The Effects of Forest Fragmentation on Microbial Communities and Substrate Metabolism at the Pierce Cedar Creek Institute in Southern Michigan

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Abstract

The potential for detrimental effects on forest health due to forest fragmentation is a growing, global concern. An understanding of the interactions between forest boundaries and forest interiors are imperative for setting ecologically sound land management strategies and preserving healthy forest ecosystems. Though a large body of research has been conducted on forest edge effects at the vegetational level, relatively little has been investigated at the microbial level. Employing permanent plot protocols set forth by EREN (Ecological Research as Education Network) for edge effect studies, we assessed data on various abiotic variables, conducted surveys of vegetational growth, and measured soil microorganism metabolic activity to determine microbial functional diversity. Our results indicated that Pierce Cedar Creek Institute's land use history played a larger role in influencing the ecosystem than the difference between edge and interior. We also found that invasive species presence significantly affected microorganisms' ability to metabolize specific soil substrates, often negatively so.

Introduction

Forest fragmentation due to human development is an increasing concern (Wade et al. 2003). Forest fragmentation exponentially increases the forest edge area exposed to human development, which in turn threatens the balance of once-stable habitats (Fahrig 2003). Forest edges, which are borders between continuous canopy and non-forest environment, crucially buffer the flow of incoming nutrients and organisms and provide a layer of stability for the forest interior. These patterns of nutrient flow and microbial activity along forest edges may serve as useful predictors of forest health

Forest edges are defined as borders between forest and non-forest environment. Forest composition differs between the interior and edge habitats at multiple levels, typically favoring greater vegetational richness at edges (Casenave et al. 1995, Guirado et al. 2006), and more variance in edge microclimate (Chen et al. 1993). Further, before any incoming biotic or abiotic factor (e.g., invasive species, weather system) can affect a forest interior, it first must pass through the forest edge. Consequently, the nature of forest edges influences the response of the forest interior to any given factor; in particular, edge structure of forest fragments determines forest microclimate and vegetational composition (Didham & Lawton 1999, Young & Mitchell 1994, Chen et al. 1993). However, relatively little is known about the differences in microbial communities between forest edge and interior soils. We do know that individual soil variables such as moisture or pH differ between the two (Riutta et al. 2012, Murcia 1995), and we also know that microorganisms play a role in determining vegetational forest health and diversity (Marcel et al. 2007), but more research is required to determine the nature of the different

interactions between individual soil variables, microbial communities, and vegetational diversity at forest edges and interiors, especially if such knowledge is to be employed effectively across broadly varying forest habitat types.

Microbial communities and consequent soil health can be efficiently and effectively monitored through measuring functional diversity (Van Bruggen & Semenov 2000, Epelde et al. 2008). This does not involve identifying every microorganism species, but rather investigates the entire community's ability to metabolize varying substrates. This collective soil enzyme activity indicates the overall functionality of microbial communities in regards to litter decomposition and other soil variables, and has been employed as a useful tool to examine the health of particular ecosystems and their response to different stressors (Bending et al. 2002).

We predicted that forest edge sites would exhibit greater microbial functional diversity than interior habitats, given that plant biodiversity is often greater on edges than interior locations (Wiens 1976), and microbial activity is often a regulator of and driving force behind vegetational biodiversity. (Marcel et al. 2007). We also expected to see correlated differences between other biotic and abiotic variables, depending on their edge and interior locations. These variables include (but are not limited to) soil moisture, pH, light, and canopy cover. (Guirado et al. 2006, Chen et al. 1993, Murcia 1995).

Methods

Site Description

Soil sample and plant data was collected at the Pierce Cedar Creek Institute property in Barry County, Michigan (USA). First settled in the 1850's, the property was used primarily for agriculture and timber. After its purchase in the late 1960's, owner Lou Batts allowed natural succession in many locations on the property. Autumn olive, an invasive species, is speculated to have been planted around the property in the 1970's. The soils in our areas of study fit the Alfisol profile, being composed of Haplic Glossudalfs and Typic Hapludalf.

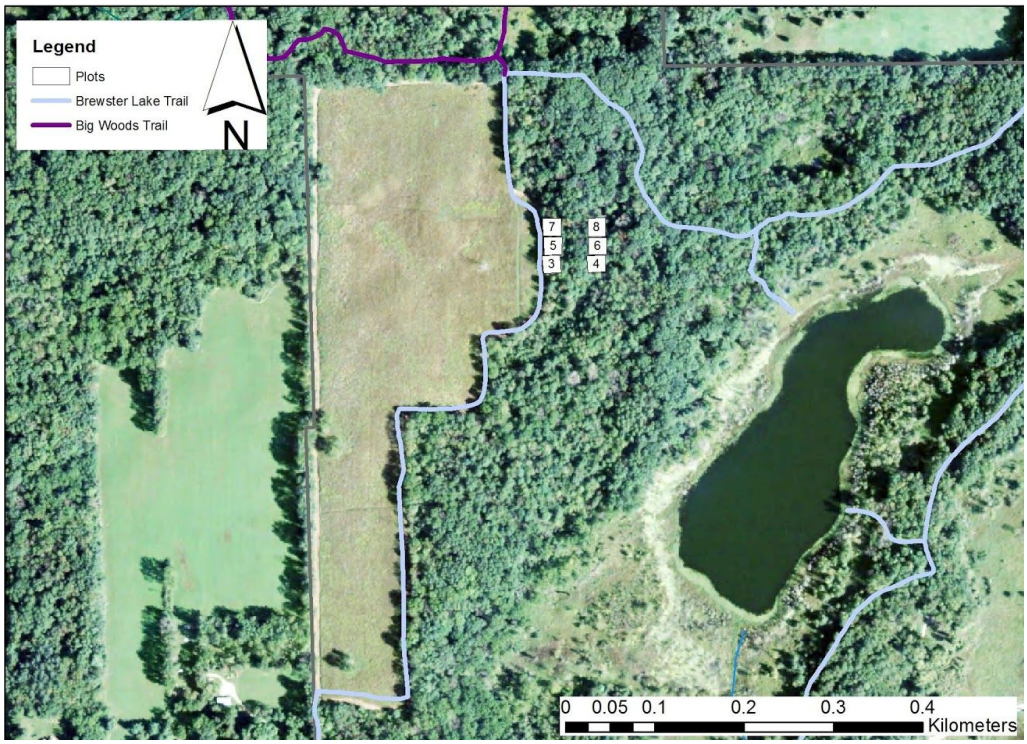


Figure 1. North PCCI Property Site Locations. Sites 2-4 are pictured, with numbered squares on the map designating individual edge and interior plots at each site.

