

Divergent soil microbial recovery in response to contrasting restoration techniques targeted against an invasive species



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Abstract

While most landscape restoration projects focus their efforts on the aboveground biota, much of what occurs belowground is overlooked. Our research seeks to understand how the soil microbiota respond to different restoration solutions due to prevailing evidence of the strong interdependency between microbial composition and plant life. At a site where European Buckthorn (*Rhamnus cathartica*) dominated for many years as an aggressive nonnative plant, we evaluated four contrasting rehabilitation treatments that had been applied the previous year. After measuring microbial biomass, fungal to bacterial ratios, and obtaining community genetic profiles, our results indicate that mulch treatments (wherein mulch is tilled into the surface layer of soil) are most effective at restoring microbial composition.

Introduction

The seemingly insurmountable rise of invasive species is considered a major threat to biological diversity around the world. European Buckthorn (*Rhamnus cathartica*) is one dominant threat in particular to the native plant populations of Northeastern Illinois and is now altering ecosystem functioning and composition significantly (Heneghan et al. 2004). In response to Buckthorn's negative influence on local plant diversity, various restoration practices have been prescribed and all with varying degrees of effectiveness. Compared to methods assessing primarily plant diversity, a potentially more comprehensive way to assess the effectiveness of different management applications is by examining that which plants rely on heavily for establishment and success: the soil microbial communities. The purpose of this study was to see how different management practices modify fungal and bacterial communities in addition to observing how the pre-restoration soil conditions may have encouraged the unwanted plant community.

In our study we assessed the microbial biomass, fungal to bacterial ratios, and used Inter-Sequence Repeat (ISSR) markers to obtain a fungal community profile. Comparisons were made between a site where several restoration treatments following Buckthorn invasion had been applied and a prairie representing the goal of rehabilitation projects due to its overall plant diversity. We hypothesized that the two treatments with mulch tilled into the surface would most closely resemble in microbial composition the model prairie due to the addition of an easily accessible bulk carbon source. This could potentially shed light on how microflora respond to both non-indigenous plant invasion and restoration efforts.

Methods

Originally a horse pasture, our primary field site was abandoned in 1995 and subsequently a massive buckthorn invasion ensued. Our secondary field site was Shaw Prairie, which is considered healthy and biologically diverse. In our study we evaluated five treatments:

- C** – Control plot (original pre-restoration Buckthorn thicket)
- M** – Municipal mulch tilled into the soil (native seeds applied on top)
- NS** – Best management practice of the region (Buckthorn cut, stumps treated with herbicide, native seeds applied)
- Tg** – Buckthorn mulch tilled into the soil
- S** – Shaw prairie (representing the goal for restoration treatments)

Slide Application and Soil Collection:

To collect fungi for molecular analysis, the slides technique was employed. Two ml of potato dextrose agar were sandwiched between two glass slides, secured at both ends, and placed in the ground just below the soil surface for 15 days.

Fungal and Bacterial Counting for F:B ratios:

Four drops of a soil suspension prepared for each sample were applied to a glass slide. Fluorescent dyes YO-PRO and propidium iodide were used to stain the smears. Photographs were taken at 100x and with a grid, live/dead bacteria hyphal intersections were counted. Methods and calculations for F:B ratios were undertaken as in Robertson et al. (1999).

Combustion Analysis for Microbial Biomass:

For microbial biomass, chloroform-fumigated and non-fumigated soils samples were analyzed for total carbon and nitrogen using combustion analysis. Methods and calculations were undertaken as in Robertson et al. (1999).

ISSR for Microbial Community Profiling:

DNA was extracted from the slides using the FastDNA Kit. Microbial community profiles were generated using five microsatellite primers and the banding patterns used to calculate Sorenson's Index of Similarity between Shaw and the other treatments. These results were also compared against a database of common local fungal species to find possible close matches.

Results

Figure 1: Live/dead fungal to bacterial ratios based on biovolume.

