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Exclusion of Overabundant White-tailed Deer (Odocoileus virginianus) Results in Shifts in Soil Microbial Communities and Abiotic Soil Condition in a Northeastern Deciduous Forest

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ABSTRACT.—Past and current anthropogenic practices have resulted in dramatic alterations to ungulate population densities worldwide. When ungulate populations are overabundant, they can alter the dynamics, structure, and function of ecosystems. White-tailed deer (*Odocoileus virginianus*), specifically, can occur at densities far greater than their historical records in parts of their native range, which includes forests in the northeastern U.S. They have been shown to alter community structure of native plants, indirectly impact animal communities, and promote the success of invasive species. Despite much research into the effects of overabundant ungulates and deer in particular, less is known about the effects of deer on soil microbial communities. Here, we utilized soil samples from inside and outside of six deer exclosures located in a regional second growth mixed hardwood forest on the Binghamton University campus in Vestal NY, U.S.A. A metagenomic analysis was conducted on DNA extracted from the soil to identify the microbes present. Soil characteristics, including soil organic matter, soil moisture, pH, and electrical conductivity were also measured. Soil samples from inside exclosures had on average lower pH, higher soil moisture and organic matter, and higher electrical conductivity. The microbial communities across all samples were dominated by *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*. However, the structure of the microbial soil community appeared to differ between samples taken inside and outside the exclosures, with those taken outside more closely resembling other outside samples and those sampled inside soils showing more variability in community structure. Overall, our results suggest that overabundant deer may have a homogenization effect on the soil abiotic environment and the soil microbial community.

INTRODUCTION

Ungulates exert strong influences on ecosystems, particularly through modifications to plant communities. As herbivores, wild ungulates directly control abundance and composition of plant species (Rooney and Waller, 2003; Boulanger *et al.,* 2018), which has corresponding indirect effects on a broad array of ecosystem functions and processes, including nutrient cycling, seed dispersal, and hydrologic regimes (Hobbs, 1996; Wolf *et al.,* 2007; Bartuszevige and Endress, 2008). Modern anthropogenic alteration to apex predator densities, habitat modification, and in some areas, protected legal status, have resulted in increased ungulate herbivore abundance, particularly in the northern hemisphere (Fuller and Gill, 2001; Augustine and DeCalesta, 2003; Côté *et al.,* 2004; Iida *et al.,* 2018). When ungulates are overabundant, grazing intensity dramatically increases, which in turn, can significantly impact the dynamics, structure, and function of ecosystems. As a result of the overgrazing and consequent alterations to plant communities, many indirect effects have been explored. Research has shown that high ungulate density corresponds to reductions in invertebrate density (Carpio *et al.,* 2014) and alterations to invertebrate community composition (Iida *et al.,* 2018); changes in soil nutrient and organic matter pools (Pastor *et*

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al., 1993; Eldridge *et al.,* 2017; Stephan *et al.,* 2017); and the acceleration of the spread of invasive plant species (Vavra *et al.,* 2007).

White-tailed deer (*Odocoileus virginianus*) specifically are considered overabundant in parts of their native range, occurring at densities that are far greater than their historical numbers (Côté *et al.,* 2004). Research into the effects of overabundant white-tailed deer has found extensive impacts on ecosystems, including alterations to vegetation dynamics and ecosystem functions, such as nutrient cycling and carbon storage (Côté *et al.,* 2004). White-tailed deer can alter both herbaceous (Holmes *et al.,* 2008) and woody plant species richness (Russell *et al.,* 2017), and can also have indirect effects on animal communities that are reliant on the same understory plants (Rooney and Waller, 2003). Through avoidance of unpalatable plants and overgrazing of palatable native plants, browsing by overabundant white-tailed deer has been shown to halt native plant regeneration (Ward *et al.,* 2017). High levels of feeding intensity have also led to measurable shifts in plant communities in some eastern forests (Shen *et al.,* 2016; Averill *et al.,* 2018).

Although much research has shown that ungulates broadly, and deer specifically, can alter aboveground plant and animal communities, few studies have directly examined whether overabundant deer alter the soil microbiome. Soil microbes are known to be susceptible to environmental disturbance; however, further quantification and comparison of how microbial communities in varying environments respond to disturbance is still needed (Shade *et al.,* 2012). Overabundant deer function as an ongoing disturbance in many ecosystems in which they alter resources, resulting in a range of effects that can impact different levels of biological organization at both the community and ecosystem levels (Habek and Shultz, 2015). Some research on herbivore impact on the soil microbiota has shown that reductions in herbivore density can change fungal communities (Burke *et al.,* 2019) and bacterial diversity (Eldridge *et al.,* 2017), and can increase litter decomposition (Kasahara *et al.,* 2016). Recent research has also found that variation in the soil microbiome can have profound impacts on plant disease tolerance (Wei *et al.*, 2019), and traits of dominant microbial taxa can drive resilience and resistance in ecosystem function with respect to climate change (Bardgett and Caruso, 2020). Thus, an understanding of if and how overabundant ungulates, such as white-tailed deer, affect the full soil microbiome is needed in order to better understand the indirect effects of this disturbance and the consequences for ecosystem dynamics.

In our study we characterized soil sampled from inside and outside of five deer exclosures located in a nature preserve and one deer exclosure located in a natural area on the Binghamton University (SUNY) campus located in New York State, U.S.A. The nature preserve at Binghamton University is impacted by a highly overabundant population of white-tailed deer. The goal of this study was to assess the effects of complete removal of the overabundant population on soil characteristics and the soil microbiome to understand soil dynamics after removal of the disturbance pressure. We measured soil characteristics, including soil organic matter, soil moisture, pH, and electrical conductivity in samples from inside and outside of the deer exclosures. We also sequenced DNA extracted from the soil samples and performed a metagenomic analysis to identify microbial taxa present in the soil. Specifically, our study addressed the question: Does complete exclusion of overabundant white-tailed deer lead to changes in soil characteristics and the soil microbiome? Given what is known about the impact of ungulates on soil nutrient availability and organic matter pools (Côté *et al.,* 2004), we hypothesized that when overabundant white-trail deer are excluded, soil characteristics will change. Specifically, soil organic matter, moisture, and electrical conductivity will all increase and pH will change relative to areas where overabundant deer

are still present. Further, because reduction in herbivore density is associated with changes in the quality of litter inputs to soils (Pastor *et al.*, 1993; Kasahara *et al.,* 2016), we also hypothesized that deer exclusion would drive differences in soil microbial community composition.

METHODS

SITE DESCRIPTIONS

All soil samples were collected on the Binghamton University campus in Vestal, NY, U.S.A. (42.0893, –75.9699). Soil was collected from five sites (College in the Woods North (CN), College in the Woods South (CS), Pond Trail (PT), Fuller Hollow Creek North (FN), Fuller Hollow Creek South (FS)) located in the campus nature preserve, a 182-acre plot of undeveloped forested land (Fig. 1). The preserve habitat is largely regional, second-growth mixed hardwood forest (List of Trees- Binghamton University, 2018). One collection site, East Gym (EG), was located in a natural area on campus, which is a patch of remnant secondary forest largely surrounded by campus infrastructure (Fig. 1). The immediate area surrounding the campus, the Town of Vestal, is composed of residential properties and is predominantly suburban.