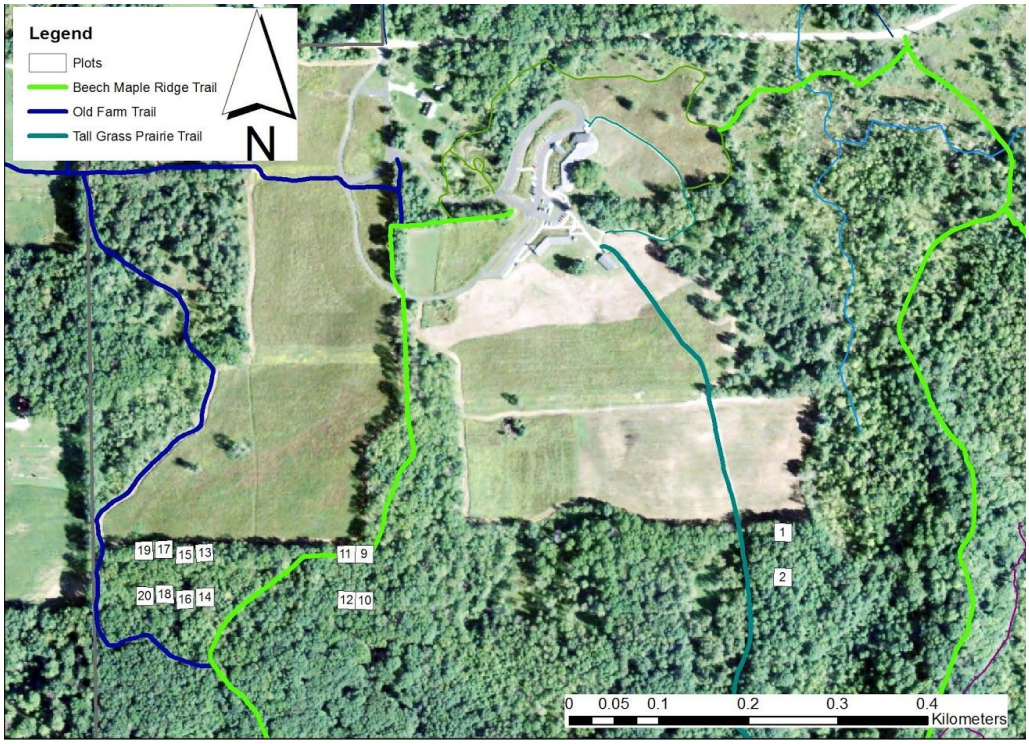


Figure 2. South PCCI Property Site Locations. Sites 1 and 5-10 are pictured, with numbered squares on the map designating individual edge and interior plots at each site.

According to EREN protocol, permanent plots were placed in various forested locations which bordered prairie previously used for farmland (Figures 1 & 2). Plots 4-9 on the northern side of the property were potentially timbered in the 1920's, but were never used for agriculture. Northern plots 4-9 have a canopy dominated by *Fraxinus americana*, *Ostrya virginiana*, and *Carya* species. The southern section of the property where plots 1-2 and 10-20 are located was used for agriculture until the 1970's. Plots 1-2 show a more mature forest than plots 10-20, and prior agricultural use in that specific area is questionable. Plots 1-2 have a canopy dominated by *Acer saccharum, Fagus grandifolia*, and *Quercus* species.

EREN Plots

Using the EREN (Ecological Research as Education Network) Forest Edge permanent plot protocol, 10 permanent plot sites were deployed at Pierce Cedar Creek Institute property in Hastings, MI in May 2017. Each site included permanent 20x20 meter edge and interior plots. Edge plots began at the forest tree line along farmland recently converted into native prairie, and continued into the forest. Interior plots were built 30 meters directly behind edge plots along a cardinal direction. Plots were permanently marked using tagged rebar stakes driven into the ground at each corner and the center. EREN protocol restrictions on plot size and distance from actively used trails inhibited the number of viable sites, and resulted in a higher representation of southern sites than northern sites.

Understory Vegetation Data Collection

Temporary 1×1 meter "miniplots" were constructed at each 20×20 meter plot's four corners and center. All species within these plots were identified and counted. Temporary 5×5 meter subplots were also constructed at each plot's four corners and center stake. Within the 5×5 meter subplots, all woody vegetation above breast height (1.37 meters) but under 2.5 centimeters (cm) in diameter at breast height (DBH) was identified and counted.

Tree Data Collection

All trees and standing dead trees above 2.5cm in diameter in each 20×20 meter plot were identified, tagged with a unique number, mapped, and measured for DBH. Canopy cover was collected using a concave densiometer at the four corner and stakes of each plot. The four stake measurements were then averaged into individual plot measurements.

Microbial and Environmental Data Collection

Soil samples were collected from the 20 plot locations on Pierce Cedar Creek Institute's property between 8am and 4pm on June 20, 2017. Three grams (g) of soil were collected using a sterile spoon within a meter of each plot stake. Spoons were sterilized using high heat treatment at Hope College, and were stored in sterile bags until use. The 3g samples from each of the five stakes per plot were aggregated for a single plot sample. This process was repeated once for each

plot, yielding 40 total 15g soil samples. Soil samples were stored in a cooler after collection until analysis or storage for future use. GPS coordinates, pH, and soil moisture content data were simultaneously collected using a Garmin Etrex 30 and Kelway Soil pH and Moisture Meter, respectively, at each stake during the sampling process.

Microbial Analysis

All large debris and root material was removed from the soil sample using sterile tweezers. Tweezers were sterilized through the same process as the aforementioned spoons. Soil was mixed thoroughly so that the stake samples were evenly distributed in the plot sample. Three grams from each of the 40 soil samples were combined with 45 mL of phosphate buffer and centrifuged for 20 minutes, settling soil granules and suspending microbes within a low turbidity buffer. The remaining 12g of soil in each sample tube were stored in an industrial freezer at 80 degrees Celcius for future physical analysis within 24 hours of collection in the field.

Prior to inoculation, any remaining debris was filtered out with a sterile cotton plug in each centrifuge tube. One-hundred microliters of the buffer and soil mixture were then pipetted into Biolog EcoPlates™ for microbial community analysis. For the next 48 hours, the plates were incubated at 25^{o}C to allow for the microbes to metabolize the plate substrates. The plates were then read at 490 nm for absorbance levels using a microplate reader at 48 hours, 72 hours, and 96 hours between incubation periods.

