Cornell University

TITLE: Microbial Basis of Disease Suppression in Composts Applied to Golf Course Turf

INVESTIGATORS:

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USGA REGION: Northeastern/Mid-Atlantic

United States Golf Association Greens Section Research
Annual Report, 1992

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Principal Investigators: Eric B. Nelson, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

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EXECUTIVE SUMMARY

Our goal in this project is to develop more effective biological control strategies with compost-based organic fertilizers by understanding the microbial ecology of disease-suppressive composts. In particular, we hope to understand the microbiology such that disease-suppressive properties of composts might be predicted and an assemblage of beneficial microorganisms useful in the development of microbial fungicides for turfgrass disease control might be discovered.

The objectives of our study are to 1) determine the spectrum of turfgrass pathogens suppressed by compost applications, 2) establish relationships between overall microbial activity, microbial biomass, and disease suppression in composts, 3) identify microorganisms from suppressive composts that are capable of imparting disease-suppressive properties to conducive composts or those rendered conducive by heat treatment, and 4) determine the fate of compost-derived antagonists in golf course putting greens following application of individual antagonists and composts fortified with these antagonists.

Over the past year, our efforts have been focussed on 1) further evaluating composts in the field for disease suppression; our goal has been to verify previous findings as well as expand the diseases for which composts are suppressive; 2) further developing laboratory assays to assess microbial activity and biomass; and 3) enumerating and recovering specific isolates of bacteria, fungi, and actinomycetes from suppressive composts. Much of our emphasis in 1992 was on objective 3.

Although 1992 was not a good year for disease development in our experimental plots, data were obtained for the suppression of dollar spot with various composts. Additional evaluations for snow mold suppression are underway

We have performed isolations of bacteria, fungi, and actinomycetes from over 20 different composts. Actinomycetes have been the most difficult group to enumerate and purify since they are extremely slow-growing and cultures can be easily contaminated with bacteria and fungi. As a means of better recovering antagonistic actinomycetes, we have employed a triple layer agar technique to recover antibiotic-producing actinomycetes. We have over 100 strains of actinomycetes that are currently being evaluated for their disease-suppressive properties. We are currently in the process of characterizing the fungal and bacterial populations from composts and we are just beginning to screen these organisms for disease suppression.

We have also been successful in refining our microbial biomass assay. We are now able to generate repeatable standard curves from both inorganic phosphate and glycerol phosphate and we are proceeding to assess biomass with this procedure in a number of different composts. During the first half of 1993 we hope to be able to assess over 25 different materials for levels of biomass and activity to determine whether this method may be suitable for assessing and thus predicting disease-suppressive properties of composts.

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The objectives of our study are as follows:

1) To determine the spectrum of turfgrass pathogens suppressed by compost applications.

2) To establish relationships between overall microbial activity, microbial biomass, and disease suppression in composts.

3) To identify microorganisms from suppressive composts that are capable of imparting disease-suppressive properties to conducive composts or those rendered conducive by heat treatment.

4) To determine the fate of compost-derived antagonists in golf course putting greens following application of individual antagonists and composts containing same antagonists.

Progress in 1992:

Objective 1) 1992 was a particularly "bad" year for turfgrass pathology research in the Northeast. Nearly all season, the weather was cool and wet. In general, conditions were more favorable for turfgrass growth than for disease development. Field plots were established at the Cornell University Turfgrass Field Research Laboratory to evaluate composts for the suppression of Pythium root rot, dollar spot, brown patch, pink snow mold, and gray snow mold. Plots are currently established for the evaluation of both of the snow mold diseases. Data should be available in the spring.

Plots were established on April 23 to evaluate a number of materials for Pythium root rot control. Plots were established in a randomized complete block design with five replications. Composted materials were applied at monthly intervals throughout the season as a topdressing mixed in with sand at the rate of 10 lb of compost/1000 ft^2. Prior to the first application, plots were

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inoculated with a cocktail of *Pythium graminicola* isolates. Subdue was applied at the rate of 2oz/1000 ft^2 as a fungicide standard. Untreated plots served as controls. The first rating was on May 7 and continued at roughly weekly intervals throughout the season.

