

ABSTRACT

Title of Thesis:

SOIL MICROBIAL COMMUNITIES IN
URBAN STREAM RESTORATIONS AND
FORESTED SITES IN FAIRFAX COUNTY,
VA

Lindsay Irene Wood, Master of Science, 2022

Thesis Directed By:

Professor Stephanie A. Yarwood,
Department of Environmental Sciences and
Technology

Urbanization is rapidly occurring worldwide and can increase hydrological flows into urban streams and alter forest structure and soil properties. Stream restoration projects are ongoing in Fairfax County, Virginia in order to reconnect the channel to the floodplain and increase nutrient removal via microbially mediated processes.

Ecological assessment of urban forests is also ongoing to understand the ecosystem services that urban forests provide. Using Illumina sequencing and qPCR, the bacterial and fungal communities were analyzed between stream riparian zones and reference sites, and between different forest qualities. Fungal communities differed significantly after stream restoration and between forest quality types. qPCR was also used to quantify denitrifying genes between restoration types. Post restoration sites had higher abundances of *nirS*, while reference sites were higher in *nirK*. The high quality forest sites were most colonized by arbuscular mycorrhizal fungi and were highest in ectomycorrhizal fungal sequences.

SOIL MICROBIAL COMMUNITIES IN URBAN STREAM RESTORATIONS
AND FORESTED SITES IN FAIRFAX COUNTY, VA

by

Lindsay Irene Wood

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2022

Advisory Committee:
Professor Stephanie Yarwood, Chair
Professor Joseph Sullivan
Dr. Denise Akob
Joan Allen

© Copyright by
Lindsay Irene Wood
2022

Acknowledgements:

This research was funded by Fairfax County, VA. I would like to thank my advisor, Dr. Stephanie Yarwood for your help and support over the years and getting me to the finish line. I could not have done it without you. I would also like to thank the members of my committee (Dr. Joseph Sullivan, Dr. Denise Akob, and Joan Allen) for their help and expertise throughout this process. I would like to offer a sincere thank you to the members of the Yarwood and Baldwin lab group who assisted me in my fieldwork and trekked through miles of forest with me and helped me through my data analyses. Lastly, I would like to thank my friends and family for their endless support along the way.

Table of Contents

Acknowledgements:	ii
Table of Contents	iii
List of Tables	v
Table 2.1: Soil Properties across treatments. N denotes the number of watersheds per treatment category	v
Table 3.3: Alpha diversity measures across quality forest types, including average ASV counts per treatment. N denotes number of samples per treatment category	v
List of Figures	vi
Chapter 1: Introduction and Literature Review	1
References	7
Chapter 2: Soil microbial community composition differs between pre and post urban stream restoration sites	10
Abstract	10
Introduction	11
Methods	13
<i>Sample Collection</i>	13
<i>Soil Chemical and Textural Analyses</i>	14
<i>Quantification of Microbial Groups</i>	14
<i>Illumina sequencing</i>	15
<i>Data analysis</i>	16
Results	18
<i>Soil properties</i>	18
<i>Quantification of Microbial Groups</i>	19
<i>Microbial community analyses</i>	20
Discussion	22
<i>Soil properties</i>	22
<i>Quantification of Microbial Groups</i>	23
<i>Microbial community analyses</i>	25
Conclusions	28
References	35
Chapter 3: Soil Microbial communities within urban forest of different qualities in Fairfax County, VA	42
Abstract	42
Introduction	43
Materials and Methods	46
<i>Site Selection and Sampling</i>	46

<i>Soil chemical and textural analyses</i>	<i>47</i>
<i>Microbial biomass</i>	<i>47</i>
<i>Quantification of Microbial Groups</i>	<i>48</i>
<i>Illumina sequencing</i>	<i>48</i>
<i>AM fungal quantification</i>	<i>50</i>
<i>Data analyses</i>	<i>51</i>
<u>Results</u>	<u>52</u>
<i>Soil Properties and i-Tree Eco Metrics.....</i>	<i>52</i>
<i>Microbial biomass and Quantification of Microbial Groups.....</i>	<i>53</i>
<i>Microbial Community Analyses</i>	<i>54</i>
<i>AM Colonization</i>	<i>56</i>
<u>Discussion</u>	<u>56</u>
<i>Soil Properties</i>	<i>57</i>
<i>Quantification of Microbial Groups</i>	<i>58</i>
<i>Microbial Community Analyses</i>	<i>58</i>
<u>Conclusions</u>	<u>63</u>
<u>References</u>	<u>74</u>
Chapter 4: Conclusions	84
<u>References</u>	<u>89</u>
Appendices	90
<u>Appendix I. Chapter 2 supplemental information.....</u>	<u>90</u>
<u>Appendix II. Chapter 3 supplemental information.....</u>	<u>94</u>
Bibliography	97