Pre-Analysis Data Normalization

Microbes

Microbial absorbances were initially normalized by subtracting the average absorbance of water for each individual EcoPlate™.

$$An = Ai - Aw$$

An = normalized absorbance (of Plate X)
Ai = Initial absorbance (of Plate X)
Aw = Average water absorbance (of Plate X)

Each plot's average absorbance levels (*Av*) (taken from each plot's two duplicate plates) were then used to calculate richness and Shannon's diversity index.

$$Av = SUM(An \text{ of Plate X and Plate Xd}) / \text{total wells (96 per plate x 2 = 192)}$$

X = Example Plate
Xd = Example Plate's duplicate
Av = Average normalized absorbance levels (of Plate X and duplicate, Xd)

The simple matching coefficient (SSM) was calculated using the formula described in Mulcahy and Edenborn et al. (2007):

$$Simple\ Matching\ Coefficient\ (SSM) = (a + d)/(a+b+c+d)$$

where:
a = Number of carbon sources used by both sample A and sample B
b = Number of carbon sources used by Sample B but not by Sample A
c = Number of carbon sources used by Sample A but not by Sample B
d = Number of carbon sources not used by bacteria in either sample

This test was used to compare the community similarity between edge and interior plots. For correlation analysis, the average normalized absorbance for each individual plot was used.

Vegetation

Tree diversity, miniplot diversity, and subplot diversity variables were calculated per plot using Shannon's diversity index. Trunk area was calculated using tree diameter readings, and totaled for each individual plot. Snags, or standing dead trees, were calculated as a percentage of the total number of trees in each plot. Invasive percentages included the total *Elaeagnus umbellata* and *Rosa multiflora* as a fraction of the total number of individuals in miniplots and subplots.

Correlation Analysis

Vegetation, microbial, and soil characteristics were normalized and initially checked for collinearity using the Spearman's rank correlation and the Hmisc package (R version 3.3.2). Tree variables included tree diversity, percent snags, total number of trees, trunk area, and canopy cover. Vegetation variables included miniplot and subplot diversity and richness. Invasive variables included number of *Rosa multiflora* in subplots and miniplots, number of *Elaeagnus umbellata* in subplots and miniplots, percent of invasives in subplots and miniplots, and number of *Persicaria virginiana* , a species which was observed to create monocultures in the field. Soil and environmental variables included in the correlational analysis were elevation, soil moisture, and soil pH.

T-*test Analysis*

All values used in the correlation analysis were analysed using an unequal variances welch two-sample *t*-test among variables and between edge and interior plot treatments.

Individual Substrate Analysis

Absorbance values by individual substrates were analysed using an independent *t*-test for edge and interior treatments. Variables which were significantly different between edge and interior plots or with another variable which had this attribute were analysed using Spearman's

correlation with individual substrate absorbance levels. Variables included tree total, trunk area, tree richness, canopy cover, subplot diversity, subplot and miniplot percentage of invasives, soil pH, and soil moisture. Subplot richness and miniplot percent invasives were tested but did not contain any significant substrates. Variables shown in results are only included if more than one substrate shows a significant correlation ($p<0.05$).

NMDS

Using the metaMDS function in the Vegan package (R version 3.3.2), non-metric multidimensional scaling ordination was used to compare each plot's normalized microbial absorbances to variables including tree total, trunk area, tree richness, canopy cover, subplot diversity, and percent subplot invasive species. These variables were chosen for the same reason as the individual substrate analysis, and for comparison between absorbances of individual substrate trends and mean absorbances of each plot overall.

Results

Microbial Variables

Community similarity as defined in Mulcahy et al. (2007) between edge and interior plots were high according to the simple matching coefficient (SSM = 0.933), where a value of 1.0 indicates identical community "fingerprints" (Mulcahy et al. 2007).

