

## **Detecting the Effects of Fungicides on Soil Bacteria Populations using Denaturing Gradient Gel Electrophoresis (DGGE)**

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- Objective** The objective was to determine the short-term impact of fungicide introduction on the indigenous bacterial community of soil.
- Rationale** Fungicide applications are a necessary practice to discourage disease development in most high quality turfgrass areas. Little is known, however, about the effects that fungicides have on resident microbial community of the soils supporting turfgrass sites. Soil bacteria are responsible for important processes including pesticide degradation, nutrient cycling, and organic matter breakdown. Thus, assessing the impact of fungicide applications on soil bacteria populations will allow for a better understanding of how the bacterial community responds to chemical inputs.
- How it was done** Three soils, a putting green root-zone sand, forest, and an agricultural soil were, incubated with technical grade chlorothalonil at rates corresponding to 0x, 1/5x, 1x, and 5x the high label rate of chlorothalonil application, respectively. The mass equivalent 15 cubic inches of soil (approximately the amount of soil within a standard cup cutter at 1 inch in depth) were incubated in plastic bags for approximately two weeks. DNA was extracted from the soils using a bead beater at the time of inoculation as well as following the two week incubation period. PCR amplification was performed using primers specific for bacterial cells only. Amplification products were analyzed for community structure changes using denaturing gradient gel electrophoresis (DGGE) which can separate a heterogeneous mixture of double stranded DNA according to differing base sequences rather than size differences.
- Results to date** It is assumed that the relative amount of biomass in a particular soil can be estimated by the quantity of extractable DNA that is present. The three soils in this study consistently produced differing levels of extractable DNA at each sampling. The sand was characterized by consistently lower quantities of extractable DNA when compared to the agricultural soil which, in turn, contained less DNA than the forest soil (Table 1). These results suggest that the sand, at this point in time, sustains a very low biomass and potentially little microbial activity when compared to other higher biomass soils. The sand was a component of a newly established putting green and may be exhibiting a low biomass based on disturbances due to construction and establishment.
- DGGE profiles indicate that the chlorothalonil application dramatically impacted the microbial community of the sand. Figure 1 shows shifts in the banding pattern of chlorothalonil treated soils which indicate a restructuring of the microbial community. Pattern shifts were evident regardless of the rate of chlorothalonil application and included both new bands forming and native bands regressing indicating populations becoming dominant or recessive, respectively. The intensity of newly formed bands correlated with the rate of chlorothalonil application which

suggests a stimulatory effect of the fungicide on certain members of the bacterial community. These members could possibly be able to degrade the fungicide and utilize the chemical as a substrate for growth or energy production.

The documentable effect of the chlorothalonil application on the microbial community of the forest and agricultural soil was minimal. Figure 1 reveals that similar banding patterns were present in both soils over time regardless of the treatment. This result illustrates a relative absence of effect of chlorothalonil on the bacterial community. Two possible reasons exist for this lack of activity. 1) We have shown that organic matter can effectively sequester organic compounds such as fungicides and may be actively decreasing the bioavailability of chlorothalonil in the forest and agricultural soils. 2) A limit of detection exists whereby small changes in an ecologically rich community are less obvious than similar changes in a community of lesser biomass and diversity. Thus, the relative richness of the forest and agricultural soil communities may tend to mask some subtle restructuring that may, in fact, be taking place.

It is important to recognize the significance of these results. Soils under the influence of managerial inputs such as regular pesticide and fertilizer applications are most likely to the ecological described above. We have indicated that of the three soils tested, the low biomass sand was impacted more than the two soils of greater biomass. The sand and other soils under similar management are at the highest risk of stress at the microbial community level and this risk is assumed to be increased under the purposely stressful conditions found on many highly maintained turfgrass sites. Research is currently underway to further characterize the nature of the population shifts through band sequencing and subsequent identification of the novel and regressing bacteria.

Table 1. DNA concentrations of soils used in this study

soil	conc (ug/g soil)
USGA mix	4.30 (1.02) <sup>a</sup>
forest	47.76 (10.20)
agricultural	18.62 (2.54)

<sup>a</sup> Standard error calculated from three replications.

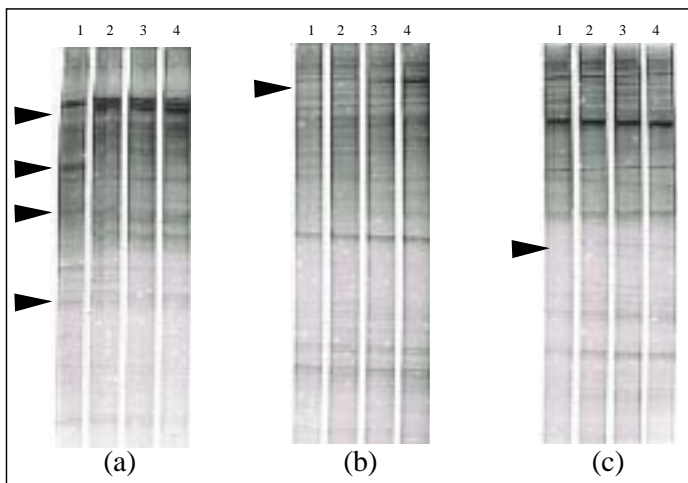


Figure 1. DGGE profiles from (a.) USGA greens mix, (b.) forest, and (c.) agricultural soils. Arrowheads mark banding pattern changes. The four lanes representing each soil are (1.) time=0 control, (2.) t=2weeks 1/5x rate, (3.) t=2w 1x rate, (4.) t=2w 5x rate. Control soils from t=2w sampling were similar to t=0 samples (data not shown).