White-tailed deer (henceforth "deer") have been recorded at densities exceeding 200 deer/km^2 in all of the areas sampled (Parisio *et al.*, 2018). This density is greater than 10 times the carrying capacity of the forest ecosystem (Parisio *et al.*, 2018). Considerable impacts to the forest ecosystem have been observed, including a total lack of understory vegetation (no wildflowers, forest shrubs, or saplings greater than 20 y in age) and highly visible browse lines. In natural forest gaps, there is little to no plant growth. Turkeys (*Meleagris gallopavo*), once present in the fall and winter, have disappeared and ground nesting birds no longer nest within the preserve (Parisio *et al.*, 2018; *D. Horvath, Steward of Natural Areas at Binghamton University,* pers. comm.*,* October 2021). Deer exclosures (high fencing with their primary purpose being to exclude deer from an area) had previously been constructed at each sample site. The oldest and smallest exclosures consist of wooden posts with 4 ft welded wire fencing attached to 4 ft polypropylene fencing. All fencing is secured to the posts to create a full 8 ft barrier. The intermediate age and size exclosures consist of 8 ft welded wire fencing supported by wooden posts and some tree trunks. The youngest and largest exclosures were constructed from 8 ft polypropylene Tenax C-flex HD Maximum Strength deer fencing. The fencing is supported through a combination of metal and wooden posts and tree trunks. The six exclosures we sampled were paired by age and size (Table 1). The two oldest exclosures (12 y) were our smallest sites, each only 9m^2. The two intermediate age exclosures (8 y) were 70m^2. Our two youngest exclosures (4 y), at 2000m^2, were over 200x larger than the oldest and over 10x larger than our intermediate age sites. We also identified the most abundant tree species growing at each location (Table 1). To prevent deer from entering, maintenance of the exclosures is done regularly by the steward of the Nature Preserve.

SOIL SAMPLE COLLECTION

To investigate differences in abiotic soil characteristics and microbial community composition between areas affected by deer (outside of exclosures) and areas where deer were excluded (inside of exclosures), in Sept 2019, a representative bulk soil sample was collected from inside and outside of each exclosure. First, the inside portion of an exclosure was visually divided into four roughly equal sections. From each section, we haphazardly

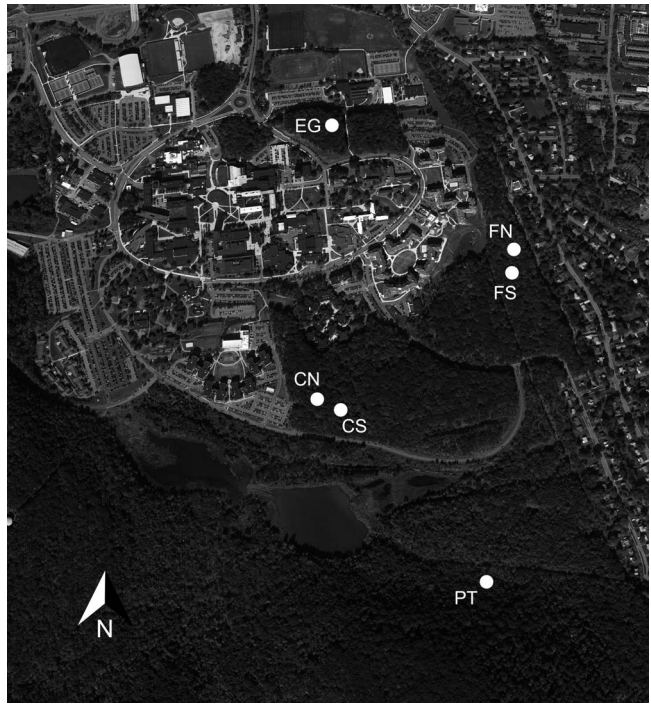

FIG. 1.—Aerial view of the Binghamton University campus with approximate locations of deer exclosure sample sites. The site abbreviations are derived as follows: College in the Woods North (CN), College in the Woods South (CS), Pond Trail (PT), East Gym (EG), Fuller Hollow Creek North (FN), and Fuller Hollow Creek South (FS). To the right, the suburban development that surrounds the campus is visible. Aerial map of Binghamton University, Vestal, NY. Google Maps, 2021, maps.google.com

collected soil to a depth of 8 cm using a small spade. We combined all this soil in a 1 gal bucket to use as a single bulk sample representative of the inside of the exclosure (deer absent). Another bulk sample was collected from outside of the exclosures. For these samples, we visually extended the lines dividing each section to the outside of the fencing to a distance of approximately 4 m, then visually extended a line parallel to the exclosure fence that connected the lines (Fig. 2). We haphazardly collected soil from outside of the exclosure to a depth of 8 cm within each of the outside sections. Again we combined all soil

TABLE 1.—Sample sites with dominant tree species, size, and age at sampling indicated

Sample site	Dominant tree species	Exclosure size	Construction year	Age at sampling (years)
CN	*Tsuga canadensis*	9.03 m^2	2007	12
CS	*Quercus sp.*			
EG	*Acer saccharum*	70 m^2	2011	8
PT	*Acer saccharum*			
FN	*Fraxinus sp., Acer sp., Quercus sp.*	2000 m^2	2015	4
FS	*Fraxinus sp., Acer sp., Quercus sp.*			

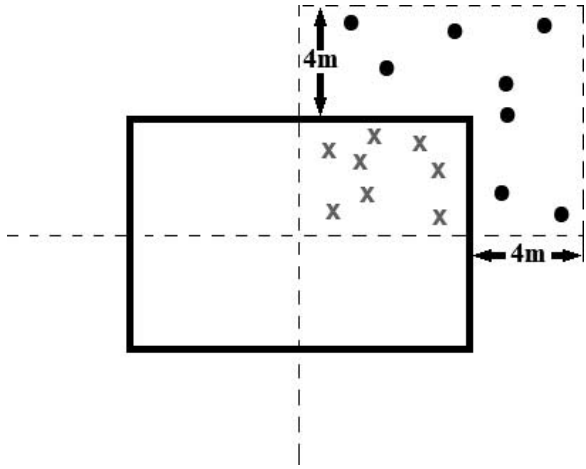

FIG. 2.—Schematic diagram of our sampling approach to deer exclosures. The exclosure is represented by the thick black line. Dashed lines are visually assessed dividers for the quadrats (smaller rectangles). Xs represent haphazard sampling inside of a quadrat within the exclosure and black circles represent haphazard sampling within a quadrat outside of the exclosure

in a 1 gal bucket to use as a single bulk sample representative of the outside of the exclosure (deer present). This process was repeated for each exclosure and all 12 bulk samples were brought back to the lab where they were processed by thoroughly hand-mixing and sorting to remove organic (*e.g.,* leaves, sticks, roots, etc.) and inorganic (*e.g.,* rocks) debris.