Correlation Analysis

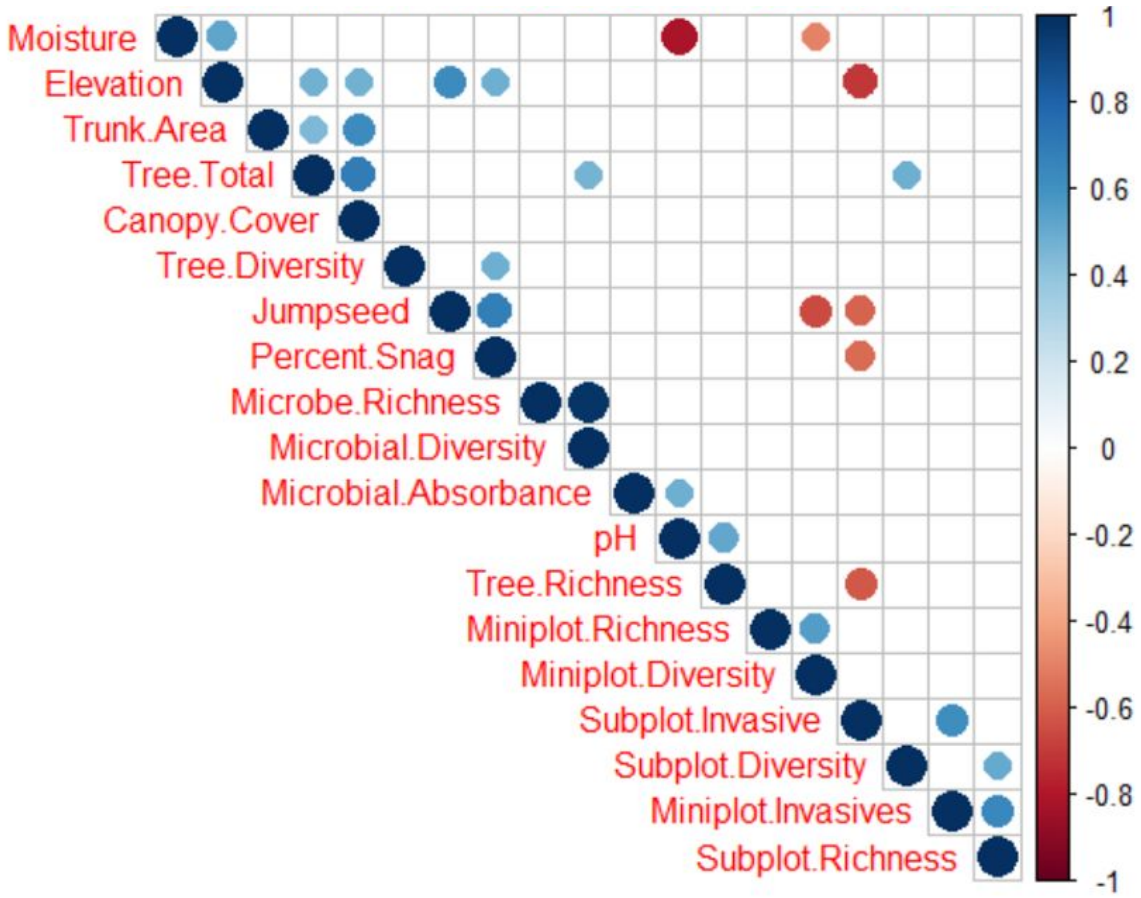
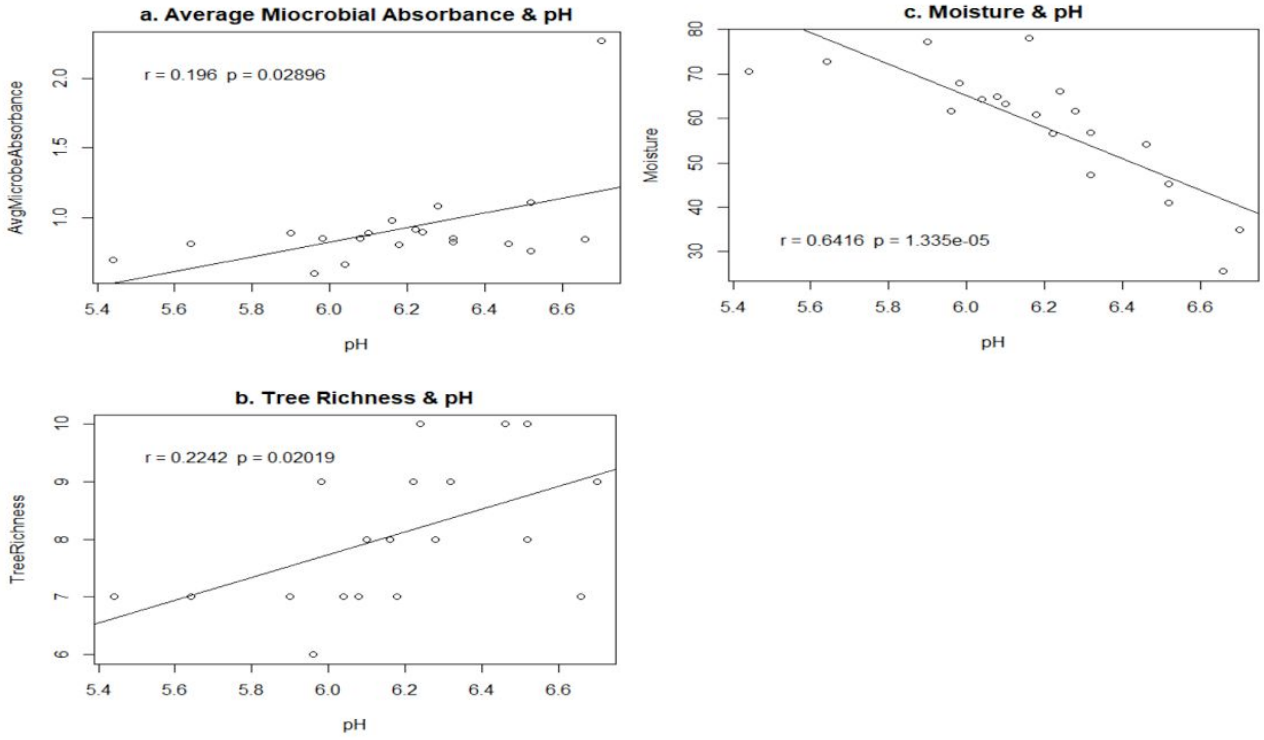


Figure 3: Correlation plot comparing various microbial, environmental, and vegetation variables. Each variable has a dot indicating where $p < 0.05$, with larger dots indicating the most significant p-values. Red to orange colors represent negative correlations and blue represents positive correlations. The greater the correlation, the darker and larger the dot indicator.

Microbial absorbance was positively correlated with pH ($r = 0.488$, $P = 0.029$). pH in turn is positively correlated with tree richness ($r = 0.515$, $P = 0.020$), and negatively correlated with moisture ($r = -0.813$, $P < 0.0001$) (Figure 4a-c).



Figures 4 (a-c). Significant linear regression correlations of overall microbial metabolic activity (a), tree richness (b), and moisture (c) with soil pH.

Microbial functional diversity was positively correlated with tree total per plot (r=0.465, *P*=0.039). Tree total in turn was positively correlated with elevation (r=0.479, *P*=0.032), trunk area (r=0.449, *P*=0.047) , canopy cover (r=0.697, *P*<0.001), and subplot diversity (r=0.484, *P*=0.031) (Figure 5a-e)

