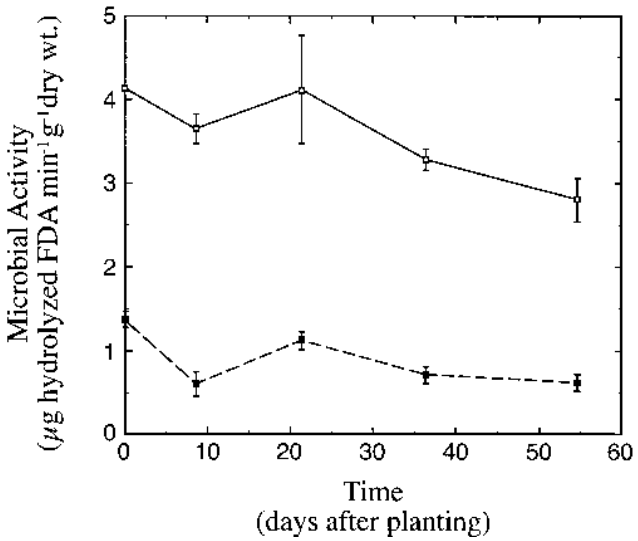


FIG. 3. Microbial activity, based on the rate of hydrolysis of FDA over time in a light ($H_3$; □) and dark ($H_4$; ■) peat mix during the production of poinsettia. Vertical bars represent standard errors ($n$ = 3).

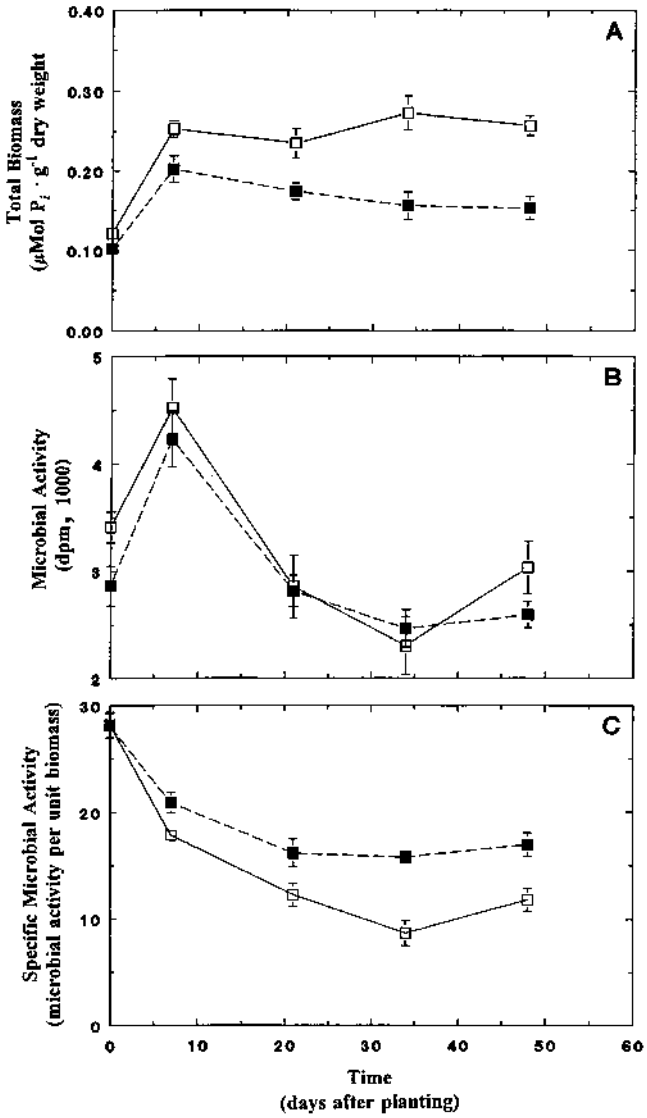


FIG. 4. Comparison of total microbial biomass, microbial activity, and specific microbial activity in a slightly decomposed suppressive ($H_3$; □) and a more decomposed conducive ($H_4$; ■) peat. (A) Total microbial biomass, based the concentration of total phospholipid phosphate; (B) microbial activity, based on the rate of [$^{14}$C]acetate uptake; (C) specific microbial activity, calculated as the level of microbial activity per unit of biomass. Vertical bars represent standard errors ($n = 6$).

After 24 days, microbial activity remained at a relatively high level even though FDA activity declined (2). Interestingly, rates of [$^{14}$C]acetate incorporation in the conducive $H_4$ peat mix did not differ significantly ($P = 0.05$) from those in the $H_3$ peat mix (Fig. 4B). Specific microbial activity (uptake of [$^{14}$C]acetate per micromole of $P_i$ per minute) in the conducive $H_4$ peat mix was consistently higher than that in the $H_3$ peat mix (Fig. 4C). In a second experiment, trends in microbial biomass, uptake of [$^{14}$C]acetate, and specific microbial activity were similar to those presented in Fig. 4.