This Table of Contents is automatically generated by MS Word, linked to the Heading formats used within the Chapter text.

List of Tables

Table 2.1: Soil Properties across treatments. N denotes the number of watersheds per treatment category.

Table 2.2: Alpha diversity measures across treatments, including average ASV counts per treatment. N denotes number of samples per treatment category.

Table 2.3: The mean relative abundances of denitrification genes from the 16S rRNA gene sequences calculated with the Tax4Fun2 R package and matched with the KEGG Orthology database for prokaryotes.

Table 3.1: i-Tree Eco plot information, including the number of tree species found at each plot, the dominant tree species per plot, as well as the average tree height per plot. Fine root samples to quantify AMF were sampled mainly from *Acer* trees and roots to quantify ECM fungal quantities were taken mainly from *Quercus* trees.

Table 3.2: Soil properties for low, mid, and high quality forest stands.

Table 3.3: Alpha diversity measures across quality forest types, including average ASV counts per treatment. N denotes number of samples per treatment category.

List of Figures

Figure 2.1: Quantitative PCR gene copy numbers for bacterial 16S rRNA gene abundance, fungal ITS gene abundance, and bacterial nitrite reductase functional gene abundances (*nirS* and *nirK*). Average copy numbers for each sample in a distinct stream watershed were taken. The means of those watershed averages were then taken for each restoration treatment, and then standard error of the mean (SEM) was calculated.

Figure 2.2: Relative abundance of the top 8 phyla from the bacteria 16S rRNA gene and fungal ITS communities. The figure is faceted by restoration treatment (post-, post-10-, pre-, and reference-).

Figure 2.3: Non-metric multi-dimensional scaling (NMDS) based on a bray dissimilarity matrix showing divergence of **a**: bacterial 16S rRNA communities and **b**: fungal ITS communities. Biplot arrows in each panel show the correlations of significant ($p < 0.05$) soil properties to the NMDS distances. Points are the averages of each sample from a stream watershed.

Figure. 3.1: i-Tree Eco plots in Fairfax County, VA. Blue circles mark the high quality sites (10 sites), green circles mark the mid quality sites (8 sites), and red circles mark the low quality sites (8 sites). High quality scores ranged from 69.5-73, mid quality scores ranged from 50-68, and low quality scores ranged from 38-49. B)

Figure 3.2: Relative abundance of the top 10 phyla from the bacteria 16S rRNA gene and fungal ITS gene communities.

Figure 3.3: The quantity of soil microorganisms as determined by A) 16S rRNA gene Q-PCR, B) ITS Q-PCR, and C) microbial biomass C determined by fumigation extraction. Means and SE bars are shown.

Figure 3.4: Non-metric multi-dimensional scaling (NMDS) based on a bray dissimilarity matrix showing divergence of **a**: bacterial 16S rRNA gene communities and **b**: fungal ITS communities. Stars show the mean of treatments with SE bars and significant environmental variables are shown as vectors in the upper left corner of panel A and lower left corner of panel B. i-Tree Eco quality scores are labels on points. Note: NMDS1 is on the y-axis.

Figure 3.5: Mean relative abundance of the primary lifestyle sequences, including ECM fungi, AMF, soil saprotrophs, and plant pathogens, subsetting from the fungal ITS sequence dataset and organized by quality type.

Figure 3.6: Percent AMF root colonization presented per i-Tree Eco plot quality score.