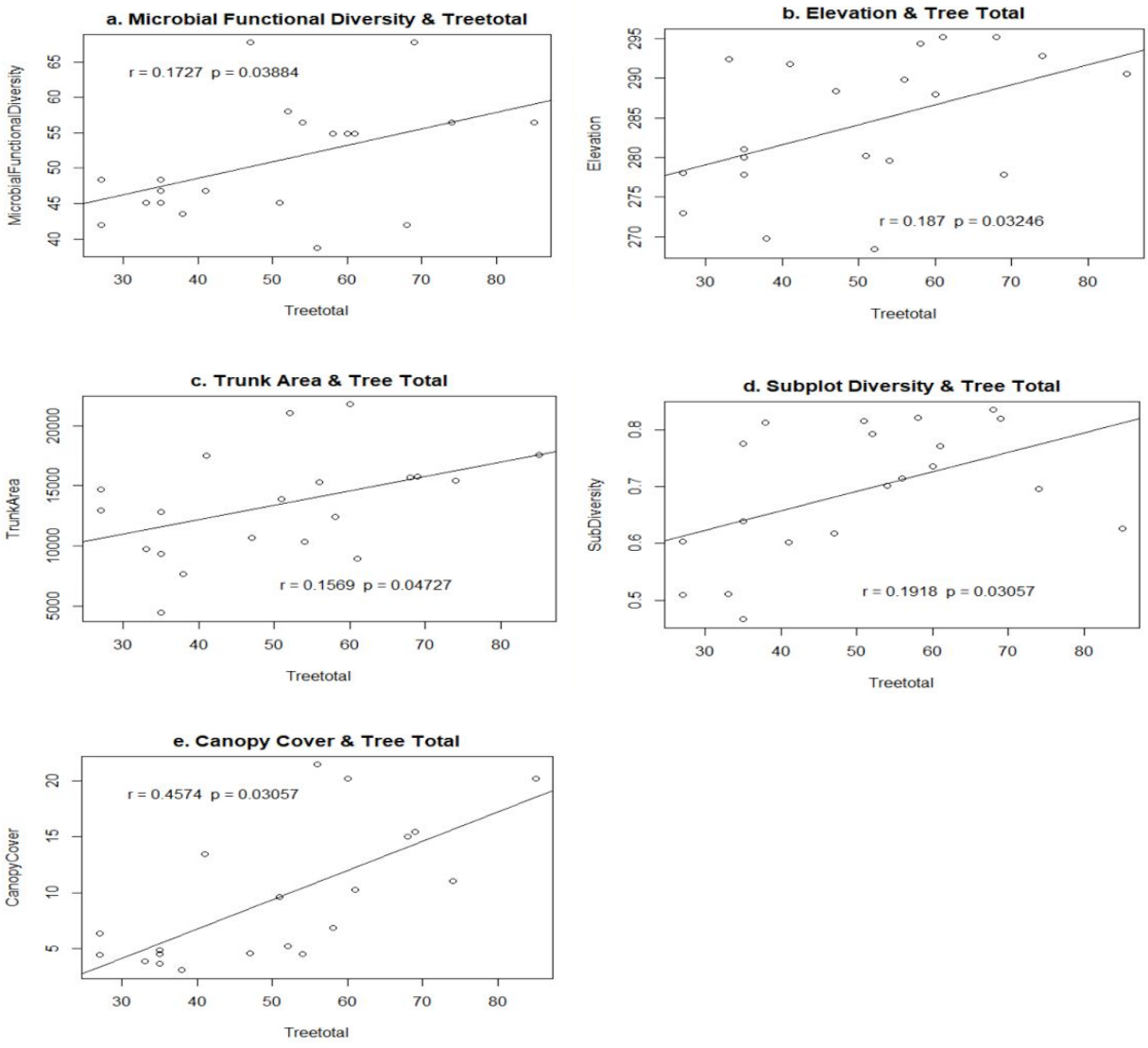
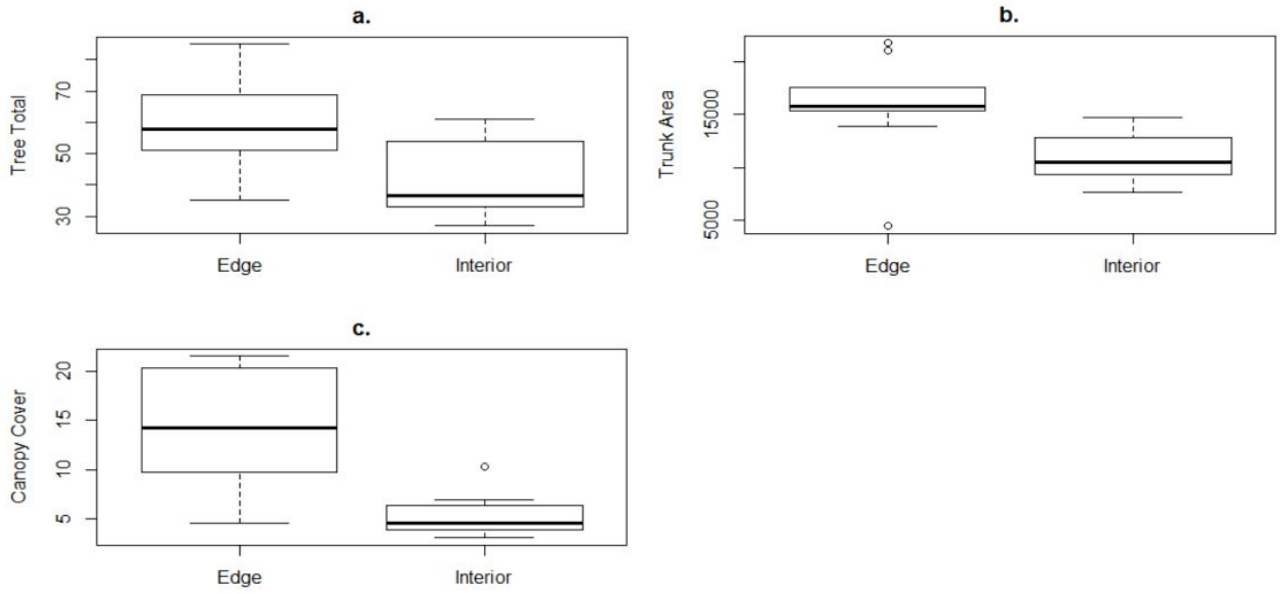


Figure 5 (a-e). Significant linear regression correlations of total tree count with microbial diversity (a), elevation (b), total trunk area (c), subplot vegetational diversity (d), and canopy cover (e).