Disease development in our plot area throughout the season was disappointing. Although conditions were fairly ideal for Pythium root rot development, no significant level of disease appeared at any time during the season. Likewise, brown patch failed to develop on our plots. However, dollar spot first became evident in our plot area after June 2.

Results of our dollar spot ratings are shown in Figure 1. Disease incidence was relatively low for the first three weeks of June after which disease incidence in untreated plots began to increase. By the end of July, numbers of spots per plot ranged from 7 to 96 spots per plot among all compost treatments with Banner-treated plots having less than one spot per plot. A number of materials were suppressive to dollar spot, most notably Sustane, Compost Plus, Greens Restore, a mature Brewery waste compost, and Earthworks 4-2-0. One yard waste compost actually enhance dollar spot development over the course of the season.

A second inoculation with *P. graminicola* was made on October 21 and results should be available by mid November. In the spring of 1993, data should be available for both pink snow mold and gray snow mold, barring any unforseen problems with the development of disease from naturally-occurring inoculum.

Objective 2) We previously refined our microbial activity assays so that they performed consistently. During the course of 1992, our efforts turned to the microbial biomass assays. Our methods of assessing total microbial biomass in both suppressive and non-suppressive composts have not been straightforward. We previously encountered a number of technical difficulties that have prevented us from being able to rely on lipid phosphate assays for the determination of microbial biomass in compost samples. The technique is dependent on the ability of malachite green, a dye, to interact with the phosphate groups of chloroform-extracted lipids. Repeated attempts to react the dye with standard phosphates failed. Additionally, we have had problems with high background phosphate levels, possibly arising from aqueous phosphate contaminants being remove in the chloroform phase. Although we have been using standard protocols developed by others, the technique has not been reliable in our hands.

We have been able to develop what we think is a more reliable method for measuring lipid phosphates. Instead of extracting phospholipids with chloroform, we have switched to methylene chloride. This solvent has similar partitioning characteristics to chloroform, however, unlike chloroform, methylene chloride is less dense than a saturated NaBr solution, thus forming a layer above the aqueous phase. This makes it easier to remove the organic phospholipid extract from the extraction vessel and keep it free of aqueous phosphate contaminants. This contamination was one of our major problems previously. Although were have not yet performed these assays on compost samples, we are now able to generate repeatable standard curves from both inorganic phosphate and glycerol phosphate (Figure 2). We are currently proceeding to assess biomass with this procedure in a number of different composts. During the first half of 1993 we hope to be able to assess over 25 different materials for levels of biomass and activity to determine whether this method may be suitable for assessing disease-suppressive properties of composts.

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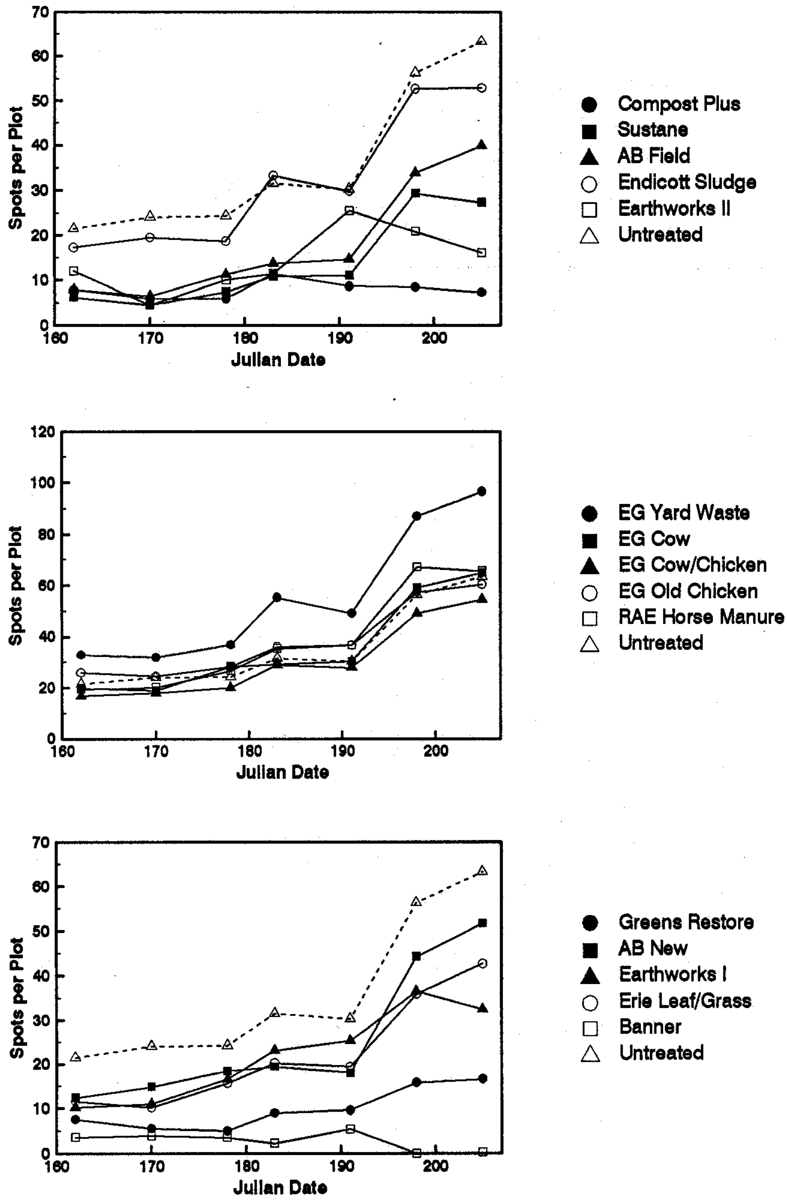

