

Closing the Loop: Alternative Land Management at  
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## Introduction

There has been an increasing emphasis on urban soil research in an effort to understand how urban development impacts soils and how soils impact urban ecosystems (Lorenz and Lal 2009). Healthy urban soils promote healthy vegetation, help mitigate storm-water surges, and lead to improved nutrient cycling that minimizes losses by leaching and run-off (Groffman, Law et al. 2004; Raciti, Groffman et al. 2008). These soil services, however, are often overlooked and neglected.

Part of this neglect comes from a lack of knowledge surrounding soil ecosystem services. Within the urban landscape, many urban lawns are treated with heavy doses of nitrogen fertilizer to maintain uniformity and color. Lawns treated with high-nitrogen fertilizers have significant implications for the health of urban soils and surrounding watersheds. Use of synthetic nitrogen fertilizers can lead to increased soil acidity, which makes soils a hostile place for beneficial soil microbes that help provide the aforementioned ecosystem services (Vitousek, Aber et al.; Groffman, Law et al.).

Practices that minimize the impacts of synthetic nitrogen fertilizers provide a means to improve the health of the urban ecosystem and promote soil ecosystem services (Cadenasso, Pickett et al.). Ecological landscaping refers to practices that enhance biodiversity, biological cycles, and soil microbial activity while utilizing local input sources to conserve water and energy resources (Byrne and Grewal 2008). Yale has identified its land management program as a key opportunity to support such practices (Yale University). In order to develop best practices, ecological landscaping implementation must necessarily involve practitioners, researchers, and policy makers. Study systems that reach across social and ecological urban landscape parameters while also emphasizing rigorous scientific experimentation are missing within the field of urban ecological landscaping (Byrne and Grewal 2008).

This research project constitutes an effort to put a rigorous scientific approach to test the science behind Yale's sustainability initiatives while also taking a multi-disciplinary approach that engages stakeholders across Yale University. This project aimed to create a "closed-loop" system by utilizing Yale's food waste to create an organic soil amendment—compost tea—that was then applied to a series of test plots monitored for soil microbial community response. Compost tea is a liquid extract of compost that contains a host of soluble and extractable nutrients that add organic material to the soil while also stimulating microbial activity (Ingham 2000).

The pilot project tested the following hypothesis: The use of a compost tea amendment will increase organic matter and microbial activity in the soil, thereby eliminating the need for synthetic fertilizers and herbicides. This hypothesis was tested through soil sampling and analysis that compared Yale's current practices with those of the sustainable land management research project.

Soil analysis focused on microbial biomass and function. Microbial activity is an indicator of soil health and fertility (Leita, De Nobili et al. 1999). Microbial biomass represents a small fraction of the soil's total organic matter, however, microbes are

essential for driving important soil processes such as decomposition, nutrient transformation, and the mineralization of organic matter, which releases nutrients essential for plant growth and soil health (Leita, De Nobili et al. 1999; Degens, Schipper et al. 2000). For this research, active microbial biomass was measured using a substrate induced respiration (SIR) technique (Fierer and Schimel 2003; Bradford, Watts et al. 2010). Microbial biomass constitutes a sensitive indicator of changing soil conditions (Leita, De Nobili et al. 1999), and was therefore chosen as an appropriate method to analyze the effects of a compost tea treatment.

Microbial functional diversity was measured through catabolic response profiles (CRP). CRPs provide an index of microbial functional diversity by measuring short-time utilization of a range of substrates through measuring CO<sub>2</sub> efflux of soil microbes (Degens, Schipper et al. 2000; Marchante, Kjoller et al. 2008). The theory behind CRP is that different microbial communities have varying capacities to metabolize substrates; therefore, microbial response to substrates provides an indicator of the functional diversity in the soil (Degens, Schipper et al. 2000; Marchante, Kjoller et al. 2008).

CRP analysis is used to create an index of catabolic evenness across microbial communities. Catabolic evenness measures the variation among soils in their responses to the different substrates. Soils with high levels of functional diversity have correspondingly high levels of catabolic evenness. High levels of evenness have been linked with higher levels of soil carbon (Degens, Schipper et al. 2000). Under my hypothesis, the addition of organic matter to the soil would create higher levels of catabolic evenness in soils treated with compost tea compared to soils under current treatment.