After processing the bulk samples, we weighed 0.5 g subsamples of soil from each for immediate DNA extraction. Additional subsamples were weighed and then dried at 60 C for a minimum of 48 h and then at 105 C for a minimum of 2 h. After each drying step, the soil was weighed for calculations of soil moisture content and the dry weight/fresh weight conversion ratio. All of the subsamples were then ashed in a muffle furnace at 550 C and weighed to calculate organic matter content.

All of the bulk samples were stored in a refrigerator for 4 w prior to large scale analysis of pH and electrical conductivity by student research teams as part of a large course-based undergraduate research experience (CURE) class at Binghamton University.

SOIL ABIOTIC CHARACTERISTICS

Soil pH and electrical conductivity were measured by 98 student teams (approximately four students per team) in the research-based course, BIOL115, at Binghamton University. All student data obtained as part of the CURE course was collected under the guidance of nine graduate student teaching assistants (TAs) and 18 undergraduate student teaching assistants. All TAs were trained by MA Kearney on equipment and protocols. In the first 5 w of class, TAs trained all student teams on equipment and protocols and students gained experience working in their research teams and practicing the protocols.

Prior to data collection, soil was taken out of the refrigerator by the TAs and allowed to warm to room temperature. Roughly one team in each of the 18 lab sections of the course (18 teams total) was assigned to one of the six sites with each team analyzing a unique site (no repeats within a section). For EG, PT, FN, and FS sites, each student team separately

weighed 10 g of soil from inside and outside of the exclosure into three 300 ml bottles. For CN and CS, teams followed this same set up (three subsamples from inside and three subsamples from outside), but used 5 g of soil. Teams measured pH and electrical conductivity of their soil subsamples by creating a 1:2 soil to distilled water slurry in each of their six 300 ml bottles. Teams thoroughly mixed their soil/water slurries for 15 min then allowed them to settle for five minutes. Electrical conductivity and pH were measured in the liquid supernatant using a Vernier LabQuest 2 and Vernier pH and conductivity probes. Three pH and three electrical conductivity readings were taken in each of the six subsamples and the average of the three readings was recorded. All data was compiled in a class spreadsheet and submitted as part of the course and is stored in an archive in Google Drive.

DNA EXTRACTION AND METAGENOMIC SEQUENCING

A DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) was used to isolate microbial genomic DNA from the 0.5 g soil subsamples according to the manufacturer's protocol. DNA extracts were checked for quality and yield by UV Spectrophotometry (VWR, UV-1600PC). We sequenced the extracted DNA samples using a minION^{TM} portable sequencer (Oxford Nanopore Technologies, Inc., Oxford, UK), the rapid barcoding library prep kit (SQK-RBK004, Oxford Technologies), and the minKNOW software package (Oxford Nanopore Technologies, Inc., Oxford, UK).

SEQUENCE ANALYSIS

Reads were quality checked using the minKNOW software. Per software parameters, reads shorter than 70 bp and with an average Q score of less than seven were removed (Jain *et al.* 2017). Passed sequences had an average Q score of 10 and mean sequence length of 2404 bp. Demultiplexed filtered sequences were then submitted to MG-RAST for classification. Taxonomy was assigned in MG-RAST, which uses the SEED environment, using a maximum e-value of $1e^{-5}$, a minimum identity of 60%, and a maximum alignment length of 15 bp.

DATA PROCESSING AND STATISTICAL ANALYSIS

All student data from BIOL 115 was screened by TAs prior to entry into the course data spreadsheet. The screening process required students to report their values directly to their graduate TAs, who would then enter the data into the course data spreadsheet. TAs were required to review the values with their student research teams before they were entered. This was done in order to flag questionable values immediately and have the TAs work with those teams to determine if the values were assessed correctly (in which case they would be entered) or if they were assessed incorrectly (in which case the team would review the proper methodology and re-measure). For taxonomic analysis, we downloaded the OTU table from the MG-RAST server and sorted OTUs using Microsoft Excel software (Microsoft Excel 365, Microsoft, Seattle, WA, USA). OTUs that matched to viral, plant, or animal sequences were removed from the OTU table. We used MS Excel to sort microbial OTU tables by abundance and then focused some of our analysis on the 10 most abundant phyla and 20 most abundant genera to better understand dynamics in the dominant taxa by site and location.

Statistical tests were performed using PAST software (PAleontological STatistics Version 4.03, Hammer *at al.,* 2001). To investigate the impact of deer exclusion on soil organic matter and soil moisture, we used a Wilcoxon signed-rank test. To investigate differences in soil abiotic characteristics (pH, soil moisture, and electrical conductivity) of inside soils and outside soils, we used Kruskal-Wallis tests with post hoc Mann-Whitney-U pairwise

TABLE 2.—Average percent soil organic matter (N = 6) and soil moisture (N = 6) of all inside and all outside soil samples. n.s. denotes a lack of significance at $\alpha = 0.05$

	Inside	Outside	P value
% Organic Matter	26.6 (±5.6)	21.0 (±2.3)	n.s.
% Soil Moisture	30.7 (±2.0)	28.5 (±1.8)	n.s.

comparisons with Bonferroni correction. For each site, we used a Mann-Whitney-U test to examine differences in soil characteristics between inside and outside. For our exploratory analysis to examine patterns in soil microbial communities from each soil sample, we used PAST to perform non-metric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarities on genus-level taxonomic data. To compare patterns among dominant community members, we analyzed raw abundance data for our first ordination. Then, to down-weigh the influence of dominant taxa on the ordination and examine patterns in less common community members, we performed a second NMDS ordination on square-root transformed abundance data. For both, we used 2D dimensionality and 50 random restarts to ensure the best solution was obtained with minimized stress. Finally, we utilized a PERMANOVA to investigate deer effect on microbial community composition. Unless otherwise noted, for all statistical analyses, significance was defined at an alpha level of 0.05. All figures and graphics were created using Microsoft Excel and PAST software.

RESULTS

SOIL CHARACTERISTICS

Soil organic matter was higher on average inside of the exclosures (26.6%) compared to outside of the exclosures (21.0%), although this difference was not significant (Wilcoxon signed-rank test, N = 6, P = 0.115, Table 2). The same trend was measured for soil moisture and was also not significant (Wilcoxon signed-rank test, N = 6, P = 0.114, Table 2). For every site except EG, organic matter was higher inside of the exclosure, and the oldest exclosures, CN and CS (both 12 y old; Table 1), had the highest organic matter measurements for inside soils (31.6% and 51.4%, respectively). Soil moisture was higher inside of each exclosure, except for EG and FS.

On average pH values were lower inside of the deer exclosures compared to outside for all sites, except EG (Fig. 3A). Median values showed the same trend and ranged from 3.9 (CS-Inside) to 5.5 (FN-Outside). A comparison of pH values across sites for outside soils was significant (Kruskal-Wallis test, $P < 0.001$). Mann-Whitney-U pairwise comparisons showed that all sites had significantly different pH values for outside soils except for EG and PT. There were also significant differences for pH across sites for inside soils (Kruskal-Wallis test, $P < 0.001$). Mann-Whitney-U pairwise comparisons showed that all sites had significantly different pH values for inside soil samples, except for EG compared to FN and PT compared to FS.

To test for a deer effect, we used Mann-Whitney U tests to examine differences in soil pH values of soil samples from inside and outside the exclosures at each site. We found significant differences for all comparisons, except for at sites EG and FN (Table 3).