Chapter 1: Introduction and Literature Review

Urbanization is occurring at a growing rate in the U.S. and globally; within the next decade, more than 60% of the world's population will live in cities (United Nations, 2010). The urban environment is typically composed of less green space and more impervious surface. Urbanization also creates more hydrological connections between streams and the watershed through the construction of stormwater flow mechanisms, such as pipes and ditches (Elmore and Kaushal, 2008). These increased flows directly impact fluxes of organic matter, nutrients, and energy into urban streams (Kaushal and Belt, 2012), causing a multitude of problems for urban streams, including erosion and decreased bank stability (Leopold, Huppman, and Miller, 2005; Bernhardt and Palmer, 2007). These impacts ultimately result in stream channels that are not connected to their floodplain (Booth, 1990; Groffman et al, 2003), where nutrient removal via microbial processes occurs (Groffman et al, 2002). Bernhardt and Palmer (2007) found that most urban ecological research revealed biotic and biogeochemical impairment in urban stream systems. In a growing effort to manage pollution entering waterways, many urban areas are investing in stream restoration projects with the goal of restoring stream connection to the floodplain where nutrient removal by microbial processes can occur. Elmore and Kaushal (2008) reported that stream restoration in Baltimore, MD resulted in increased denitrification due to the restoration reducing incision and therefore allowing for more water flow into the riparian floodplain. Forested stream reaches can have greater stream width, nutrient input, and lower stream velocity (Sweeney et al, 2004) and therefore can have greater habitat availability for nutrient processing and denitrifying organisms (Vannote et al, 1980). Restoration projects, however, can initially impact ecosystem

function as they often involve removing sediment from the bank and vegetation from the riparian zone. The floodplains are replanted, but the microbial function of the restored floodplain is not well understood.

Organic matter content in the restored riparian zones of stream restorations is often lower when compared to natural reference sites (Gift et al, 2010), as restoration projects in urban streams tend to use materials that are low in carbon to accomplish bank stabilization and reducing incision, or they remove sediment from the bank and expose soil horizons low in organic matter (Bernhardt and Palmer, 2007). Organic matter buildup in the riparian soil is limited by plant production and microbial mediated litter decomposition, but itself is an important limiting factor for other microbial mediated processes like denitrification (Gift et al, 2010). Stream incision associated with urbanization can reduce the water table, creating aerobic conditions in soils that were hydric and anaerobic, no longer supporting denitrification (Groffman et al, 2003). Organic carbon (C) is essential for denitrification, as most denitrifying organisms are heterotrophic and require C (Burchell et al, 2007). Organic matter, and therefore organic C in the soil can also lower the oxygen levels in the floodplain, as the increased microbial activity creates a higher demand for oxygen, creating anaerobic conditions suitable for denitrification (Tiedje, 1983; Gift et al, 2010). Restored riparian floodplains are most likely to have lower levels of organic matter, and therefore organic C, and subsequently lower levels of denitrification when compared to reference floodplain soils. Overtime, however, restored riparian zones have been shown to have higher levels of soil organic matter compared to younger restoration sites (McMillan and Noe, 2017).

In addition to their importance in stream ecosystems, urban forests are increasingly being studied for their roles in mitigating urban problems, such as cooling urban heat islands (Moss et al, 2019), storing carbon (Nowak and Crane, 2002), slowing stormwater flow, and reducing energy demand in the built environment (Roy, Byrne, and Pickering, 2012). According to the United Nations, quantifying ecosystem services of urban forests is increasingly important, in regards to natural capital accounting in the global fight against climate change. U.S. federal and state resources and environmental policies have seen an uptake in ecosystem based management (EBM) strategies, but they remain largely undefined for urban forestry (Steenburg, Dulkner, and Nitoslawski, 2019). The i-Tree Eco tool is part of the USDA i-Tree software suite, based on the UFORE (Urban Forest Effect) model by Nowak and Crane (2000) and is increasingly being used in urban forest management, as it is capable of quantifying forest factors and valuing their ecosystem services (Moss et al, 2019). Song et al (2020) used the i-Tree Eco tool to evaluate the ecosystem services of green spaces in a Chinese city. They found that the trees in those spaces could store over 50,000 t of C and prevent over 120,000 cubic meters of runoff. Using i-Tree Eco in urban areas could aid in informing policy makers of the optimal structure and composition of urban greenspaces in new development projects.