The purpose of this project was to establish an applied research study on campus with important management implications (Redman 1999). The parameters analyzed provide sensitive indicators of potential shifts in microbial communities under different management routines. The results of this project will provide a scientific basis that can help inform Yale's decision on integrating ecology into its land management regime. The emphasis of the project focused on the ecological framework of Yale's land management program; economic considerations were not addressed under the scope of this project.

## **Methods**

### *Plot Identification, Design, and Maintenance*

Before the experiment could begin, plot locations around Yale's campus had to be identified and permission granted by the University. Rigorous research experiments demand a large degree of local control and replication, which is often difficult to secure in an urban setting.

A significant amount of negotiation had to take place to ensure the robustness of the experiment. A large number of stakeholders were critical to implementing this project on Yale's campus. The Office of Sustainability took the lead in negotiating efforts between the Yale University Planning Office, Yale Landscaping and Maintenance Services, and the project leaders. The number of experimental locations was agreed upon at 12, with 8 plots on central campus and 4 plots at Marsh Botanic Gardens (Figure 1).

The experimental plots were each 6 meters by 6 meters. This larger plot was then divided into 2 m by 2 m quadrants. Quadrants were surrounded with a buffer (.6 meters wide) to protect treatments from cross contamination. Plots were marked out using blue whiskers (see Figure 2). Each quadrant received a different treatment:

- 1) Current Treatment: synthetic fertilizer and herbicide applied under current management
- 2) Compost Tea Intensive Application: soil amendment applied every two weeks
- 3) Compost Tea Efficient Application: soil amendment applied every six weeks
- 4) Experimental Control: no treatment

Incorporating two different rates of compost tea application was intended to have important management implications; the theory being if soil response of the lesser application was similar to that of the more intensive treatment, then Yale Grounds would not have to apply treatment as often.

Establishing a plan for control over the experimental plots required strong communication between project leaders and the Yale Grounds crew. A strategy was developed that employed a notched tarp to protect quadrants 2-4 from routine fertilizations while enabling quadrant 1 to receive current treatment.

### *Soil Sampling*

The first soil sampling took place July 1<sup>st</sup> 2010 before any treatments had been applied. This sampling established a baseline set of analyses from which to compare future samples. Ensuing sampling took place every six weeks after the onset of compost tea application, which began August 1<sup>st</sup> 2010. The first round of experimental sampling took



Figure 1: Map of plot locations across campus. Note: the map shows 8 of the 12 plot locations; 4 additional plots are located within Marsh Botanic Gardens.

place on September 15, 2010 and the last round of soil sampling took place on October 31, 2010.

At each sampling date, fifteen soil cores from each treatment quadrant were collected. Soil was sampled to the first 5cm of the soil horizon. Surface soils were used since this tends to be the area of highest microbial activity (Bradford, Watts et al. 2010). The soil cores were pooled on site, yielding a total of 48 soil samples from across the research plots. Soils were then taken to the lab and refrigerated until lab analysis.

#### *Compost Tea Application*

Compost tea was applied every two weeks on the “Compost Tea Intense” plots and every six weeks on the “Compost Tea Efficient” plots. Compost tea was made every two weeks utilizing a homemade compost tea bubbler. Finished compost was steeped in bubbling water to maintain aerobic conditions; additional ingredients to boost microbe presence and activity were mixed in with the water (Ingham 2000). The tea was made with finished compost provided by New Milford Farms, the composting facility to which Yale sends its food waste. The compost tea was made in 60 liter batches for each application using the following recipe:

- 1500 g finished compost
- 115 g molasses
- 230 g organic fish and seaweed fertilizer
- 25 g vegetable oil

Compost tea was applied at a rate of 1.2 liters per plot; the tea was mixed with equal parts water.

#### *Lab Analysis*

Soils were analyzed for pH by mixing water to soil in 2:1 ratio, gravimetric soil moisture (24 hours at 105C), substrate induced respiration, and catabolic response profiles.