Edge and Interior T-*tests: Overall Edge and Interior Comparisons*

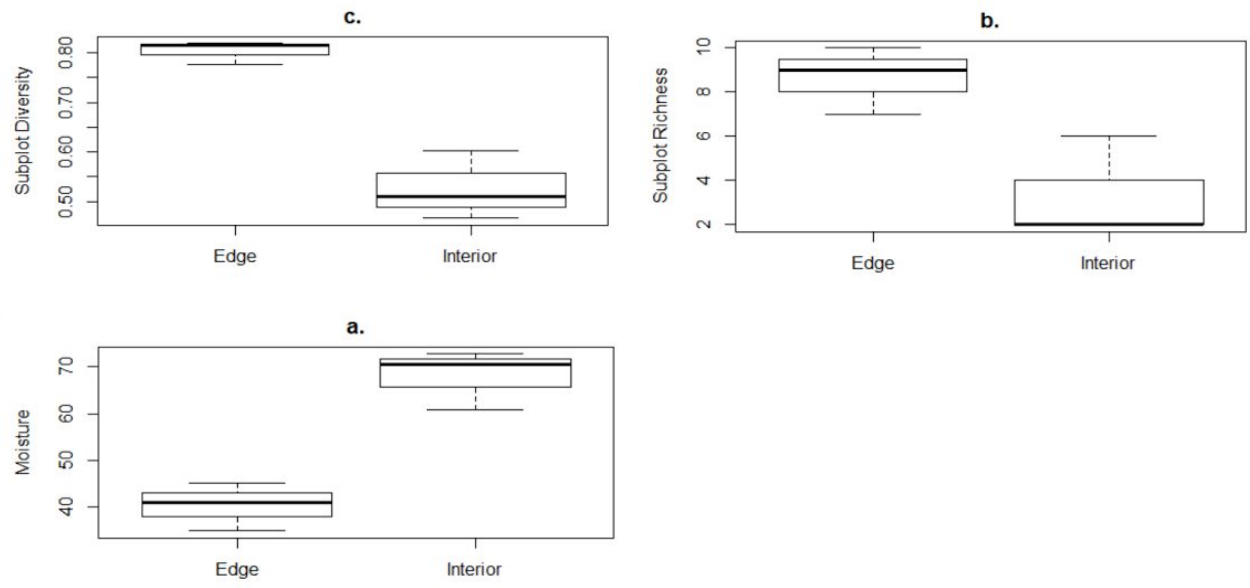
Three variables differed significantly between edge and interior treatments. Tree total (t = 2.8089, df = 17.364, P = 0.012), total trunk area (t = 2.9629, df = 12.663, P = 0.011), and canopy cover (t = 4.1355, df = 11.125, P = 0.0016) variables were higher in edge compared to interior plots (Figure 6a-c)



Figures 6 (a-c). Boxplot comparisons of total tree count (a), total trunk area (b), and canopy density (c) between edge and interior test groups. Bold lines represent variables' median. All were higher along the forest edge ($P<0.05$).

Edge and Interior T-*tests: Northern Site Edge and Interior Comparisons*

Northern edge and interior plots were compared separately from southern sites. The differences in forest composition and management history between northern and southern sites presented potential for distinct interactions. Northern plots were defined by its separate location on the north side of Cloverdale Road and their management history. Moisture content was significantly higher in interior plot treatments (t = -5.8503, df = 3.8207, P = 0.0049). Subplot richness (t = 3.3362, df = 3.4689, P = 0.036) and subplot diversity (t = 6.4986, df = 2.467, P = 0.013) were significantly higher in edge plots than interior (Figure 7a-c).



Figures 7 (a-c). Boxplot comparisons of moisture (a), subplot vegetation richness (b), and subplot vegetational diversity (c) between edge and interior test groups (in northern sites only). Moisture was higher in the forest interior ($P<0.01$), while diversity and richness were higher along the forest edge ($P<0.05$).

Edge and Interior T-*tests: Southern Site Edge and Interior Comparisons*

Southern edge and interior plots were also compared separately from northern sites. Southern plots were defined by its separate location on the south side of Cloverdale Road and differing management history. Tree total ($t = 2.2356$, df = 11.332, $P = 0.046$), total trunk area ($t = 6.8379$, df = 9.4044, $P = 6.12e-05$), and canopy cover ($t = 4.0686$, df = 7.9444, $P = 0.0036$) variables were significantly higher in edge plot treatments. These trends were also evident in analyses which included both northern and southern sites.

Individual Substrate Analysis

Individual substrate analysis according to edge and interior plot treatments was performed using an unequal variances *t*-test. Five substrates contained higher absorbances in edge plots and 7 substrates contained higher absorbances in interior plot treatments (Table 1).

Further individual substrate analysis was performed using Spearman's rank correlation test. Of the 15 significantly correlated substrates, 13 were positively correlated with tree variables, and only two were negatively correlated (D-Mannitol and Glycyl-L Glutamic Acid) (Table 2). pH was correlated positively with 11 substrates and negatively with 8 substrates (Table 3). Moisture was positively correlated with 6 substrates and negatively correlated with 10 substrates (Table 3). Out of 19 total significant substrates, 15 had opposite correlations with pH and moisture (Table

3). Significant substrates were evenly split between positive and negative correlations with subplot diversity (Table 4). Out of 12 substrates, 11 were negatively correlated with percent invasive species in subplots and miniplots combined (Table 5).

Table 1. Substrates whose absorbance values varied significantly according to edge or interior plot locations. Higher mean in each E-I pair is indicated by a grey cell.

Substrate	Edge Mean	Interior Mean
4-Hydroxy Benzoic Acid	1.4144902	0.9856491
D,L-Glycerol Phosphate	0.1018431	0.4500175
D-Cellobiose	1.903039	1.499596
D-Galactonic Acid Lactone	0.8897451	1.3909649
D-Mannitol	0.6938627	1.2725088
D-Xylose	1.0374902	0.5362632
Glycyl-L Glutamic Acid	-0.379451	0.09082456
i-Erythritol	0.6448431	0.1461228
L-Arginine	2.093294	1.499158
L-Asparagine	1.426392	1.991246
L-Phenylalanine	0.62015686	0.07114035
L-Serine	0.967902	1.485105

Table 2. Significantly correlated substrate absorbances with tree variables.

Substrate	Tree Total	Trunk Area	Tree Richness	Canopy Cover
Cyclodextrin				+
D-Lactose	+	+		
4-Hydroxy Benzoic Acid	+			
D-Cellobiose		+		
D-Galacturonic Acid			+	
D-Malic Acid			+	
D-Mannitol		-		
Glucose-1-Phosphate	+			
Glycyl-L Glutamic Acid			-	
Itaconic Acid	+			
L-Arginine	+	+	+	
L-Asparagine			+	
Phenylethylamine			+	
Pyruvic Acid Methyl Ester			+	
Tween 40	+			

Table 3. Significantly correlated substrate absorbances with soil pH and moisture.

Substrate	pH	Moisture
?-D-Lactose	+	
?-Hydroxybutyric Acid	+	
D,L-?-Glycerol Phosphate	-	-
D-Cellobiose	+	-
D-Galacturonic Acid	+	-
D-Glucosaminic Acid	-	+
D-Malic Acid	+	-
D-Mannitol	-	+
D-Xylose	+	-
L-Phenylalanine	+	-
L-Serine	-	+
L-Threonine	+	-
N-Acetyl-D-Glucosamine	-	+
Phenylethylamine	+	-
Putrescine	-	+
Pyruvic Acid Methyl Ester	+	-
Tween 40	-	+
Tween 80	+	-

Table 4. Significantly correlated substrate absorbances with subplot diversity.