While the use of i-Tree Eco for natural resource management is increasing, including in Fairfax County, VA where these studies take place, the analyses typically focus on the above-ground factors and not the soil properties or microbial cycling. Microbial communities can have large effects on forest ecosystem services; they influence rates of litter decomposition and nutrient cycling within the soil (Bodelier,

2011) and can impact soil quality, as the microbial composition and activity can alter biogeochemical cycling and the organic matter turnover within the soil (Tajik, Ayoubi, and Lorenz, 2020). Microbial processes are essential in the ecosystem processes that urban forests can provide, however, they are often overlooked in ecological studies. Balser et al (2006) concluded that understanding the role of microorganisms in ecosystem functioning and response to disturbance is limited by methodological differences between microbial and ecosystem ecologists. The practice of evaluating the ecosystem functions of the urban forest should therefore not overlook the role of the microbial communities.

Arbuscular mycorrhizal fungi (AMF) are found globally, forming symbiotic relationships with around 75% of extant plant species worldwide, and can regulate carbon, nitrogen, and phosphorus cycling in the soils and plants (Kivlin et al, 2011). AMF can indirectly influence soil organic matter through their influence on soil aggregation and plant communities, but have also been found to directly contribute to soil organic C and nitrogen (N) pools through production of glomalin (Rillig et al, 2001). Kivlin et al (2011) found that AMF species clustered phylogenetically based on site location and plant community, suggesting that shifts in plant communities due to climate change, or perhaps urbanization, can alter AMF communities, which in turn could alter plant and soil nutrient cycling processes. Ectomycorrhizal (ECM) fungi are also ubiquitous in forests worldwide and are drivers of forest ecosystem processes (Read et al, 2004). They distribute C, water, and other nutrients to the surrounding soil (Hogberg and Hogberg, 2002; Hobbie et al, 2006; Anderson and Cairney, 2007). Linde-Medina and Marcucio (2018) revealed that ECM fungal diversity in forests can be explained by environmental and host variables and concluded that the current environmental thresholds

used in ecosystem assessment tools undervalue the importance of soil factors relating to ECM fungal diversity.

The overall goals of our two studies were to 1) understand what changes in the microbial communities' composition after stream restoration in the riparian floodplain; and 2) understand how forest quality impacts the microbial communities. We aimed to understand if this knowledge can help us determine better riparian management strategies in future development projects, including stream restorations.

The first study examines the differences in the riparian floodplain microbial communities, including denitrifying gene abundance between restored, un-restored, and reference stream sites in Fairfax County, VA. We compared the bacterial 16S rRNA gene and fungal ITS communities within the floodplain soils of these sites in relation to the soil chemical properties, as well as assessed the bacterial and fungal gene quantities. We also assessed the gene quantities of the functional genes, *nirK* and *nirS*; two gene homologs for the enzyme nitrite reductase involved in microbial denitrification. We hypothesized that: 1) the composition of bacterial and fungal communities based on 16S rRNA gene and ITS sequences would differ between post-restored, pre-restored, and reference areas; 2) bacterial 16S rRNA gene and fungal ITS gene abundances would be higher in reference and restored sites compared to pre-restored sites; 3) we would observe an increase in denitrification functional genes (*nirK* and *nirS*) in the post-restoration compared to pre-restoration soils.

The second study investigates the relationship between urban forest qualities and the soil microbial communities, as well as arbuscular mycorrhizal fungal root colonization. We selected i-Tree sites previously analyzed by Fairfax County and

categorized them into quality types: high, mid, and low quality. We compared the soil bacterial 16S rRNA gene and fungal ITS communities between the quality types, as well as collected fine roots from each site to calculate and compare the percent colonization by arbuscular mycorrhizae fungi (AMF) at each site. First, we hypothesized that the soil bacterial and fungal communities would differ in quantity and composition between low, mid, and high-quality plots. Secondly, we predicted that there would be an increase in mycorrhizal fungi, detectable in sequencing libraries matched to fungal functional guilds and by the percentage of roots colonized by AMF.