Substrate induced respiration (SIR) provides an index of microbial biomass by measuring rates of CO<sub>2</sub> efflux over a given incubation time (Fierer and Schimel 2003). Each of the 48 soil samples was weighed in 50mL test tubes to a dry-weight equivalent of 4 g. Soil samples were mixed into a slurry with 4g of an autolyzed yeast solution; soils were then capped and flushed of CO<sub>2</sub>. After 4 hours of incubation at 20C, the CO<sub>2</sub> that had accumulated within the headspace of the test tube was measured using an Infra-Red Gas Analyzer. CO<sub>2</sub> efflux is used to estimate the active microbial biomass within each soil sample (Fierer and Schimel 2003).

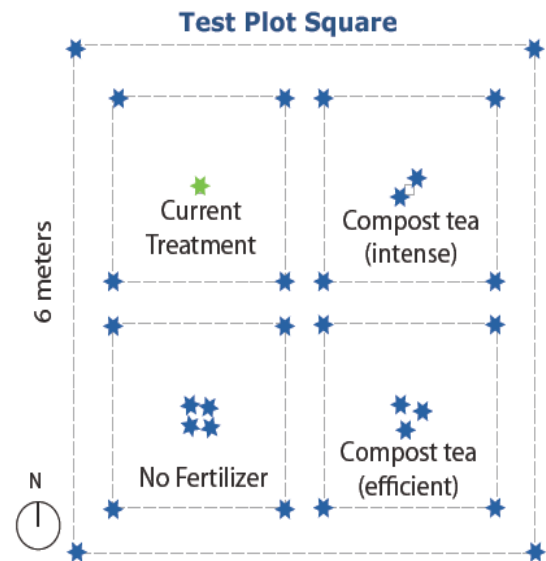


Figure 2: Schematic of research plot design with 4 treatment quadrants and a buffer protecting the plots from cross contamination from within the plot and from outside of the

Catabolic response profiles employ similar techniques to those listed above. Soils were weighed to a dry weight equivalent of 4 g and then mixed into slurries with ten different substrates (a control of water, autolyzed yeast, lignin, cellulose, chitin, glucose, sucrose, glycine, citric acid, and oxalic acid). All substrates were pH adjusted to a pH of 6 before they were added to soils. Soils receiving labile substrates (water, glucose, sucrose, glycine, and the acids) were capped, flushed, and incubated at 20C for four hours; and those receiving recalcitrant substrates (lignin, chitin, and cellulose) were capped, flushed, and incubated for 24 hours. After incubation, the CO<sub>2</sub> efflux of all soils was measured using the IRGA.

### *Statistical Analysis*

To analyze the effects of compost tea treatment across time and space, I used a linear mixed effects model. The baseline experimental data was identified as a covariate in the model as a means to compare treatment effects to this initial pre-treatment round. Fixed effects were the covariate, treatment (current treatment, compost tea intense, compost tea efficient, and no treatment), and sampling round (baseline, experimental round 1, and experimental round 2), with interactions between treatment and round built into the model. Site was identified as a random effect.

## **Results and Discussion**

### *Microbial Biomass*

The original hypothesis predicted that soils receiving compost tea would exhibit increases in microbial biomass. The baseline covariate was identified as a significant predictor within the statistical model indicating a large degree of initial variation among plots (P=.0074). Treatment had a marginal effect (P=.1069), and to examine this effect, log response ratios were used to see how soils receiving tea treatment compared to soils under current treatment over the length of the study. Because of the initial variation among plots, a measure of relative change over time was calculated to see how soils compared to each other. I calculated the mean relative change in each treatment over the experimental timeline (treatment/baseline) and then calculated the natural log of the relative change of each treatment compared to the baseline (ln[relative change of treatment/relative change of current treatment]).

The compost tea treatments/current treatment ratios are both less than zero, indicating that they had overall decreases in microbial biomass compared to current treatment (Figure 3a). Looking at averages in microbial biomass over time (Figure 3b), one can see there are evident fluctuations associated with sampling round, and round was identified as the most significant predictor of microbial biomass (P<.0001).