**Bacterial species composition.** The composition of culturable bacterial species was affected dramatically as the substrate decomposed (Tables 1 and 2). The first of two distinct shifts occurred sometime between 8 and 57 days after potting. Pseudomonads and other facultative oligotrophic gram-negative genera such as *Hydrogenophaga* predominated during the initial 57 days of the growth period. A second shift was observed much later (100 to 172 days). After 172 days, gram-positive pleomorphic bacterial taxa such as *Micrococcus* spp., nonidentifiable strains, and strains lost upon subculturing (possibly oligotrophs) predominated in the rhizosphere and edaphic niches (Tables 1 and 2). Less than 1% of the bacterial

TABLE 1. Relative population density of rhizosphere bacteria isolated on 0.1× tryptic soy broth agar from cucumber seedling root tip segments after potting in a slightly decomposed light ($H_3$) peat potting mix

| Taxon[a] | Relative population density at[b]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8 Days in: | | | 57 Days in: | | | 172 Days in: | | |
| | SU 1 | SU 2 | SU 3 | SU 1 | SU 2 | SU 3 | SU 1 | SU 2 | SU 3 |
| *Aeromonas* spp. | | | | 1.7 | | | | | |
| *Agrobacterium* spp. | | | | 3.4 | 5.6 | 8.3 | | | |
| *Alcaligenes* spp. | 3.0 | | | 3.4 | | | | | |
| *Arthrobacter* spp. | | | | 1.7 | 1.9 | 2.1 | | | |
| *Aquaspirillum* spp. | | 2.4 | | | | | | | |
| *Aureobacterium* spp. | | | | 1.7 | | 2.1 | | | 2.2 |
| *Bacillus* spp. | | 2.4 | | | | 2.1 | 2.3 | | |
| *Bordetella* spp. | | | | 1.7 | | | | | 4.4 |
| *Clavibacter* spp. | | | | 1.7 | | | | | 2.2 |
| *Comamonas* spp. | | | | | | | 2.3 | | 2.2 |
| *Curtobacterium* spp. | | | | | | | 2.3 | | |
| *Cytophaga* spp. | | | | | | | 2.3 | | 2.2 |
| *Flavobacterium* spp. | | 4.6 | | | | | 4.5 | 10.0 | 2.2 |
| *Hydrogenophaga* spp. | | 46.6 | 20.4 | 20.7 | 24.1 | | | | |
| *Klebsiella* spp. | | | 1.9 | 5.2 | | | | | |
| *Microbacterium* spp. | | 2.4 | | | | | 2.3 | | |
| *Micrococcus* spp. | 3.0 | | | | | | | 4.0 | 26.4 |
| *Morganella* spp. | | | 3.7 | | | | | | |
| *Ochrobacterium* spp. | | | | | | | | | 4.4 |
| *Pantoea* spp. | | | 7.4 | 3.4 | | | | | |
| *Phyllobacterium* spp. | | | | 3.4 | 9.3 | 8.3 | | | |
| *Pseudomonas* spp. | 60.6 | 23.2 | 40.7 | 36.2 | 11.2 | 4.2 | 4.5 | 6.0 | 6.6 |
| *Salmonella* spp. | | | 5.6 | | | | | | |
| *Sphingobacterium* spp. | 21.2 | | | | | | | 2.0 | |
| *Vaxiovorax* spp. | | | | | | | | | 4.4 |
| *Xanthomonas* spp. | | | | | | | | 2.0 | 6.6 |
| GC similarity groups | | | | | | | | | |
| 1 | | 2.4 | 3.7 | | 9.3 | 12.5 | | | |
| 2 | | | 1.9 | 1.7 | | 10.4 | | | |
| 4 | | | 1.9 | | | 14.6 | | 2.0 | |
| 5 | | 2.4 | 7.4 | | 1.9 | 2.1 | 27.3 | 28.0 | 4.4 |
| 6 | | | | | 9.3 | | | 2.0 | |
| 7 | 9.1 | | 1.9 | 3.4 | 3.7 | 10.4 | | | |
| 9 | | | 1.9 | 1.7 | 7.4 | | 2.3 | | |
| 10 | | | | 1.7 | 3.7 | 6.3 | | | |
| 12 | | | | | | | 4.5 | | |
| 13 | | | | | | | | | 13.2 |
| 14 | | | | | | | 9.1 | 16.0 | |
| 15 | | | | | | | | | 2.2 |
| 16 | | | | | | | 6.8 | 4.0 | 2.2 |
| 17 | | | | | | | | | 4.4 |
| Lost[c] | 3.0 | 13.8 | 1.9 | 7.3 | 12.6 | 16.7 | 29.5 | 24.0 | 8.8 |

[a] Strains were identified by GC-FAME analysis based on the similarity of their phospholipid fatty acid profiles. Strains with a similarity index ≥0.5 were assigned to a taxon, values <0.5 and ≥0.1, were assigned to a genus, and strains with values <0.1 were assigned to a GC similarity group. Taxa often were identified to the species level, but only genus results are shown.