On average electrical conductivity (EC) values were higher inside of the deer exclosures compared to outside for all sites, except PT (Fig. 3B). Median values showed the same trend and ranged from 124 $\mu S\ cm^{-1}$ (PT- Outside) to 255 $\mu S\ cm^{-1}$ (CS- Inside). A comparison of

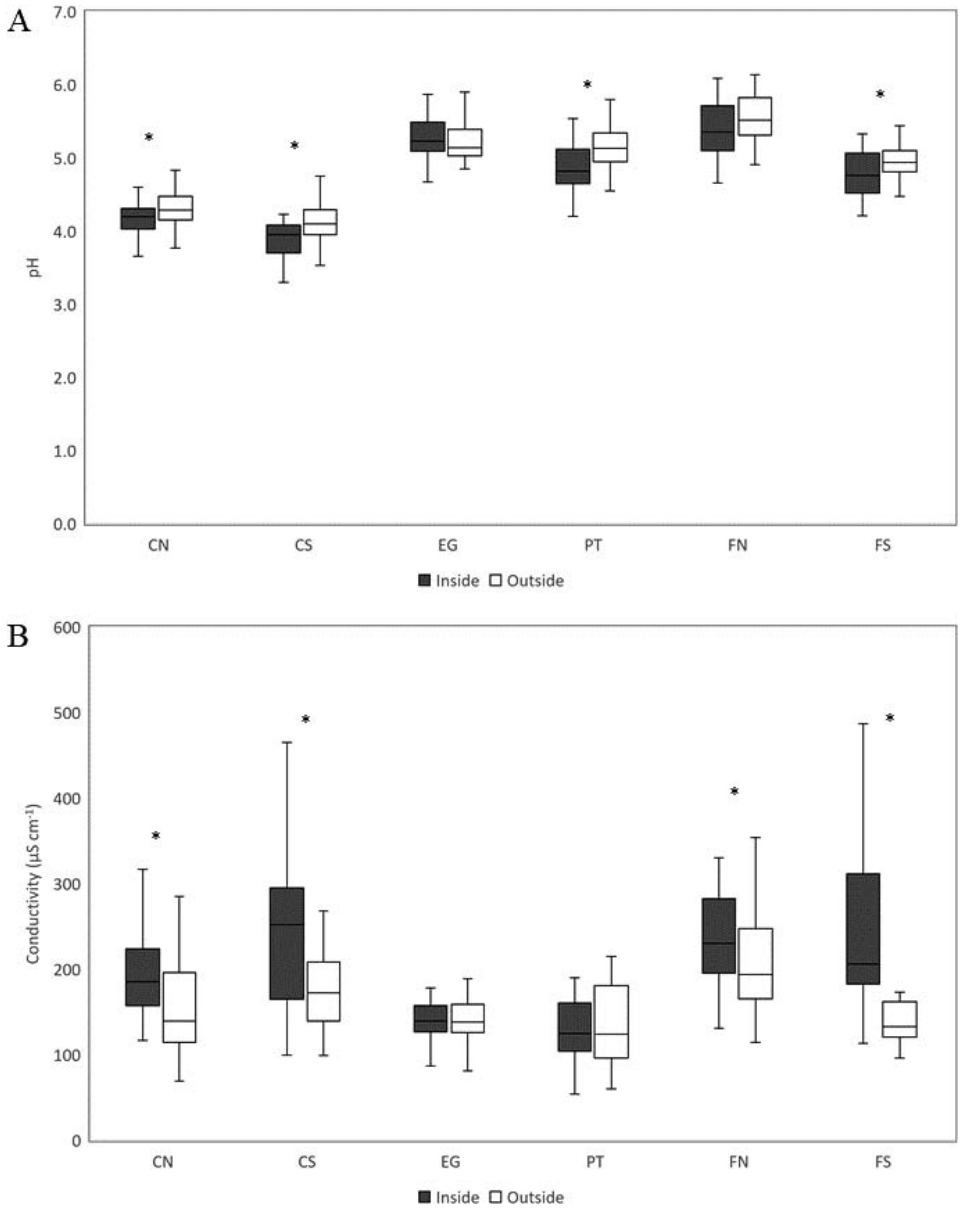

FIG. 3.—Box plots showing **A.** soil pH and **B**. soil electrical conductivity inside (gray) and outside (white) of the deer exclosures by site. Samples are listed from left to right as they increase in size of exclosure and decrease in age at sampling. Median is shown as solid line and * indicates a significant difference ($\alpha = 0.05$) using Mann-Whitney U testing between inside and outside.

TABLE 3.—Results of Mann-Whitney-U comparisons of inside and outside soil samples for pH and electrical conductivity by site. n.s. denotes a lack of significance at $\alpha = 0.05$

Site	pH P value	Electrical conductivity (μS/cm) P value	Sample size (N)
CN	0.0074*	<0.001*	52
CS	<0.001*	<0.001*	42
EG	n.s.	n.s.	51
PT	<0.001*	n.s.	47
FN	n.s.	0.021*	48
FS	0.0026*	<0.001*	53

EC values across sites for outside soils was significant (Kruskal-Wallis test, $P < 0.001$). However, Mann-Whitney-U pairwise comparisons showed that EC values at all sites were the same for outside soils except for FN, which differed significantly from all other sites. There was a significant difference in electrical conductivity values across sites for inside soils (Kruskal-Wallis test, $P < 0.001$). Mann-Whitney-U pairwise comparisons showed that electrical conductivity values at PT and EG were the same, but differed significantly from all other sites, and that inside soils at FN differed from CN.

To test for a deer effect, we used Mann-Whitney U tests to examine differences in soil EC values comparing inside soil samples to outside soil samples at each exclosure site. We found significant differences for all comparisons, except for at sites EG and PT (Table 3).

COMMUNITY STRUCTURE

Taxonomic analysis showed a community structure similarity at the domain level with *Bacteria* dominating samples at 97.9% inside the exclosures and 98.0% for outside. Remaining sequences were matched with *Eukaryota* (1.34% inside, 1.26% outside), and *Archaea* (0.74% for inside, 0.73% for outside).

Comparison of total individuals at all sites showed that overall inside samples (237,421) had fewer hits (the number of unique database sequences that were found in the similarity search in MG-RAST) than outside samples (267,047), although variation was site dependent. There were dramatic differences in the percent of total hits recorded for soil samples taken inside compared to outside the exclosures collected at CN, where 7% of total site hits came from inside and 93% hits from outside . FN had a similar distribution of hits with 11% from soil samples from inside the exclosures and 89% from outside. For the remaining four sites, we found that the percent of hits was either roughly equal (EG, FS) or slightly higher in the outside soil (CS, PT).

A total of 35 unique phyla were identified across all sites. All 35 phyla occurred in each sample except for CS-Inside (33 phyla) and CN-Inside and FN-Inside (each with 30 phyla). *Proteobacteria* were the most abundant microbial phylum by far at all sites for both inside (50.1%) and outside (50.4%) soils. The abundance of *Proteobacteria* ranged from 46.6% in CNO to 59.6% in FNI. *Actinobacteria* (Inside- 14.3%, Outside- 15.4%) and *Acidobacteria* (Inside-13.9 % and Outside- 13.3%) were the next most abundant microbial phyla. The majority of the remaining phyla each comprised less than 5% of the community (many less than 1%) and sequences that were unclassified at the phyla level were rare for all sites and locations.