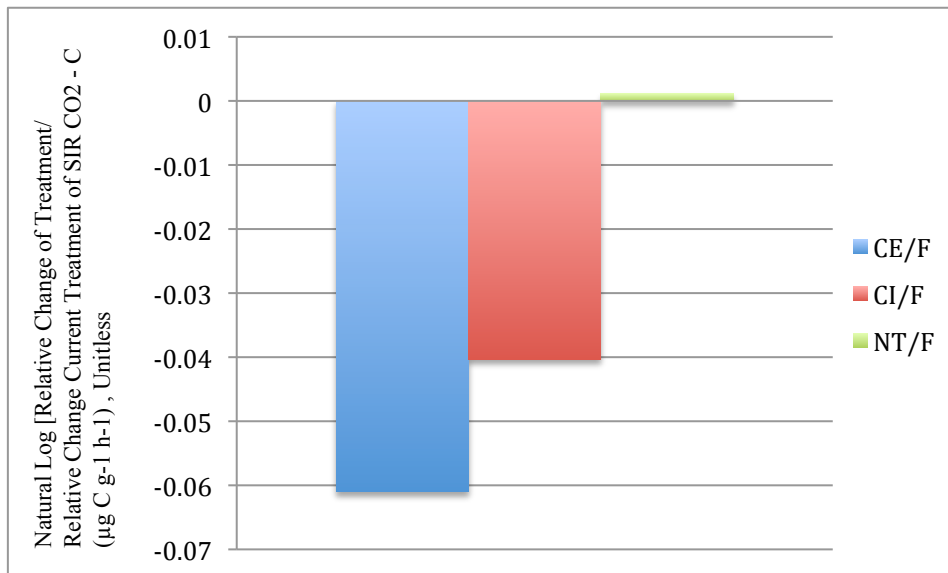


Figure 3a: Natural log Ratios of Relative Change of Treatment/Relative Change Current Treatment of SIR CO<sub>2</sub> - C ( $\mu\text{g C g}^{-1} \text{h}^{-1}$ ) over time; CE is the efficient application of tea every 6 weeks; CI is the intense application of tea every 2 weeks; NT is no treatment; and F is fertilized plots under current treatment. CE experienced a 6.5% decrease in microbial biomass as compared to current treatment while CI experienced a 4% decrease.

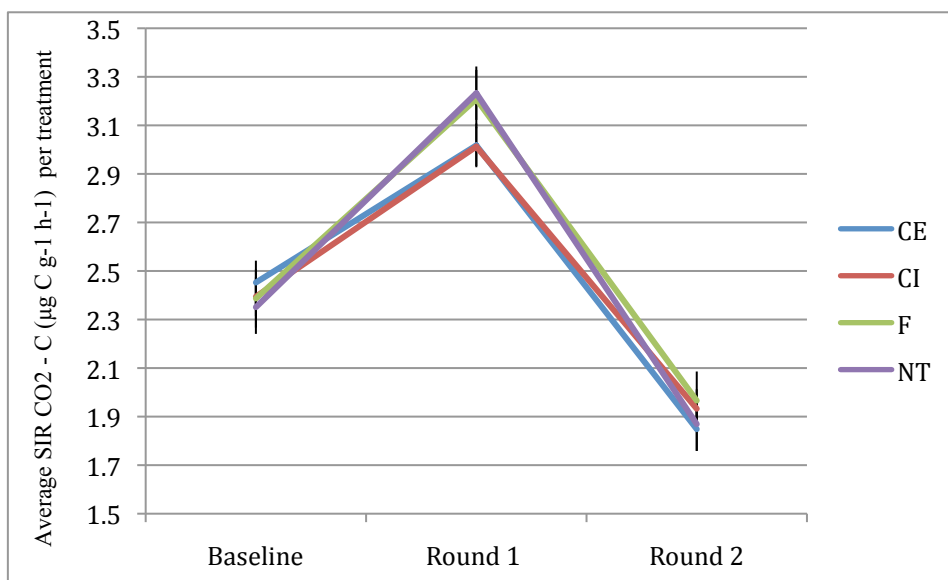


Figure 3b: Mean average SIR CO<sub>2</sub> - C ( $\mu\text{g C g}^{-1} \text{h}^{-1}$ ) at each sampling time. Seasonal fluctuations are evident with sampling taking place in July, September, and October.