[b] The relative population density of bacterial taxa on each root tip segment (SU) was calculated as follows: $100n_i/N$, where $n_i$ is the number of strains assigned to the $i$th taxon and $N$ is the total number of strains for a given root tip segment.

[c] Strains lost upon subculturing.

TABLE 2. Temporal dynamics in bacterial species composition as OM decomposed and suppressiveness collapsed during production of poinsettia in an $H_3$ decomposition level peat substrate

| Group | Relative population density[a] at: | | | |
|---|---|---|---|---|
| | 8 Days[b] | | 172 Days[c] | |
| | Mean | Range | Mean | Range |
| Rhizosphere | | | | |
| Gram negative | 72 | 49–84 | 19 | 14–22***[d] |
| Gram positive | 7 | 0–17 | 16 | 4–36** |
| *Pseudomonas* spp. | 38 | 23–61 | 6 | 5–6*** |
| GC similarity groups[e] | 13 | 5–23 | 47 | 34–54*** |
| Lost | 8 | 3–11 | 21 | 8–30** |
| Peat mix | | | | |
| Gram negative | 27 | 26–30 | 23 | 11–32 |
| Gram positive | 13 | 10–16 | 7 | 4–10* |
| *Pseudomonas* spp. | 8 | 4–13 | 11 | 7–13 |
| GC similarity groups | 53 | 49–60 | 65 | 54–79** |
| Lost | 7 | 2–10 | 8 | 4–13* |

[a] The relative population densities of bacterial taxa on root tip segments and peat samples were calculated as follows: $100n_i/N$, where $n_i$ is the number of strains assigned to the $i$th taxon and $N$ is the total strains isolated from a given root tip segment or peat sample.

[b] $H_3$ peat 8 days after potting prior to the decline in disease suppression.

[c] $H_3$ peat 172 days after potting after the decline in disease suppression had taken place.

[d] Significant change in the frequency of strains with a given character between 8 and 172 days: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

[e] Strains with similarity index values <0.1 were placed in GC similarity groups.

[f] Strains lost during subculturing.

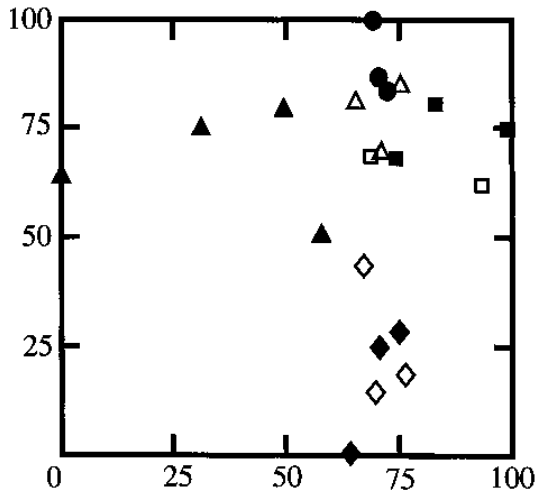


FIG. 5. Bray-Curtis polar ordination plot for separation of rhizosphere (closed symbols) or peat (open symbols) SUs based on similarity of bacterial species composition. Data were collected over time as the concentration of biologically available energy reserves decreased and suppressiveness was lost. Sixty to seventy bacterial strains were isolated on 0.1× tryptic soy broth agar at each sampling time throughout the growth period and identified by GC-FAME analysis. Symbols represent days after potting: △, 6; ▲, 8; ●, 38; ■, 57; □, 100; ◇ and ◆, 172. Dice resemblance functions (31) assessed the relative similarity between bacterial populations. Axes represent percent dissimilarity (31). Differences between points in the two ordination dimensions can be used to access degree of dissimilarity.

strains recovered from the cucumber rhizosphere after 172 days, after the collapse in FDA activity and suppressiveness, were effective as biocontrol agents when applied as cucumber seed treatments against *P. ultimum.*

The Bray-Curtis polar ordination plot (31) based on Dice resemblances (Fig. 5) clearly revealed this shift in composition. SUs representing rhizosphere and niches on day 8 (suppressive) clustered in the upper left portion of the plot. SUs representing bacterial populations recovered from the nonrhizosphere peat mix as suppressiveness collapsed (days 38, 54, and 100) clustered in the upper right portion. Those SUs representing bacterial populations harvested after 172 days, long after the collapse in suppressiveness had occurred, were clearly distinct and clustered in the lower right corner.