To understand the dominant microbial taxa within the community, we examined the 10 most abundant phyla in each sample. We found *Acidobacteria, Actinobacteria, Bacteroidetes,*

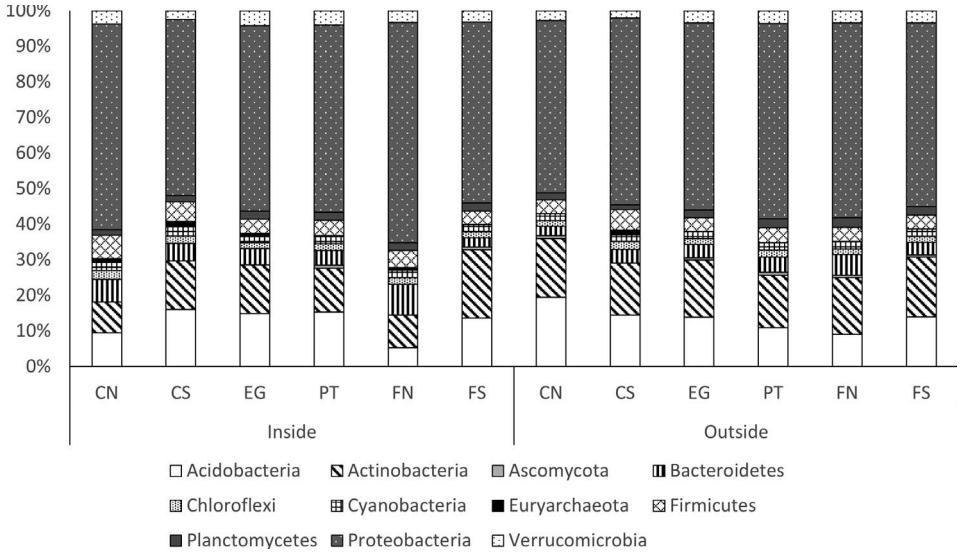

FIG. 4.—Stacked column bar graph of the top 10 most abundant microbial phyla across soil samples. Samples are grouped by sample location (inside exclosures on the left, outside of exclosures on the right). Paired sites (CN & CS, EG & PT, FN & FS) are listed from left to right as the size of exclosure increases and age of exclosure at sampling decreases. Taxa are reported in alphabetical order moving from the bottom to the top of each column. Relative abundance data was analyzed by using MG-RAST against the SEED database and then visualized using Microsoft Excel Software (Microsoft, Seattle, WA, U.S.A.)

Chloroflexi, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria, and *Verrucomicrobia* present in all samples. However, the *Ascomycota* taxon was found to be within the 10 most abundant phyla in five of the six outside samples (CN, EG, PT, FN, and FS) and only two of the six inside samples (PT and FS). Conversely, the *Euryarchaeota* taxon was found to be within the 10 most abundant phyla in four of the six inside samples (CN, CS, EG and FN) and only one of the six outside samples (CS) (Fig. 4).

Across all sites and locations, a total of 754 unique genera were identified. CNI contained the fewest genera classified (460), whereas PTI and FSI contained the most (715 and 716, respectively) (Table 4). To explore the dominant microbial taxa within the communities, we examined the 20 most abundant genera in each sample and found 35 distinct genera represented. Some overlap in the dominant microbial community was observed with 11 of the 35 dominant genera distributed across all sites and locations (Table 5). These 11 genera comprised on average, 27% of the total observed microbial community abundance. However, 12 of the 35 genera were only found as part of the dominant microbial community of inside samples, five of which were found only in one sample (FNI). Two samples (EGI and PTI) did not have any dominant genera that did not also overlap with outside samples. The remaining 12 dominant taxa were present in at least one inside and one outside sample.

Non-metric multidimensional scaling ordinations highlighting the dominant microbial community members (Fig. 5A, stress = 0.0045) and the less common microbial community members (Fig 5B, stress = 0.0149) showed similar patterns. There was distinct clustering in both ordinations of outside soil samples compared to the inside samples, and inside samples

TABLE 4.—Microbial genera richness, evenness, and Shannon Diversity Indices for all soil samples by site and location. Results from Hutcheson *t*-test by location are also indicated; n.s. denotes a lack of significance at $\alpha = 0.05$

Site	Genera richness	Evenness	Shannon Diversity	Hutcheson *t*-test P value
CNI	460	0.46	5.35	<0.001
CNO	685	0.23	5.04	
CSI	520	0.35	5.22	0.03
CSO	614	0.32	5.27	
EGI	677	0.27	5.20	n.s.
EGO	686	0.26	5.19	
PTI	715	0.26	5.23	<0.001
PTO	700	0.29	5.30	
FNI	518	0.45	5.46	<0.001
FNO	683	0.30	5.32	
FSI	716	0.23	5.12	<0.001
FSO	697	0.25	5.17	

were more widely distributed in both ordination patterns. For both, the environmental vectors overlaid on the ordination plots revealed that soil organic matter and average pH had the closest associations with the microbial assemblages. When we compared microbial community composition by location to examine the deer effect (inside and outside), we found a marginally significant difference (PERMANOVA, Bray-Curtis, $P = 0.09$). Shannon diversity, genera richness, and evenness were variable between sites and locations (Table 4). Hutcheson *t*-tests showed significant differences in diversity between inside and outside soil samples at all sites, except EG.

DISCUSSION

Although the effect of overabundant deer has been extensively studied in forest plant and animal communities (Côté *et al.,* 2004; Webster *et al.,* 2005; Holmes *et al.,* 2008; Goetsch *et al.,* 2011; Shen *et al.,* 2016; Averill *et al.,* 2018; Russell *et al.,* 2017; Maillard *et al.,* 2021), less is known about their effects on soil microbial communities. Contemporary research on the subject has been limited to fungal community structure (Burke *et al.,* 2019), focused on other vertebrate herbivores (Eldridge *et al.,* 2017), or examined deer effects on soil ecological functions (*e.g.,* litter decomposition (Kasahara *et al.,* 2016)). Here we attempted to directly examine the impact of overabundant deer exclusion on the community structure of the soil microbiome. In our study, soils affected by overabundant deer had overall more similar microbial communities compared to the more variable microbial communities found in soils where deer had been excluded. We also found that soils where overabundant deer were excluded had higher soil organic matter and moisture and significantly higher electrical conductivity and lower pH values.