Substrate	Subplot Diversity
?-Cyclodextrin	+
2-Hydroxy Benzoic Acid	+
D-Mannitol	-
D-Xylose	+
Glycyl-L Glutamic Acid	-
i-Erythritol	+
Itaconic Acid	-
L-Asparagine	-
L-Phenylalanine	+
L-Serine	-

Table 5. Significantly correlated substrate absorbances with percent invasive species in subplots and miniplots.

Substrate	Subplot Invasive	Miniplot Invasive
D-Lactose	-	
Hydroxybutyric Acid	-	
D,L-Glycerol Phosphate	-	
D-Glucosaminic Acid	-	-
D-Malic Acid	-	
D-Mannitol	+	
Glycyl-L Glutamic Acid	-	
Itaconic Acid	-	
L-Arginine	-	
L-Asparagine		-
N-Acetyl-D-Glucosamine		-
Putrescine	-	-

NMDS Analysis

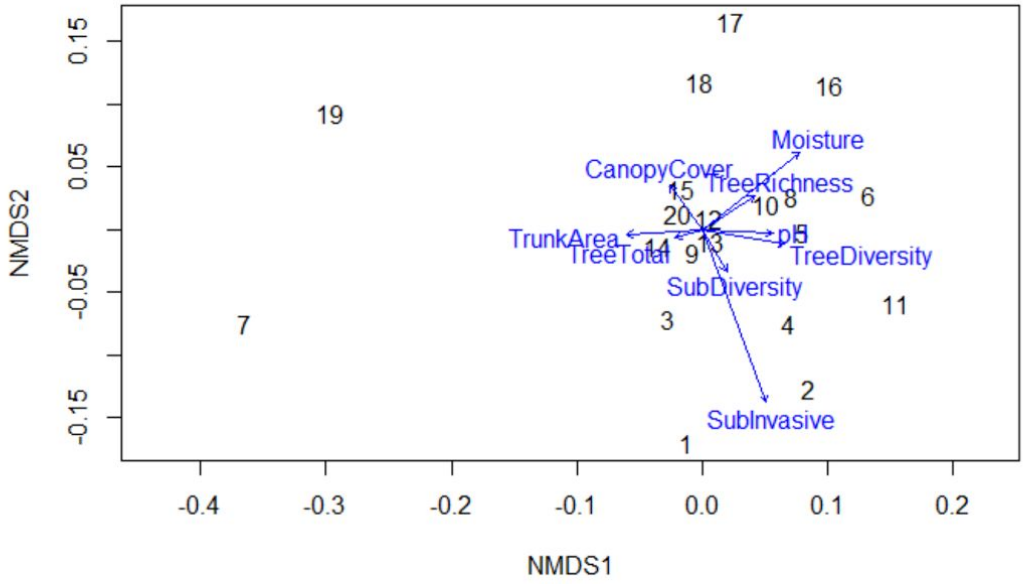


Figure 8: NMDS ordination of tree variables, subplot variables, pH, and soil moisture. Odd numbers denote edge plots, and even numbers denote interior plots.

Mean microbial absorbance per plot (indicated by number) against tree variables, invasive percentages, and soil variables were plotted using non-metric multidimensional scaling (Figure 8). Little clustering is evident around particular variables, but the greatest density of plots occurs at the intersection of all variables save for the percent of invasive species in subplots.

Discussion

The combined NMDS ordination, correlation, and t-test analyses among habitat variables, edge and interior treatments, and microbial communities do not directly support the hypothesis that microbial and plant diversity are uniformly higher along forest edges. The varied forest types and land use histories across PCCI's grounds appear to complicate the comparison between forest edges and interiors at our chosen sites.

Edge and Interior Plots

Our primary question was whether there were significant differences in environmental and microbial aspects between the forest edge and interior. Overall, three variables were significantly higher in edge plots, all of them vegetational: tree total, total trunk area, and canopy cover (Table 6). Southern and northern sites were compared for significant factors, due to their different management histories (mapped in Fig. 1 and Fig. 2). While the northern site locations were potentially timbered in the 1860's and again in the 1920's, they were not used for agriculture. In contrast, much of the forest housing our southern sites was only allowed to regrow from agricultural property after the 1970's. Southern sites also had greater tree densities, greater total trunk areas, and denser canopy cover in edge plot treatments. This is a logical conclusion, given that the southern forests were relatively young and had not yet reached the canopy breakup phase described in late-successional forest stands (Spies & Franklin 1988). The higher forest interior moisture content, greater herbaceous diversity, and greater herbaceous richness found at the northern sites are all consistent with prior research findings that mature forests tend to have higher moisture and vascular plant diversity (Qian et al. 1997). Due to the restrictions imposed by EREN protocols about plot size and distance from actively used trails, we were unable to have an equal representation from northern and southern sites. Therefore, equivalent t-test results of southern sites and overall sites are likely due to the bias introduced by the greater sampling in the southern forest. This bias was reflected in our statistical analysis as northern and southern site differences were not initially recognized and accounted for. It is highly recommended that future analysis of these results should make statistical amendments to prevent this bias.

Table 6. Summary Table of Edge and Interior Comparisons

Edge vs. Interior	
Combined and Southern Plots	
Larger Edge Mean	Tree Total
Larger Edge Mean	Total Trunk Area
Larger Edge Mean	Canopy Cover
Northern Plots	
Larger Edge Mean	Subplot Diversity
Larger Edge Mean	Subplot Richness
Larger Interior Mean	Moisture

Looking more specifically into the interactions among microbial, soil, and plant variables, our results revealed dynamic correlations and further environmental inferences.

Table 7. Summary of Environmental Correlations

Environmental Correlations	
Microbial Absorbance	
correlation (+) with	**pH**
pH	
correlation (+) with	Tree Richness
correlation (-) with	Moisture
Microbial Functional Diversity	
correlation (+) with	Tree total
Tree Total	
correlation (+) with	Elevation
correlation (+) with	Trunk Area
correlation (+) with	Canopy Cover
correlation (+) with	Subplot Diversity

Microbial Absorbance and pH

Higher total mean microbial absorbance levels per plot, indicating more effective substrate metabolization by microbial communities, were positively correlated with higher soil pH levels. Higher soil pH could indicate more functionally diverse microbial communities. Because individual substrate analysis was not plot-based (as were mean microbial absorbance levels per plot), but the data were rather aggregated for edge and interior groups, the results of these analyses are not directly comparable to each other. However, initial correlational analyses

of microbial absorbance and diversity informed us of potential factors driving individual microbial communities indicated by substrate presence. More indirect factors that could influence microbial absorbance levels include variables that significantly correlated with pH levels, namely tree richness, moisture, and percent invasive species per subplot (Table 7).