While my hypothesis predicted increases in microbial biomass in tea-amended soils, the results demonstrate the opposite effect. Does this mean that compost tea is ineffective at increasing microbial activity in the soil? Not necessarily. These results signify that the compost tea amendment is transforming the dynamics of the microbial communities within the soil. Soil communities contain complex suites of microorganisms that respond differently to additions of organic matter (Fontaine, Mariotti et al. 2003). There are r-strategists that quickly grow and consume fresh organic matter (FOM) upon its addition to the soil; and k-strategists, slower-growing microbes that live on more recalcitrant soil organic matter (Fontaine, Mariotti et al. 2003; Fierer, Bradford et al. 2007). In theory, soils with low resource availability have greater relative abundance of k-strategists, as these microorganisms can outcompete the r-strategists that need inputs of FOM to remain active. With the introduction of FOM into the soil, the relative abundance of k-strategist



populations has been shown to decline as conditions turn to favor faster growing r-strategists (Fierer, Bradford et al. 2007).

The addition of compost tea potentially stimulates populations of r-strategists that outcompete k-strategists for organic food sources; these dynamic cycles may provide an explanation for the reduced microbial biomass measured in the lab. The FOM addition leads to changes in microbial community structure that enable enhanced activity and growth of r-strategists while the k-strategists can't compete.

Long-term, we'd expect an increase in microbial biomass as the build-up of soil organic matter continues. With consistent applications of organic material through compost tea application, populations of r-strategists would have a healthy supply of labile carbon compounds while more recalcitrant soil organic matter would also increase, which would provide a food source for k-strategists as well (Fontaine, Mariotti et al. 2003; Nendel and Reuter 2007).

Studies analyzing the effect of compost addition on microbial biomass generally encompass years of application and analysis after which increases in microbial biomass over time are recorded (Leita, De Nobili et al. 1999; Nendel and Reuter 2007). This study, after just 12 weeks of treatment, provides an early look into the dynamics of shifting soil communities under differing treatments.

#### *Catabolic Evenness*

There's a significant amount of variation in microbial diversity and function across Yale's landscape. Performing CRP and analyzing results demonstrates that microbial

communities differ greatly across space. This seems to suggest that different landscape practices across campus have significant effects on the catabolic functioning of microbial communities. Figure 4 shows how soil communities within the 12 research plots around campus cluster differently according to their function.