Metagenomic sequencing of soils from all sites showed that samples from inside the exclosures had fewer overall hits than outside samples. However, when examined by site, there was considerable variation in the total hits recorded and the relative proportion of hits for soil samples inside and outside the exclosures. Terrestrial ecosystems intrinsically possess heterogeneity in the apportionment of soil resources that drive the organization of biological communities and the interactions within them (Farley and Fitter, 1999), especially within soil microbial communities (Treves *et al.,* 2003; Young and Crawford, 2004; Or *et al.,*

TABLE 5.—Dominant microbial community genera by location and site. The top 20 genera by relative abundance (% of the total genera identified) in each sample are shown. A total of 35 genera are represented

	Inside						Outside						Total samples with taxon present
	CN	CS	EG	PT	FN	FS	CN	CS	EG	PT	FN	FS	
Anaeromyxobacter	1.3	0.9	1.0	1.1	1.0	0.8	0.9	1.0	0.8	1.0	1.0	0.9	12
Bradyrhizobium	2.8	2.9	4.4	4.5	1.9	5.3	4.0	3.3	5.0	4.7	4.7	5.1	12
Burkholderia	3.9	3.4	2.8	3.0	3.7	2.9	2.9	3.2	2.9	3.1	2.8	2.8	12
Candidatus Koribacter	2.5	4.6	4.2	4.4	1.1	3.9	5.9	4.1	4.0	3.1	2.3	4.1	12
Candidatus Solibacter	4.3	4.8	6.6	6.5	3.1	5.3	6.2	3.8	5.6	4.6	4.3	5.5	12
Geobacter	2.0	1.4	1.2	1.0	1.6	0.9	1.1	1.7	1.0	1.0	1.0	0.9	12
Methylobacterium	0.9	0.9	1.5	1.5	0.9	1.6	1.3	1.1	1.5	1.7	1.5	1.5	12
Pseudomonas	1.2	1.3	1.0	1.2	1.6	1.1	1.1	1.5	1.1	1.2	1.4	1.2	12
Rhodopseudomonas	2.0	2.3	2.9	2.9	1.4	3.3	2.8	2.2	3.2	3.2	3.2	3.2	12
Streptomyces	1.5	1.7	2.3	2.1	1.2	3.2	2.3	1.9	2.6	2.5	2.5	2.6	12
unclassified (Verrucomicrobia subdivision 3)	1.2	0.9	1.7	1.7	1.1	1.1	1.1	0.8	1.3	1.3	1.1	1.2	12
Acidobacterium	1.6	4.8	2.8	2.9	–	2.9	5.2	4.5	2.8	2.1	1.6	2.9	11
Cupriavidus	1.3	0.8	0.9	0.9	1.5	–	0.7	0.7	0.9	0.9	1.0	0.8	11
Mycobacterium	–	3.3	2.1	1.5	1.1	3.0	4.4	4.3	2.4	1.9	2.2	2.6	11
Frankia	–	1.1	1.3	1.2	–	1.8	1.4	1.4	1.4	1.4	1.5	1.6	10
Nitrobacter	–	1.0	1.3	1.3	–	1.8	1.4	1.0	1.6	1.6	1.4	1.6	10
Rhizobium	–	–	0.8	0.8	–	1.1	0.9	0.8	0.9	1.0	1.0	1.0	9
Terriglobus	–	1.1	0.9	1.0	–	1.0	1.6	1.5	1.0	–	–	1.0	8
Mesorhizobium	0.8	–	–	–	–	0.9	0.8	–	0.8	1.0	0.8	0.9	7
Caulobacter	–	1.1	1.0	0.8	–	–	1.0	0.9	0.8	–	–	–	6
Chthoniobacter	0.9	–	0.9	0.8	–	–	–	–	–	0.9	0.9	0.9	6
Sinorhizobium	–	–	–	–	–	0.9	–	–	–	0.8	0.8	–	3
Clostridium	0.9	–	–	–	–	–	–	0.8	–	–	–	–	2
Acidovorax	0.9	–	–	–	1.1	–	–	–	–	–	–	–	2
Polaromonas	0.9	–	–	–	1.6	–	–	–	–	–	–	–	2
Bacillus	–	1.0	–	–	–	–	–	–	–	–	–	–	1
Bacteroides	1.2	–	–	–	–	–	–	–	–	–	–	–	1
Bordetella	–	–	–	–	1.0	–	–	–	–	–	–	–	1
Chitinophaga	–	–	–	–	1.2	–	–	–	–	–	–	–	1
Flavobacterium	–	–	–	–	1.2	–	–	–	–	–	–	–	1
Methylibium	–	–	–	–	1.0	–	–	–	–	–	–	–	1
Ralstonia	–	–	–	–	0.8	–	–	–	–	–	–	–	1
Rhodococcus	–	–	–	–	–	0.8	–	–	–	–	–	–	1
Roseiflexus	1.0	–	–	–	–	–	–	–	–	–	–	–	1
Xanthomonas	–	0.9	–	–	–	–	–	–	–	–	–	–	1
Total	33.3	40.3	41.5	41.0	28.9	43.9	47.0	40.4	41.8	38.9	36.9	42.2	

2007; Carson *et al.*, 2010; O'Brien *et al.*, 2016). The inherent heterogeneous nature of the soil environment at each of the sites may account for some of the variability in sequencing outputs found. The observed variation in total hits may also be related to the size and/or age of the deer exclosures utilized. In the oldest and smallest sites (CN and CS), there were fewer hits in soil samples from inside the exclosures compared to outside, and especially extreme differences at CN where 93% of the total hits were from outside samples.