References

- Anderson, I. C., & Cairney, J. W. G. (2007). Ectomycorrhizal fungi: Exploring the mycelial frontier. *FEMS Microbiology Reviews*, 31(4), 388–406. <https://doi.org/10.1111/j.1574-6976.2007.00073.x>
- Balser, T. C., McMahon, K. D., Bart, D., Bronson, D., Coyle, D. R., Craig, N., Flores-Mangual, M. L., Forshay, K., Jones, S. E., Kent, A. E., & Shade, A. L. (2006). Bridging the gap between micro—And macro-scale perspectives on the role of microbial communities in global change ecology. *Plant and Soil*, 289(1), 59–70. <https://doi.org/10.1007/s11104-006-9104-5>
- Bernhardt, E. S., & Palmer, M. A. (2007). Restoring streams in an urbanizing world. *Freshwater Biology*, 52(4), 738–751. <https://doi.org/10.1111/j.1365-2427.2006.01718.x>
- Bodelier, P. (2011). Toward Understanding, Managing, and Protecting Microbial Ecosystems. *Frontiers in Microbiology*, 2, 80. <https://doi.org/10.3389/fmicb.2011.00080>
- Booth, D. B. (1990). Stream-channel Incision Following Drainage-Basin Urbanization. *Journal of the American Water Resources Association*. <https://onlinelibrary-wiley-com.proxy-um.researchport.umd.edu/doi/abs/10.1111/j.1752-1688.1990.tb01380.x>
- Burchell II, M. R., Skaggs, R. W., Lee, C. R., Broome, S., Chescheir, G. M., & Osborne, J. (2007). Substrate Organic Matter to Improve Nitrate Removal in Surface-Flow Constructed Wetlands. *Journal of Environmental Quality*, 36(1), 194–207. <https://doi.org/10.2134/jeq2006.0022>
- Elmore, A. J., & Kaushal, S. S. (2008). Disappearing headwaters: Patterns of stream burial due to urbanization. *Frontiers in Ecology and the Environment*, 6(6), 308–312. <https://doi.org/10.1890/070101>
- Gift, D. M., Groffman, P. M., Kaushal, S. S., & Mayer, P. M. (2010). Denitrification Potential, Root Biomass, and Organic Matter in Degraded and Restored Urban Riparian Zones. *Restoration Ecology*, 18(1), 113–120. <https://doi.org/10.1111/j.1526-100X.2008.00438.x>
- Groffman, P. M., Bain, D. J., Band, L. E., Belt, K. T., Brush, G. S., Grove, J. M., Pouyat, R. V., Yesilonis, I. C., & Zipperer, W. C. (2003). Down by the riverside: Urban riparian ecology. *Frontiers in Ecology and the Environment*, 1(6), 315–321. [https://doi.org/10.1890/1540-9295\(2003\)001\[0315:DBTRUR\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2003)001[0315:DBTRUR]2.0.CO;2)
- Groffman, P. M., Boulware, N. J., Zipperer, W. C., Pouyat, R. V., Band, L. E., & Colosimo, M. F. (2002). Soil Nitrogen Cycle Processes in Urban Riparian Zones | Environmental Science & Technology. *Environmental Science & Technology*, 36(21), 4547–4552.