Figure 1. Development of dollar spot on a creeping bentgrass putting green at the Cornell University Turfgrass Field Research Laboratory. Disease development arose from natural inoculum and was monitored through June and July. Dashed line in all three figures represents untreated plots.

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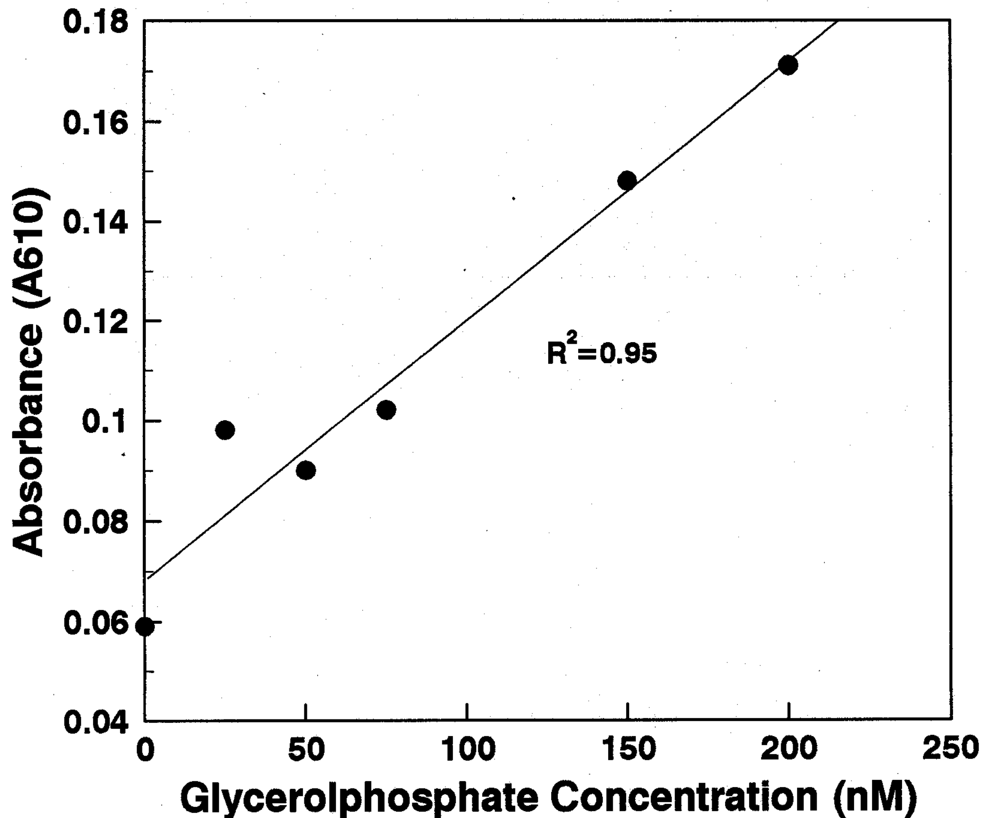

Figure 2. Relationship between extractable concentrations of glycerolphosphate (standard) and absorbance at 610 nm.