## DISCUSSION

Differences in carbohydrate content between the slightly decomposed $H_2$ and the more decomposed $H_4$ peat samples (Fig. 1) demonstrated here by CPMAS $^{13}$C-NMR spectroscopy verify previous findings by others interested in plant material transformations to peat and coal (11, 19, 36). The slightly decomposed $H_2$ peat samples clearly contained higher carbohydrate concentrations compared to the more decomposed $H_4$ peat. The increases in *P. ultimum* population and root rot and the decrease in FDA activity in the $H_3$ peat mix, as described previously by Boehm and Hoitink (2 [Fig. 1]), were accompanied by a decrease in NMR-detectable carbohydrate in the fine-particle fraction of this substrate (Fig. 2). The concentration of CPMAS $^{13}$C-NMR-detectable carbohydrates after 172 days in the now-conducive $H_3$ peat mix was similar to that in the consistently conducive $H_4$ peat mix (Fig. 1 and 2). Lack of a detectable decline in the coarse particles suggests that these particles at days 14 and 77 were similar in composition. Carbohydrate content in the fines would most likely be more accessible to degradation by soil microorganisms because of a greater surface area available for colonization. Although adequate carbohydrate reserves were present in the coarse particles, even after 77 days, the total surface area of these particles relative to that of the fines may have been too low to support a level of microbial activity adequate for effective biological control of pythium root rot of poinsettia. Alternatively, lack of availability of carbohydrates present in the interior components of the coarse particles with decomposed outer surfaces may also have accounted for the decline in microbial activity. A direct spectroscopic procedure, such as near-infrared reflectance spectroscopy, capable of assessing in situ surface chemistry may provide additional information (35).

A significant shift in culturable rhizosphere bacterial species composition accompanied the decline in carbohydrate content in the $H_3$ peat mix (Tables 1 and 2). At potting in this mix, 10% of the rhizosphere microflora (mostly pseudomonads and *Pantoea* spp.) was capable of inducing biological control of pythium damping-off and root rot (3), compared to <1% on day 172. This is similar to the percentage of bacteria recovered from the conducive $H_4$ peat mix capable of causing biocontrol at 10 days after planting (3). Pleomorphic gram-positive bacteria and oligotrophs typical of those recovered from highly mineralized soil fractions (24) and the conducive $H_4$ peat mix (3) were abundant in the conducive $H_3$ peat mix at 172 days (Tables 1 and 2). In the $H_3$ mix, gram-negative bacteria had significantly declined in populations after 172 days, whereas gram positives and oligotrophs increased (Table 2). As the carbohydrate content of the $H_3$ mix decreased (Fig. 2) and suppressiveness declined (2), a microflora capable of inducing biological control was replaced by another that could not. This finding, in conjunction with that presented previously regarding the effect of OM decomposition level on the efficacy of introduced biocontrol agents (3), suggests strongly that OM is critical to maintenance and activity (sustained activity) of a microflora capable of inducing biological control.

In light of the previous work done with this $H_3$ peat mix (2, 3), it is interesting that suppressiveness was lost before bacteria capable of inducing biocontrol of pythium root rot had declined to low relative population densities. The $H_3$ peat mix was conducive after approximately 50 days, when FDA activity was low (2). At this time after potting, the population of pseudomonads, and other facultative oligotrophic gram-negative genera often described as biocontrol agents, was still relatively high (Table 2) even though a significant shift in species composition had already occurred (Fig. 5). This indicates, quite possibly, that the nutritional status of the biocontrol agents at that time was inadequate to sustain biological control activity.

The lack of a difference in microbial activity (based on the rates of incorporation of [$^{14}$C]acetate into phospholipids) in the $H_3$ peat mix (Fig. 4B) indicates that lack of competition for nutrients could not solely explain the loss of suppressiveness observed previously (2). This observation supports an earlier report by Liebman and Epstein (28), which showed that competition for nutrients is not the principal factor limiting germination of fungal spores when microbiostasis prevails but rather that other factors played a significant role. The significantly ($P = 0.05$) higher level of specific microbial activity observed in the conducive $H_4$ peat mix (Fig. 4C), relative to that in the $H_3$ peat mix, suggests that the microflora in the conducive environment utilized the nutrients more efficiently.

The overall conclusions of this work are that energy availability in soil OM critically determined survival and efficacy of bacteria in biological control of pythium damping-off and root rot. Rhizodeposition products such as root exudates, mucigel, and sloughed root cells, although important as energy sources for rhizosphere bacteria (8, 25, 33), appeared inadequate to sustain prolonged suppressiveness in the conducive mixes. It appears that an adequate supply of biologically available energy must be available within the soil system prior to the introduction of a plant to maintain or carry a critical threshold level of biocontrol agent activity through the cropping period as roots grow through the soil. The foregoing offers an explanation for the lack in efficacy often associated with introduced biocontrol agents in field soils where this role of OM is usually not taken into consideration.

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