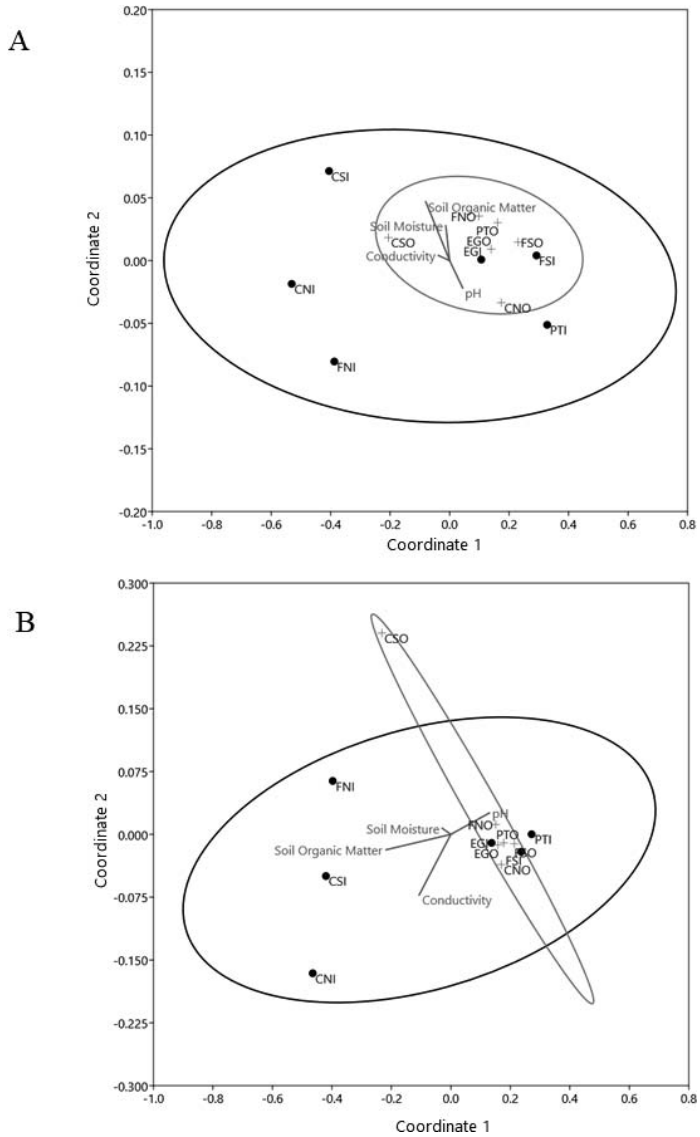

FIG. 5.—Nonmetric multidimensional scaling (NMDS) ordination plots of the distance between microbial communities based on the Bray-Curtis index. **A**. Compares patterns among dominant community members (Stress = 0.0045) and **B**. shows patterns when dominance is minimized (Stress = 0.0149). Inside samples are denoted by black dots and outside samples are denoted by grey crosses. Abundance of taxa were used for comparisons. Environmental vectors are shown. The increasing gradient of the environmental variable is indicated by the direction of the vector and vector length is proportional to the correlation between the variable and the ordination pattern. The ellipses represent the samples which were within a 75% confidence limit of the centroids

In other studies that utilized exclosures in their design, exclosure size and age were highly variable ranging in size from $4m^2$ to $4{,}000m^2$ and in age since exclusion from 2 to 18 years (Webster *et al.,* 2005; Rooney, 2009; Goetsch *et al.,* 2011; Bressette *et al.,* 2012; Habeck and Schultz, 2015; Shen *et al.,* 2016; Averill *et al.,* 2018; Burke *et al.,* 2019). A meta-analysis by Habeck and Schutlz (2015) found no relationship between exclosure area and plant community responses to deer, but found that increased time since deer exclusion had a strong influence on plant community through effects on woody species richness. Given temporal effects exist for plant communities and other research has shown that plant and soil communities are connected (Kulmatiski *et al.*, 2008; Wei *et al.*, 2019), temporal changes in plant communities in response to deer exclusion may be driving the differences in the soil microbiome observed in this study. However, in our literature review, we found no research exploring exclosure size effects on microbial assemblages, and we know soil is spatially heterogeneous across even small distances (Ettema and Wardle, 2002); therefore, we cannot rule out that exclosure size could also impact soil communities. In our study, the size and age of exclosures were variable across sites (Table 1). Without suitable replication in our study ($N = 2$ for each size and age class), we cannot decouple whether the differences observed were due to effects of one or both factors. More research is required to determine if and how age and exclosure size influence microbial community structure.

Bacterial OTUs had the highest relative abundances in all soil samples (both inside and outside the exclosures) at the domain level. In other soil environments, similar relative abundances of bacterial taxa of greater than 90% have been reported (Delmont *et al.,* 2012; Uroz *et al.,* 2013; Castañeda and Barbosa, 2017) and highlight the functional importance of bacteria in soil communities (Delgado-Baquerizo *et al.*, 2016). At the phyla level, *Proteobacteria* was the most dominant taxa at all sites and locations. The relative abundance of *Proteobacteria* at sites in this study (from 46.6% to 59.6%) resembles those found in forest, grassland, and agricultural soils (approximately 40% according to Janssen 2006). *Protobacteria* is a diverse phylum and has been shown to contribute to several global soil ecosystem functions, such as carbon, nitrogen, and phosphorus cycling (Spain *et al.,* 2009; Kersters *et al.,* 2006). *Actinobacteria* and *Acidobacteria* were the next most abundant microbial phyla across pooled soil samples inside and outside exclosures at our sites. Alongside *Proteobacteria,* both *Actinobacteria* and *Acidobacteria* are generally considered dominant phyla in soils (Janssen *et al.,* 2006; Fierer *et al.,* 2012b) and have been found regularly at high relative abundances in different soil environments (Janssen *et al.,* 2006; Fierer *et al.,* 2012a; Shange *et al.,* 2012; Ferrenberg *et al.,* 2013; Zeng *et al.,* 2016). The remaining phyla observed each comprised less 5% of the microbial community, with the majority scarcely contributing (less than 1%). Because recent research has shown that microbial community resilience and resistance can be driven by dominant community taxa (Bardgett and Caruso, 2020) and an aim of our research project was to explore the response of soil microbial communities to a release from the overabundant deer disturbance, we decided to focus on the top ten most abundant phyla and the top 20 most abundant genera at each site to identify the dominant microbial taxa in each community and measure variation of these taxa between site and location.

At all sites *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria,* and *Verrucomicrobia* were ubiquitous. *Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Planctomycetes, and Verrucomicrobia* are also commonly found across different soil environments at similar relative abundances to what our study found (Fierer *et al.,* 2012a). However, among the top ten most abundant phyla, there was variation in the presence of *Ascomycota* (present inside PT, FS; outside CN, EG, PT, FN, and FS) and

Euryarchaeota (present inside CN, CS, EG and FN; outside CS). The lack of representation of *Ascomycota* within the dominant phyla groups of most inside soils may reflect variability in the plant community. Ascomycetes have been found in association with different plant species when functioning as saprophytes and decomposers (Chee-Sanford, 2008), and have been found to be most abundant at intermediate levels of tree species richness (Zhang *et al.*, 2018). This difference in the dominant microbial assemblage may also reflect differences in the stage of decomposition of leaf litter (Purahong *et al.,* 2016). On the other hand, the higher proportion of *Euryarchaeota* in the four inside samples, may be indicative of the mycorrhizal fungal communities at those sites. In an analysis of boreal forest soil archeal communities, Bomberg and Timonen (2007) observed greater *Euryarchaeota* frequency and diversity within the mycorrhizosphere, while soils outside the mycorrhizosphere had no *Euryarchaeota*. The exclusion of deer can significantly alter the arbuscular mycorrhizal community in soils and these effects may be dependent on time (11.5 y studied in Burke *et al.,* 2019). This may operate directly through impact from deer on herbaceous plant hosts of mycorrhizal fungi or indirectly through influences of other abiotic or biotic factors (Burke *et al.,* 2019). In our study, the higher frequency of soil samples containing *Euryarchaeota* as part of the dominant community inside exclosures may be indicative of a relationship of changing mycorrhizal communities as a result of deer exclusion.