Objective 3) We have continued isolations of bacteria, fungi, and actinomycetes from various suppressive composts. These organisms have been enumerated using conventional microbiological techniques with selective media. To isolate these microorganisms, 10 g of compost was placed in 90 ml of distilled water and placed on a shaker for 10 min. at room temperature. A dilution series was prepared and 0.1 ml aliquots plated onto appropriate culture media. For bacterial isolations, suspensions were plated onto 1/10-strength trypticase soy agar. For fungi, suspensions were plated onto 1/3-strength potato dextrose agar amended with rifampicin to inhibit unwanted bacterial growth. After incubation of isolation media at 27C, developing colonies of each respective group of microorganisms were then enumerated and expressed as colony forming units (CFU) per gram dry wt of compost.

Our methods for actinomycete isolations have changed. Previously, suspensions were plated onto 2% water agar amended with cycloheximide, nystatin, and polymyxin B to inhibit fungal and bacterial growth. However, despite out efforts to improve the selectivity of this medium, we were unable to completely eliminate problems with bacterial contamination. We have since gone to a

triple layer agar technique developed by L.J. Herr in 1959. This method consists of placing a thin layer of water agar on the bottom of the petri plate followed by a layer of agar containing the compost suspension. This is followed 48 hr later by a layer of agar containing chopped mycelium of *P. graminicola*. After an additional 4 days plates appear as in Figure 3, with distinct clearing zones around the colonies of antibiotic producing actinomycetes. These colonies are then picked and transferred to appropriate culture media.

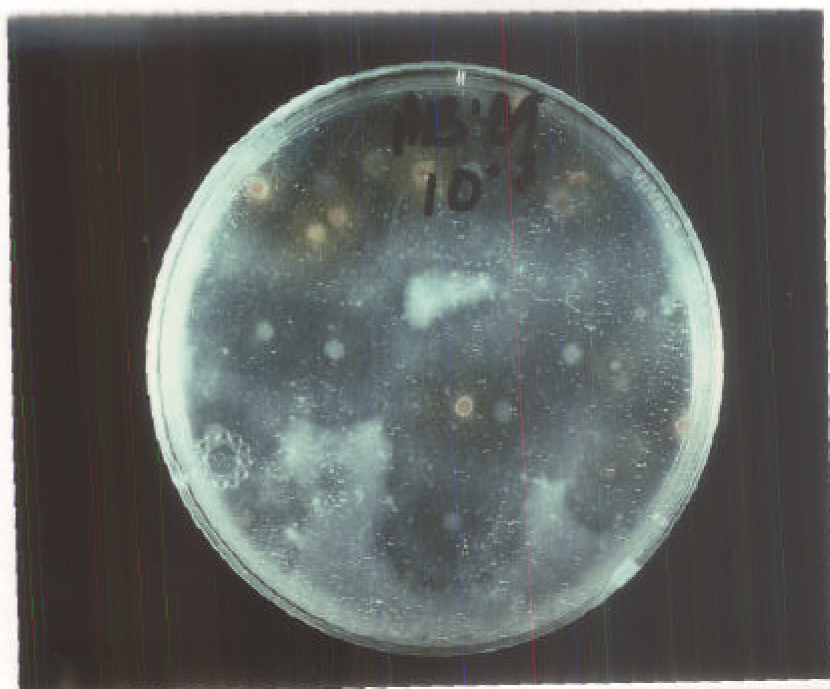

Figure 3. Triple layer agar technique for isolating antibiotic-producing actinomycetes. Colonies in the center of clearing hyphal zones (*P. graminicola*) represent the actinomycetes of interest. The petri plate shown in this figure represents isolations from a brewery waste compost.