- Hobbie, E. A. (2006). Carbon Allocation to Ectomycorrhizal Fungi Correlates with Belowground Allocation in Culture Studies. *Ecology*, 87(3), 563–569. <https://doi.org/10.1890/05-0755>
- Högberg, M. N., & Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, 154(3), 791–795. <https://doi.org/10.1046/j.1469-8137.2002.00417.x>
- Kaushal, S. S., & Belt, K. T. (2012). The urban watershed continuum: Evolving spatial and temporal dimensions. *Urban Ecosystems*, 15(2), 409–435. <https://doi.org/10.1007/s11252-012-0226-7>
- Kivlin, S. N., Hawkes, C. V., & Treseder, K. K. (2011). Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 43(11), 2294–2303. <https://doi.org/10.1016/j.soilbio.2011.07.012>
- Leopold, L. B., Huppman, R., & Miller, A. (2005). Geomorphic Effects of Urbanization in Forty-One Years of Observation. *Proceedings of the American Philosophical Society*, 149(3), 349–371.
- Linde-Medina, M., & Marcucio, R. (2018). Living tissues are more than cell clusters: The extracellular matrix as a driving force in morphogenesis. *Progress in Biophysics and Molecular Biology*, 137, 46–51. <https://doi.org/10.1016/j.pbiomolbio.2018.01.009>
- McMillan, S. K., & Noe, G. B. (2017). Increasing floodplain connectivity through urban stream restoration increases nutrient and sediment retention. *Ecological Engineering*, 108, 284–295. <https://doi.org/10.1016/j.ecoleng.2017.08.006>
- Moss, J. L., Doick, K. J., Smith, S., & Shahrestani, M. (2019). Influence of evaporative cooling by urban forests on cooling demand in cities. *Urban Forestry & Urban Greening*, 37, 65–73. <https://doi.org/10.1016/j.ufug.2018.07.023>
- Nowak, D. J., & Crane, D. E. (2000). The Urban Forest Effects (UFORE) model: Quantifying urban forest structure and functions. In: Hansen, Mark; Burk, Tom, Eds. *Integrated Tools for Natural Resources Inventories in the 21st Century. Gen. Tech. Rep. NC-212. St. Paul, MN: U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station. 714-720., 212.* <http://www.fs.usda.gov/treearch/pubs/18420>
- Nowak, D. J., & Crane, D. E. (2002). Carbon storage and sequestration by urban trees in the USA. *Environmental Pollution*, 116(3), 381–389. [https://doi.org/10.1016/S0269-7491\(01\)00214-7](https://doi.org/10.1016/S0269-7491(01)00214-7)
- Read, D. J., Leake, J. R., & Perez-Moreno, J. (2011). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*. <https://doi.org/10.1139/b04-123>

Rillig, M. C., Wright, S. F., Nichols, K. A., & Schmidt, W. F. (n.d.). *Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils*. 11.

Roy, S., Byrne, J., & Pickering, C. (2012). A systematic quantitative review of urban tree benefits, costs, and assessment methods across cities in different climatic zones. *Urban Forestry & Urban Greening*, 11(4), 351–363. <https://doi.org/10.1016/j.ufug.2012.06.006>

Song, P., Kim, G., Mayer, A., He, R., & Tian, G. (2020). Assessing the Ecosystem Services of Various Types of Urban Green Spaces Based on i-Tree Eco. *Sustainability*, 12(4), 1630. <https://doi.org/10.3390/su12041630>

Steenberg, J. W. N., Duinker, P. N., & Nitoslawski, S. A. (2019). Ecosystem-based management revisited: Updating the concepts for urban forests. *Landscape and Urban Planning*, 186, 24–35. <https://doi.org/10.1016/j.landurbplan.2019.02.006>

Sweeney, B. W., Bott, T. L., Jackson, J. K., Kaplan, L. A., Newbold, J. D., Standley, L. J., Hession, W. C., & Horwitz, R. J. (2004). Riparian deforestation, stream narrowing, and loss of stream ecosystem services. *Proceedings of the National Academy of Sciences*, 101(39), 14132–14137. <https://doi.org/10.1073/pnas.0405895101>

Tajik, S., Ayoubi, S., & Lorenz, N. (2020). Soil microbial communities affected by vegetation, topography and soil properties in a forest ecosystem. *Applied Soil Ecology*, 149, 103514. <https://doi.org/10.1016/j.apsoil.2020.103514>

Tiedje, J. M., Sexstone, A. J., Myrold, D. D., & Robinson, J. A. (1983). Denitrification: Ecological niches, competition and survival. *Antonie van Leeuwenhoek*, 48(6), 569–583. <https://doi.org/10.1007/BF00399542>

van der Linde, S., Suz, L. M., Orme, C. D. L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrman, J., Grebenc, T., Gweon, H. S., Hansen, K., Jacob, F., Kristöfel, F., ... Bidartondo, M. I. (2018). Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature*, 558(7709), 243–248. <https://doi.org/10.1038/s41586-018-0189-9>

Vannote, R. L., Minshall, W. G., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*. <https://doi.org/10.1139/f80-017>

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & II, R. P. M. (2015). The urban stream syndrome: Current knowledge and the search for a cure. *Journal of the North American Benthological Society*. <https://doi.org/10.1899/04-028.1>

Chapter 2: Soil microbial community composition differs between pre and post urban stream restoration sites