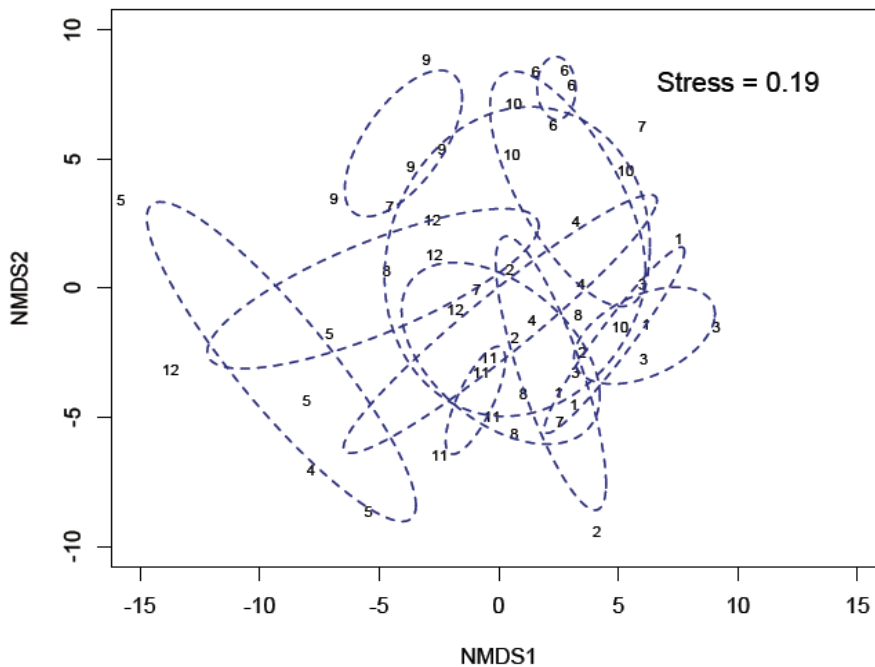


Figure 4: NMDS plot depicting how plots functionally differentiate across campus. The graph is a visual representation of a distance matrix that used CRP proportional respiration values to analyze functional similarities across all experimental plots. Numbers represent plot locations. Plots 1-4 are grouped at Marsh Botanical Garden; 5 & 6 are located at Kroon Hall; 7 & 8 are located along Hillhouse Ave; 9 & 10 are located along Grove St; 11 & 12 are located within Old Campus.

CRP is a measure of the functional diversity of microbial populations. Soils with high levels of functional diversity have correspondingly high

levels of catabolic evenness; a high level of evenness is linked with higher levels of soil carbon (Degens, Schipper et al. 2000). Under my hypothesis, compost tea additions would add organic matter to the soil thereby increasing catabolic evenness. Catabolic evenness was measured using the Simpson-Yule index,  $E = 1/\text{SUM}(p_i^2)$ , where  $p_i$  equals the response of the soil to an individual substrate as a proportion of total respiration activity across all the substrates (Degens 1998). For this experiment, the maximum evenness was 10.

There was a significant interaction between treatment and round on catabolic evenness ( $P = .0428$ ). Again, the covariate was a significant effect in the model, indicating a large degree of initial variation among plots. I used log response ratios again to understand how compost tea treatments relate to current treatment over the length of the experimental timeline (Figure 5a,b). Separate log response ratios for each experimental round were calculated due to the significance of interaction between treatment and round. The ratios, all negative, indicate that soils receiving compost tea experienced relative decreases in catabolic evenness when compared to relative changes of current treatment. To see how changes in evenness relate to each other across time, the average catabolic evenness at each sampling point for each treatment was calculated and plotted (Figure 5).

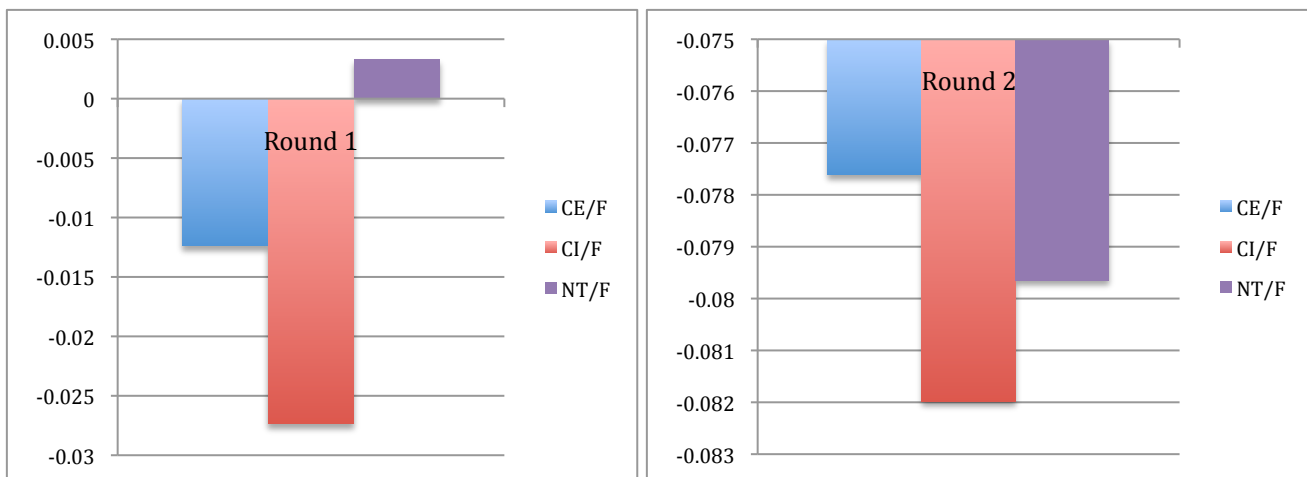


Figure 5a,b: Log response ratio of Relative change of catabolic evenness for treatments/current treatment. Two separate graphs are necessary because of a significant interaction between treatment and time. Figure 5a represents changes after 6 weeks of treatment; Figure 5b represents changes after 12 weeks of treatment. Note: Graphs are natural log ratios and therefore unitless.