We have performed isolations from over 20 different composts. Our most intensive isolations have come from different ages of brewery waste compost and from a 3.5 yr-old Endicott compost. These latter composts have been our most consistently-suppressive composts to *P. graminicola*. Populations of selected groups of microorganisms recovered from the various composts tested are listed in Table 1. Actinomycetes have been the most difficult group to enumerate and purify since they are extremely slow-growing and cultures can be easily contaminated with bacteria and fungi. We have over 100 strains of actinomycetes that are currently being evaluated for their disease-suppressive properties. We are currently in the process of characterizing the fungal and bacterial populations from these composts. We currently have nearly over 500 cultures of bacteria and fungi

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Table 1. *Pythium* suppression and populations of fungi, bacteria, and *Pythium*-suppressive actinomycetes from composts

	Microbial Populations (log CFU/g dry wt. compost)			*Pythium graminicola* Suppression Disease Rating[d]	
Compost	Heterotrophic Bacteria[a]	Heterotrophic Fungi[b]	Antibiotic-producing Actinomycetes[c]	Uninoculated	Inoculated
Brewery Waste 1989	8.65	7.53	9.86	1.0	2.3
Brewery Waste 1991	9.85	5.73	8.00	1.5	3.5
Brewery Waste 1992	5.04	4.72	NA	1.0	2.3
Endicott Sludge 1989	9.65	6.54	6.43	1.0	1.8
Leaves A4-21-92	8.99	5.87	NA[e]	1.0	2.0
Leaves A5-20-92	9.34	5.04	6.34	1.0	3.3
Leaves B6-4-92	8.61	5.59	6.79	1.5	1.8
Leaves/Chicken Manure A4-21-92	8.90	3.77	NA	1.0	1.5
Leaves/Chicken Manure A5-20-92	9.23	5.23	6.91	1.0	4.8
Leaves/Chicken Manure A6-4-92	9.38	4.00	NA	1.0	2.0
Chicken Manure A6-4-92	8.80	3.65	<2.26	1.0	1.0
Chicken Manure B6-4-92	9.18	4.87	NA	1.0	1.0
Chicken/Cow Manure A6-4-92	9.36	5.04	NA	2.0	2.0
Yard Waste Grind B4-21-92	8.67	4.41	NA	1.0	1.5
Yard Waste Grind B5-20-92	9.43	5.04	6.68	1.0	3.3
Yard Waste Grind A5-20-92	9.34	5.04	6.89	1.0	4.5
Food Waste B6-4-92	9.36	3.90	NA	1.5	2.0
Food Waste B7-1-92	9.18	4.15	NA	1.0	2.0
Food Waste A6-4-92	8.70	3.83	5.23	2.3	2.8
Food Waste A7-1-92	8.62	3.49	NA	2.5	2.0

[a] Heterotrophic bacterial populations determined by plating on 1/10-strength trypticase soy agar (TSA).
[b] Heterotrophic fungal populations determined by plating on 1/3-strength potato dextrose agar (PDA).
[c] *Pythium*-suppressive antibiotic-producing actinomycetes determined by a triple-layer agar plating procedure described by Herr (1959).
[d] Compost mixed with sand at the rate of 80 mg dry wt/cm^3 sand. Rated after 5 days on a scale of 1-5 for which 1= no disease and 5=completely dead or unemerged seedlings of *Agrostis palustris*. Controls consisted of sand to which no compost was added (Disease rating 1.0 and 5.0 for uninoculated and inoculated, respectively).
[e] NA = data not yet available

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from these composts. We are just beginning to screen these organisms for disease suppression.

Upon preliminary analysis, the generic and species composition of microbial communities appears to be fairly non-diverse. Fungal populations in all of the composts tested consist predominantly of dark, dematiaceous unidentified fungi, as well as numerous *Penicillium* spp., *Mucor* spp., and *Trichoderma* spp. Bacteria populations are typically predominated with only two or three different morphological types in any given compost. Despite the low diversity, population sizes are extremely high, ranging from 10^5 to greater than 10^9. In contrast, all composts tested to date, with the exception of a chicken manure compost and a food waste compost, contained very high levels of actinomycete populations, particularly antibiotic producers.

Objective 4) No progress to date. Field experiments were originally planned for the autumn be continued in the spring of 1993 following results of laboratory screeenings of isolated microorganisms. However, due to the unusually cool weather and premature frost, we have chosen to wait until spring 1993 to launch this part of the study. Additionally, more complete screenings of isolated organisms will be complete by the spring.