Microbial Absorbance, pH, and Invasive Species Presence

Perhaps the most intriguing result was that greater presence of invasive species correlated both with lower pH and lower microbial metabolic activity in 9 out of the 10 substrates that displayed significant correlations one way or the other. Percent invasive species in miniplots were investigated for a more inclusive investigation. All 4 substrates were negatively correlated with higher invasive species presence in miniplots. NMDS results showed very low clustering of plots (assessed using mean total absorbance levels in comparison to environmental variables) around higher percentages of invasive species in subplots. Together, these data support the possibility that higher invasive species presence, particularly that of Rosa multiflora and Elaeagnus umbellata, decreases the overall metabolic potential of microbial communities in forest soils. This is consistent with a study by Gibbons et al. (2017) that invasive plants significantly altered soil chemistry and microbial diversity, with different species of invasives effectively priming the soil for some bacterial taxa over others.

Microbial Diversity and Tree Total

Microbial diversity, an important indicator of soil health that was measured as their overall ability to metabolize multiple substrates, was only positively correlated with the total number of trees per plot. Indirect factors that could influence higher microbial diversity include variables which correlate with higher total tree numbers per plot: lower elevation, greater trunk area, greater canopy cover, and greater subplot diversity (Table 7).

All factors which are potentially influenced by edge and interior plot treatments (tree total, total trunk area, denser canopy cover, moisture content, subplot richness, and subplot diversity) were taken into consideration for further analysis. Although edge and interior plots did not show any overall significance regarding microbial absorbances or diversity, individual substrate analysis in comparison to significant variables and edge vs. interior treatments were used for a closer investigation.

Individual Substrate analysis and Environmental Factors

Table 8. Summary of Individual Substrate Results

Individual Substrate Comparisons		
	Substrates with greater mean absorbance	
Edge	5	
Interior	7	
	Positive Correlations	Negative Correlations
Tree Total	**6**	0
Trunk Area	**3**	1
Tree Richness	**6**	1
Canopy Cover	**1**	0
Total Tree Variables	**16**	2
pH	**11**	8
Moisture	6	**10**
Subplot Diversity	5	5
Subplot Invasive	1	**9**
Miniplot Invasive	0	**4**

All 6 significant substrates were positively correlated with tree total, 3 out of 4 substrates were positively correlated with trunk area, and 1 substrate was positively correlated with canopy cover. Trunk area, canopy cover, and tree total positively correlate to one another and collectively indicate higher microbial absorbances when they have higher presence in plots. Subplot diversity and moisture content however, contained more conflicting results. Greater subplot diversity was correlated to 5 substrates with higher absorbance, and 5 lower. As highlighted previously, moisture was negatively correlated with 10 out of 16 substrates. Given that edge plots overall had greater tree totals, trunk area totals, and canopy cover, it was expected that edge plots would have overall greater microbial absorbance levels. However, the varied subplot diversity and moisture correlated absorbance levels complicate edge and interior comparison results for individual substrates. Edge and interior mean absorbance levels were compared for each substrate, revealing 5 higher absorbances in edge plots than interior and 7 higher in the interior than the edge (Table 8).

Individual Substrate analysis and NMDS Ordination

These results complement the initial NMDS ordination (Figure 8). Figure 8 clearly shows a higher collection of plots surrounding the tree variables than surrounding the higher percent invasive, which is to be expected based on our individual substrate analysis. The vast majority of significant substrates with higher absorbances correlated with higher tree variables. Conversely, almost all significant substrates had lower absorbances in correlation with higher percent invasive species.

If substrates had greater absorbance, meaning they were more effectively metabolized by microbial communities, in correlation with higher tree variable values as well as edge plots (which they are overall correlated with), then we would expect odd numbered plots to collect near tree variables. However, our individual substrate analysis of significant absorbances shows an almost equal division of substrate types that are either higher in edges or higher in interior plots. This is why the NMDS graph shows a more random collection of both edge and interior plots correlating with higher tree variables.

Microbial Methodology

We have reason to believe that our soil samples accurately reflect the bacterial community structure within our plots because bacterial communities are fairly represented by soil samples ranging anywhere from 1.0-10.0 g, and likely higher (Kang & Mills 2005). Because each one of the five soil samples per plot is an accurate representation of the local microbial community, the aggregated samples should represent the plots' overall microbial community with similar accuracy, while simultaneously mitigating any potential effects of microhabitat differences.

While the Biolog EcoPlates™ were an effective tool to measure microbial metabolic activity, future studies may benefit by also employing some form of DNA analysis so that more may be postulated about specific plant and microbe interactions. The split results from the substrate-wise analysis could indicate that EREN's standard classifications of edge and interior are not well represented at Pierce Cedar Creek Institute's property. The aforementioned differences in land use history between the northern and southern sites provide the most likely explanation for the conflicting data; at both the biotic and abiotic level, forest edges and interiors on PCCI property are similar and ill-defined. It is also possible that the mature forest on the northern edge of PCCI property differs little between its edge and interior because of shifting light environments in the forest interior caused by the late-successional canopy breakup phase, a phenomenon that has been noted in prior edge effect studies (Harper et al. 2005).

Future Directions

There is potential for future forest edge research at PCCI, courtesy of the EREN Edge Effect sites now permanently installed on the institute property. The most compelling results from any study limited to PCCI grounds will likely be seen at least several years, if not decades, into the future, after forest maturity in the southern site locations increases significantly enough to be directly comparable to maturity in the northern region. However, the connection between invasive species presence and microbial community functional diversity cries for exploration, and can be explored at any time. It would be interesting to examine this more fully, paying particular attention to invasive species-specific correlations to the presence of individual microbes, in an effort to determine which way causality runs. To that end, we would suggest that both Biolog EcoPlates™ and some form of DNA analysis be employed to discover the specific species makeup of microbial communities. DNA analysis may yield interesting results pertaining to what species are interested in particular substrates, and could also reveal correlations between

specific microbial species or communities and their environment. This will help future studies be more precisely indicative of the soil nutrient environment. We know from prior studies that invasive plant species alter soil chemistry (Gibbons et al 2017), and we also know that microbial communities have a strong impact on plant health (Marcel et al. 2007), but we do not yet know how those two impacts interact. As fragmentation continues and the amount of forest edge area grows, understanding site- and species-specific interactions at the vegetational and microbial level will be crucial to employing effective forest management strategies.

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