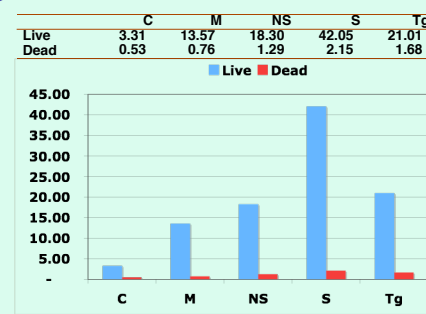
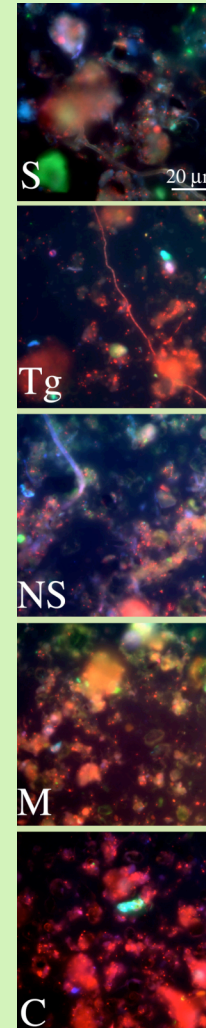
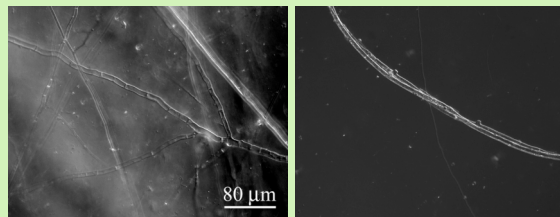
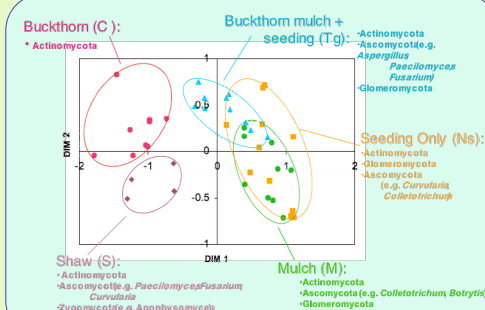


Figure 2: Measured values for microbial biomass carbon:nitrogen, nitrate and ammonia concentration, and % microbial community similarity to Shaw.

	MBC:MBN	Nitrate (ppm)	Ammonia (ppm)	% Similar to Shaw
C	10.39	0.37	1.08	32.00
M	11.70	3.66	5.39	31.94
NS	10.07	1.44	1.05	51.01
S	12.57	3.51	1.57	63.71
Tg	11.48	1.85	1.78	51.73

Figure 3: Non-metric multidimensional scaling plot showing community similarities between treatments based on ISSR banding patterns. Also shown are the most common fungal taxa within each treatment.



Images

Above: Representative fluorescent microscopy images used for fungal to bacterial ratio calculations. In red are dead bacteria and fungi and in green are the living microbes. Purple or blue-stained organisms are autofluorescing and are also living.
Far left: Image of fungi collected in agar slides. Visible are fungal hyphae with septations and branching typical of saprophytic species.
Left: Two hyphal strands of basidiomycetous fungi.

Conclusions

In agreement with our hypothesis, our studies conclude that microbial communities are recovering most appropriately in the mulch treatments (M & Tg). Between the four treatments, fungal to bacterial ratios, microbial biomass C:N, nutrient concentration and % similarity to Shaw were greatest for Tg, suggesting a trend toward ideal Shaw-like conditions (Fig. 1,2). These parameters for determining this possible shift toward the ideal state are weakest for the control treatment containing the original Buckthorn thicket. In particular, the significantly low fungal to bacterial ratio in the control may address the underlying processes behind invasive plant dominance—with a relatively smaller proportion of fungi to bacteria, there is a slower turnover of nutrients (Fig.1). This conclusion is supported by our nutrient test results, which indicate that nitrate and ammonia concentrations are significantly low in the control treatment. Native plant populations, as a result, will find it more challenging to recover under these conditions and invasive species such as Buckthorn will continue to thrive.

Our studies reveal strong relationships between prescribed restoration treatment and overall ecosystem recovery. Due to the many complexities of aboveground biota, it may be more challenging or at least require more time to determine such relationships. This certainly highlights the possibility that rehabilitation success could more effectively be correlated with the microbial rather than the aboveground response. Thus more immediate judgments of contrasting restoration treatments can be drawn and their evolution toward the ideal state tracked more reliably.

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