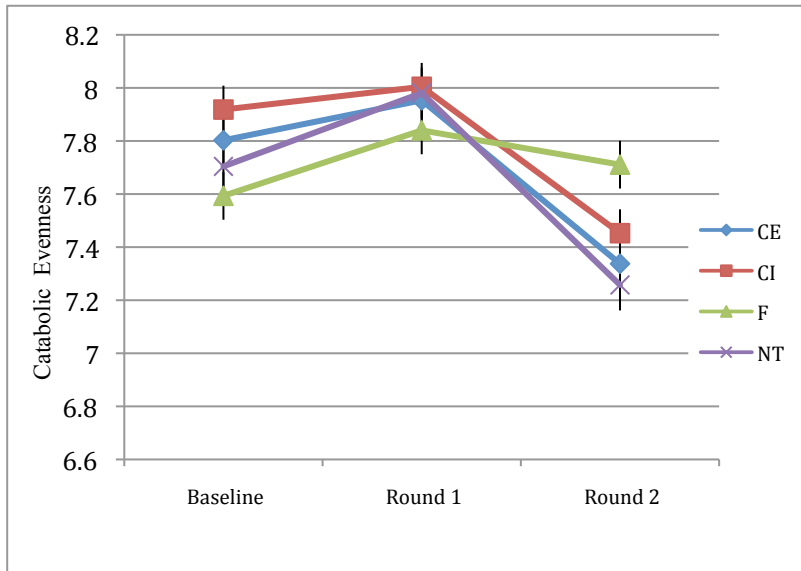


Figure 5c: Mean average catabolic evenness for each treatment over the length of the treatment timeline; fluctuations with round of sampling are evident.

Compost tea treatment, when coupled with sampling round, has a significant effect on soil functional diversity. The original hypothesis was based on the premise that high levels of catabolic evenness are associated with soils high in organic matter content. Soils receiving application of compost tea exhibited a shift in catabolic evenness, however, not in the direction hypothesized.

As a potential explanatory factor for shifts in microbial biomass and catabolic evenness, changes in pH were analyzed across time. An initial increase in hydrogen ion concentration after the six weeks of treatment might have constituted a perturbation to soils that resulted in decreases in biomass and evenness; however, after 12 weeks there is a resulting decline in H<sup>+</sup> ion concentration to similar levels of those soils under current treatment (Figure 6).