Abstract

Ongoing stream restoration projects in Fairfax County, VA aim to reconnect the degraded streambed to the riparian floodplain, with the goal of nutrient removal and bank stabilization. The microbial communities of the riparian floodplains along stream restorations, however, are not well studied. The bacterial (16S rRNA gene) and fungal (ITS) quantities and community composition, as well as denitrification functional gene quantities (*nirK* and *nirS*) were measured in soil samples collected from four different stream restoration treatments: pre-restored sites, post restored sites, 10 year old post restored sites, and reference stream sites. Soil chemical properties were measured for the soil samples as well. The reference sites had significantly higher quantities of bacterial 16S rRNA genes, as well as *nirK* genes. Bacterial and fungal alpha-diversity measures did not differ between restoration groups, while the beta-diversity measures revealed the restoration treatment significantly altered the fungal communities.

Introduction

Billions of dollars are spent annually to restore urban streams that have experienced bank erosion, channel incision, and disconnection from the floodplain (Leopold, Huppman, and Miller, 2005; Bernhardt and Palmer 2007). Human activities have doubled nitrogen (N) input into terrestrial and aquatic ecosystems over that last century (Vitousek et al. 1997). In areas such as the Mid-Atlantic, USA, water quality concerns have led to new targets for reductions in maximum daily loads and served as the catalyst for extensive urban stream restorations. Natural floodplain soils experience periodic inundation, due to their connection to the channel and the groundwater and are conducive for N removal via denitrification (Kaushal et al, 2008; Gift et al, 2010; Roley et al 2012; McMillen and Noe, 2017). Although early stream restoration focused on stabilizing the channel (Bernhardt et al, 2005; Bernhardt and Palmer, 2007), today restoration methods such as natural channel design (NCD) reestablish the stream-floodplain connection.

Although stream restoration is considered an effective best management practice for improving water quality, metrics for gauging restoration success have had mixed results. There is good evidence that the restored streams have less runoff and channel incision (Bernhardt and Palmer, 2007), but traditional ecological metrics such as plant community composition or benthic invertebrates can be slow to show improvement post-restoration (Dosskey et al. 2010, Violin et al, 2011; Moreno-Mateos et al. 2017). Measuring 10 different ecological parameters, including canopy cover, Violin et al. (2011) found no improvement over degraded urban systems after 7 years in at least one NCD stream. However, the degree and type of disturbance can play a large role in limiting the time needed for ecosystem recovery. McNeish et al. (2017) documented recovery of benthic macroinvertebrates following removal of *Lonicera maackii* from the riparian buffer within a few months. It is unclear how successful restorations are at meeting long-term goals such as nutrient retention and restoration of floodplain function

(Thompson et al, 2018; Griffith and McManus, 2020) Unlike vegetation and benthic macroinvertebrates, microbial communities may reset quickly and more consistently to changing ecosystem conditions (Harris 2003, Anderson et al. 2013), potentially making them a more responsive indicator of restoration trajectories.

Soil microbial communities directly and indirectly influence floodplain soil nutrient dynamics (Phillips et al. 2013) and water quality (Bossio et al. 2006). For example, a wide variety of soil bacteria carry out denitrification (~10% of the population), such as from *Pseudomonas* species, and can be measured by quantifying functional genes. Increasing stream and floodplain connectivity can lead to increased soil organic matter (McMillan and Noe 2017). Both bacteria and fungi decompose plant inputs and mediate soil organic matter formation (Contrufo et al. 2013). Soil bacteria and fungi also influence plant productivity and health. Molecular methods have been used to assess the quantity and composition of soil microbial community across many ecosystems. These approaches have been used in a mid-western prairie restoration chronosequence, in which decade-scale post restoration microbial communities saw declines in richness and diversity (Barber et al. 2017) and in restored wetlands, in which microbial communities dramatically differed from their pre-restored agricultural counterparts (Bossio et al. 2006).