Community assemblage at the genera level similarly showed extensive variation across all sites and between soil samples taken inside and outside exclosures. The top 20 most abundant genera by relative abundance were identified for each sample, which revealed 35 unique genera across all samples and sites (Table 5). Some of the variation in dominant genera was attributable only to inside soil samples (12 out of 35, five of which at FNI), whereas some were ubiquitous across all sites and locations (11 out of 35, 27% of microbial abundance), and the rest remained variable between samples taken inside and outside exclosures (12 out of 35). The microbial communities we examined in soil samples from inside and outside the exclosures seemed to differ in their dominant microbial genera, except for EG and PT, which had complete overlap in their most abundant genera. Our results show significant differentiation between soil microbiomes exposed to overabundant deer and released from deer disturbance; however, there was no singular pattern in terms of the direction of response by soil microbial communities. This may be attributable to how deer directly and indirectly alter edaphic variables alongside natural variation in inputs to soil from a heterogeneous dominant plant community. Soil pH, for example, is a known environmental constraint on microbial species and has been demonstrated to drive microbial community assemblage and diversity across varying spatial scales and soil environments (Högberg *et al.,* 2007; Lauber *et al.,* 2009; Rousk *et al.,* 2010; Osborne *et al.,* 2011). Additionally, there is a strong correlation between bacterial abundance and soil pH (Fierer and Jackson, 2006). In our study, we found that at all sites, with the exception of EG, soil pH was lower inside compared to outside of the exclosures, suggesting that overabundant deer may alter soil acidity. Interestingly, other studies have found the opposite relationship with the directionality of pH differences; with more acidic soils observed in areas disturbed by deer compared to areas where deer were not present (Hiernaux *et al.,* 1999; Yong-Zhong *et al.,* 2005; Pei *et al.,* 2008; Kumbasli *et al.,* 2010; Maillard *et al.,* 2021). However, many of those studies examined deer effect in systems with more natural population sizes (Hiernaux, *et al.,* 1999; Yong-Zhong, *et al.,* 2005; Pei *et al.,* 2008; Kumbasli *et al.,* 2010) or represented sites that had much lower baseline pH levels in soils that were sampled on naturally, deer-free islands (Maillard *et al.,* 2021). The variation in soil pH across our sites and between samples taken inside and outside exclosures may have

contributed to the observed differences in the abundance and richness of microbial taxa. The composition of the forest vegetative community can also drive microbial community composition in soils (Carney and Matson, 2006; Dukunde *et al.,* 2019). Therefore, the site-by-site variation observed may be explained by the heterogeneous composition of the forest vegetation in those areas (Table 1). We found significant variability, when we compared pH across all soil sampled inside and outside exclosures, which we propose is at least in part, a result of the vegetation differences at each site. The dominant tree species present at our sampling locations were clearly different and past research has established that variability in litter type and quality can influence soil abiotic conditions (Satti *et al.,* 2003; Kohzu 2004; Wardle *et al.*, 2012; Prieto *et al.*, 2019; Veen *et al.,* 2019).

Microbial diversity and composition are also dependent on nitrogen availability and access to carbon resources. (Fierer *et al.,* 2007; Treseder, 2008; Fierer *et al.,* 2012; Siciliano *et al.,* 2014; Zhou *et al.,* 2017). Generally, herbivory will decrease the quantity and quality of litter inputs available, because palatable nitrogen-rich plant species are replaced by chemically protected nitrogen-poor counterparts (Bardgett and Wardle, 2003). It has also been proposed that deer may alter nutrient cycling and organic matter content in soils through the changes they impose on litter quality and quantity (Côté *et al.,* 2004) and additions from excrement and the change in soil bulk density through compaction (Butler and Kielland, 2008). Potential impacts of inputs from excrement and urine include the creation of nutrient hotspots that can serve as a source of nutrient flow (Pastor *et al.,* 1993*;* Moe *et al.,* 2008). The increase of nutrient availability affects plant community composition (Pastor *et al.,* 1993) and chemical intake (Moe *et al.,* 2008) as well as sodium (Liebig *et al.*,2006), nitrogen (Pastor *et al.,* 1993; Moe *et al.,* 2008), and carbon mineralization in the soil (Pastor *et al.,* 1993). Animal urine can cause the soil chemical and physical composition to vary as well as create elevated soil pH (Somda *et al.,* 1997; Kumbasli *et al.,* 2010). Deer excrement at our study sites were presumably abundant given that deer pellet count surveys from Feb 2012 estimated far greater deer compared to the actual number observed at Binghamton University (Parisio *et al.*, 2018). Therefore, it is possible that nutrient inputs from deer excrement and urine contributed to the observed differences in soil abiotic and biotic environments in our study. However, because we did not quantify deer excrement and urine at our sites, or their potential interactions with the soil microbial community, further research is required to understand their effects on the soil environment.

Additionally, overgrazing removes plant matter that would otherwise be deposited onto soils during senescence periods and recycled through decomposition. With a high density of grazing, plant material is consumed, and the nutrients are redeposited elsewhere through urine and fecal pellets or lost to annual cycling as they are incorporated into deer biomass. In our study, we found that on average, soil organic matter was higher inside compared to outside exclosures at all sites, with the exception of EG. Further, although we did not measure nutrients directly, soil electrical conductivity (EC) is often used as a proxy for nutrient availability in soils as it is used as a measure of soluble salt ions (*e.g.*, Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, NO_3^{-}; Adviento-Borbe *et al.,* 2006). When we compared soil conductivity, we found significant variability predominantly in soil samples from inside exclosures, whereas soil samples from outside exclosures were far more homogeneous. We also found that on average (with the exception of PT), EC was higher inside of the exclosures compared to outside, and at all sites and locations the recorded levels were low enough to be considered indicative of non-saline soils. These findings suggest that deer may be playing a role in altering the nutrient content of soils at our sites, potentially through (1) a reduction in plant litter quality driven by overgrazing, (2) the reduction of annual senescence-driven inputs of

plant nutrients, (3) the deposition of nutrients in "hotspot" locations elsewhere in the forest through urine and fecal pellets, or (4) a combination of all three. Subsequently, the microbial community, dependent on the quantity and quality of resource inputs (Eldridge *et al.,* 2017; Krishna and Mohan, 2017), can be expected to respond to deer exclusion.

There were exceptions to the general trends we recorded in this study, however, and this may be related to the length of time since deer were excluded from our study sites and/or the size of the exclosures themselves. Temporally, soil inside of our exclosures may not have fully recovered or diverged from the high deer intensity it was exposed to prior to the construction of the exclosures. Further studies are needed to explore the impacts of age and exclosure size on soils affected by overabundant deer, as our study was limited in this important aspect. Additionally, a consistent exception to many observed trends was site EG, which was the one site in our study that was part of the small isolated natural area on the lower campus, and may have been more greatly affected by the surrounding human activities. Additional research qualifying the soil microbial community in forest systems impacted by overabundant deer is needed and more detailed analysis of the role of nutrient additions and resource changes from deer and how they relate to microbial community structure and function may elucidate the mechanisms driving the homogenization effect of deer on the soil microbiome.

CONCLUSION

Overall, overabundant deer appear to have a homogenization effect on soil abiotic conditions and the soil microbial community. Homogenization at the vegetative community level as a result of overabundant deer has been demonstrated, especially in favor of deer resistant flora (Rooney *et al.,* 2004; Wiegmann and Waller, 2006; Rooney, 2009). Our study suggests that overabundant deer may also be having a similar homogenization effect on the soil microbiome, likely mediated through the regulation of nutrient and plant litter resources accessible to microbial communities. Biotic homogenization of soil microbial communities has been demonstrated in response to anthropogenic land-use changes (Rodrigues *et al.,* 2013; Goss-Souza *et al.,* 2017; Tian *et al.,* 2018). Further, our study shows that when overabundant deer are excluded, the microbial communities begin to shift, suggesting that there is the potential for recovery of highly affected soils or perhaps, a shift towards alternative stable states. These two possibilities require further analysis to determine the directionality of the changes to the soil microbiome upon deer exclusion.

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