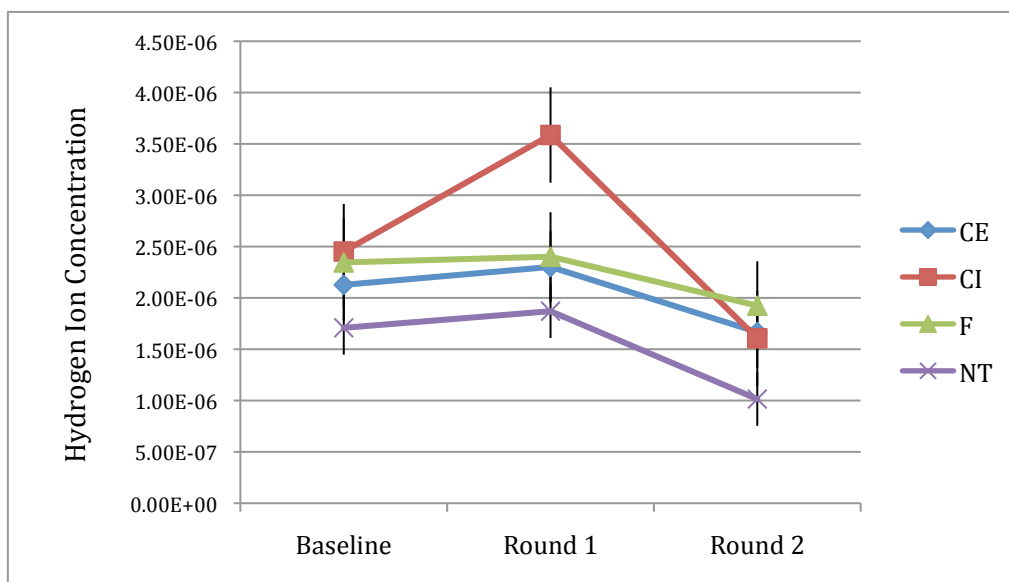


Figure 6: Mean average hydrogen ion concentrations across the treatment timeline. The compost intense application initially increased H<sup>+</sup> ion concentrations; however, after 12 weeks concentrations decreased to similar levels of those in plots under current treatment.

An early shift in microbial functional diversity may also be explained by shifts in community structure resulting from periodic doses of the tea amendment. As mentioned above, resource availability can impact the structure of microbial populations (Fontaine, Mariotti et al. 2003; Fierer, Bradford et al. 2007). A decrease in catabolic evenness may be a result of shifting abundances of r-strategists and k-strategists.

#### *The Future of Alternative Land Management at Yale*

These results provide interesting insight into the shifts microbial communities undergo in response to alternative treatments. Although different from original expectations, these results may shed light on the common critique of organic land management programs: They take a significant amount of time to actually see effects on soil biological and physical characteristics. Should the university maintain this experiment and continue tea applications across the length of a whole growing season, it will be interesting to see if there are changes in microbial biomass and catabolic evenness that comply with initial hypotheses.

This study provided an important step in directing the university towards the importance of incorporating ecology into its land management program. After twelve weeks of compost tea treatment, there are evident responses among the microbial community, however, it's too early to draw conclusions about the efficacy of the compost tea treatment.

#### *Feasibility*

With the uncertain future of alternative land management at Yale, it's important to consider the feasibility of adopting new practices. Compost tea application across the university would require significant investments in infrastructure and manpower. Current fertilizer consists of dry pelletized material that can be spread with a few passes of a broadcast sprayer that covers widths of approximately 25 feet per pass. Compost tea application on a large scale would require spreaders that can handle a wet fertilizer. Spreading compost tea would require increased manpower, as it simply can't be broadcast as far as a dry fertilizer, requiring more passes per application.

Additionally, increased maintenance concerns may arise with the use of compost tea. The tea is more apt to clog spreaders, and sufficient cleaning after application is required to prevent clogging and build-up. Making the compost tea is an intensive process: It takes twenty-four hours to brew and must be used within four hours before the liquid turns anaerobic (Ingham 2000). From a management perspective, these issues are critical to consider in implementing a new management plan.

#### **Conclusion**

We hear the word "sustainability" thrown around environmental discourse without knowing *exactly* what the word is referring to. What is the scientific basis behind claims promoting more sustainable initiatives? This project put rigorous scientific research behind Yale's decision to take a more ecological approach to its landscaping program.

The project is one that not only has implications for Yale's own operations, but also for

the education of faculty, staff, students and the greater New Haven community. Research projects within the public realm provide ample opportunity for public education. This alternative land management project has been coupled with an educational campaign that utilizes digital media to inform the public about the project and why such research is important to environmental health. A website is currently in development as a part of Yale's "Campus as a Living Laboratory" Initiative (<http://sustainability.yale.edu/compost-tea-study>).

The website will provide information about the research conducted on Yale's campus, allowing visitors to interact with graphics that will help them understand the dynamics occurring in the soil. The website aims to encourage public discourse and understanding of this research, and an effort will be made to educate people about the exact parameters measured and how these parameters provide an index for urban ecosystem health. In this way, the scientific results will be disseminated to a broad audience that will help those outside the scientific research community understand the important role that soil microbes play in urban ecosystem health.

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