Proposed Research Schedule for 1993 and Beyond:

Objective 1. Of the turfgrass pathogens examined to date in this laboratory, composts have been suppressive to *Pythium graminicola, P. aphanidermatum, P. ultimum, Rhizoctonia solani, Sclerotinia homoeocarpa, Typhula incarnata* and *Laetisaria fuciformis*. Future studies will continue to emphasize *Pythium* spp., particularly others involved in the Pythium root rot complex. However, studies are planned to determine the suppressiveness of composts to *Microdochium nivale* (pink snow mold) and *Cochliobolus sativus* (leaf spot). These will be examined, where practical, in the field as well as in the laboratory. We expect to find composts that will be suppressive to some degree to all of these pathogens.

Objective 2. Our goal with this objective is to develop predictive assays for disease suppression in composts. Our focus thus far has been on FDA hydrolysis as an indicator of microbial activity and on microbial biomass. We have been hampered by undue technical difficulties which we feel we have resolved. In 1993, we will be screening over 25 composts for microbial activity and microbial biomass in an attempt to establish relationships between these parameters and *Pythium* suppression. We plan to look further at specific activity of the entire compost microbial community. Specifically, we plan to examine single source carbohydrate utilization by the intact microbial community as a means of distinguishing predominant microbial activities in suppressive and conducive composts. In order to define a number of the technical aspects of microbial activity assays based on FDA hydrolysis, we have focussed on *Pythium* spp. We plan to develop such relationships with *R. solani* (brown patch).

Objective 3. Individual organisms are currently being tested in plant assays to identify those that are able to restore suppressiveness in heat-treated compost to levels found in the naturally recolonized suppressive compost. Suppressive samples are made conducive by heating to 60 C for five days prior to inoculation (the extended heating period was chosen as representative of temperature/time exposures in the center of a typical compost windrow). Composts are then inoculated by culturing

candidate organisms in the laboratory on an appropriate liquid culture medium for two to five days. The suspension (containing cells, spores, etc.) are then poured into a mixture containing 80 mg compost/ml sand and incubated at room temperature for 24 hr. The inoculated mixture is then placed in wells of a tissue culture plate, seeded with creeping bentgrass and allowed to grow for 7 days. Wells are then inoculated with the target pathogen. Our emphasis has been on *P. graminicola*, but we plan to expand this to also include *R. solani*.

We will be continuing these screenings for the greater part of the remainder of the project. We are currently giving most of our attention to the actinomycetes since we have a useful prescreening technique. However, over the next year, we plan to give particular emphasis to bacterial strains since we feel that our best candidates suitable for integration into greens and fairway management practices will come from this group, as well as from the actinomycete group.

Objective 4. Our final year of funding on the current USGA grant will conclude with a field test examining the fate of selected antagonists in golf course putting greens. Emphasis will be on sand-based putting greens. To determine how well select antagonists establish in the rhizosphere of plants following field applications of an antagonist-amended compost, we will inoculate both suppressive and non-suppressive compost samples with cultures of known antagonists recovered from suppressive compost samples. Individual antagonists shown to be effective in laboratory experiments will be cultured in the laboratory on an appropriate culture medium for two to five days. The suspension of the culture (containing cells, spores, etc.) will be poured into a mixture containing 80 mg compost/ml sand and allowed to incubate at room temperature for 24 hr prior to application. Plots will be treated and monitored for disease development as in previous experiments. Untreated and fungicide-treated plots as well as plots treated with composts not fortified with antagonists will serve as controls.

Population dynamics of select bacterial antagonists will be monitored weekly from the time of application through the end of the season by removing 20 random cores (1 cm-diameter) from each replicate plot. Cores for each treatment will be pooled and 10 g of core samples placed in 90 ml of distilled water and comminuted in a blender for 1 min. A dilution series will then be prepared and 0.1 ml aliquots plated onto selective media. Rifampicin-resistant derivatives of parental strains will be selected from the wild-type population and used to monitor populations. After incubation at 27C, colonies of the target organism will be enumerated and expressed as CFU/g dry wt of sample. Soil temperature (3 cm depth) and moisture will be monitored throughout the experiment and soil analyses will be performed before and after applications are made. Cultural inputs such as mowing heights, aerification, fertilization, pesticide applications, etc. will also be recorded.

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