Over the last 10 years, 42 stream restoration projects, averaging 225 m each, have restored a total of 10,609 m of stream in Fairfax County, VA. We aimed to characterize the microbial communities within some of these stream restorations and compare them to non-restored analogs and to non-degraded reference streams. Stream restoration design is predominately NCD. Since 2004, Fairfax County has carried out ecological monitoring focusing on benthic invertebrates, water quality, vegetation, and soil physical and chemical parameters. Pairing the soil microbial work with Fairfax County's existing monitoring network, allowed us to directly measure ecosystem function at the scale of stream restoration interventions. We expected that the composition of bacterial and

fungal communities based on 16S rRNA gene and ITS sequences would differ between post-restored, pre-restored, and reference areas. We hypothesized that bacterial 16S rRNA and fungal ITS gene abundances would be higher in reference and restored sites compared to pre-restored sites and that we would observe an increase in denitrification functional genes (*nirK* and *nirS*) in the post-restoration compared to pre-restoration soils.

Methods

Sample Collection

Riparian floodplain soils were collected in the summers of 2017 and 2018 along 18 stream branches of the Potomac River (Table S1) at upstream, downstream, and central locations with each floodplain. Each sampling site consisted of 4-5 subplots per stream branch for a total consolidated area of 0.04 hectares. Soil samples for microbial analysis from each plot were composites of 6 randomly located points within each plot, making up a total of 83 consolidated soil samples from 18 different stream branches.

Sample sites were characterized as restored sites that are 3-4 years old (post), one restored site that was 10 years-old (post-10), degraded sites that will be restored (pre), and reference sites from Prince William Forest Park (ref); a protected forested area that serves as a reference floodplain of a natural stream system. Soils in Fairfax County are split amongst three geographical and physiological regions: the coastal plain in the eastern part of the county, the piedmont upland in the central part of the county, and lastly the triassic basin in the western portion of the county. Fairfax County has widely adopted natural channel design as their stream restoration approach with the aim to restore the function of the stream.

At each sampling location a 1 x 1 m plot was established approximately 1 m from the stream bank, in upstream, downstream, and central locations from the restoration, in an area that should represent a floodplain. A 7/8"-outer diameter, nickel plated soil probe

(AMS 33"L Nickel-Plated Soil Probe) wiped off with 70% ethanol was used to sample a 15 cm depth soil core. Six cores were taken in a plot and composited to make a single representative sample per site. A total of 83 soil samples were consolidated and living invertebrates and large seeds (e.g. acorns) removed. Samples were stored at -80°C until used for DNA extractions.

Soil Chemical and Textural Analyses

Approximately 200 ml of soil was sent to Waypoint Analytical, a laboratory in Richmond, VA. Parameters including, organic matter content (%OM) (Sparks, 1984), estimated nitrogen release (ENR), Mehlich-3 phosphorus (Mehlich, 2008), soil pH, Ca (ppm), Mg (ppm), and K (ppm) were measured for each soil sample, as well as a textural analysis to determine the textural classes and percentages of sand, silt, and clay.

Quantification of Microbial Groups

Soil DNA was extracted using QIAGEN'S DNeasy PowerLyzer PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) with the recommended 0.25 g of soil. This methodology has been adopted by the Earth Microbiome working group (www.Earthmicrobiome.org). Extractions were eluted to a final 100 μ l volume. The eluted DNA concentrations were determined using a Qubit Fluorometer (Life Technologies, Grand Island, NY) with high sensitivity fluorescent dye and buffer.

DNA samples were diluted to 2.5 ng/ μ l for quantitative PCR (qPCR). Four marker genes were quantified for each sample. The four genes of interest were 16S eubacterial rRNA (used to compare total bacterial biomass), the fungal ribosomal ITS region (used to compare total fungal biomass), and *nirK* and *nirS* (homologous genes coding for nitrogen reductase involved in microbial denitrification). The primers, thermal cycler run conditions, and run efficiencies can be found in supplementary table 2. A 2 μ l

aliquot of each dilution was added to a reaction mixture of SYBR green fluorescent dye (with Rox), 1 μ M forward and reverse primers, and sterilized nano-pure water for a total reaction volume of 20 μ l in each well of a 96-well plate. Each sample was run in triplicate with a 10-fold diluted plasmid standard set per plate for each of the functional genes.

Illumina sequencing

DNA samples were diluted to 5 ng/ μ l and amplified with the F515+adapter primer (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTA A-3') and the R806+adapter primer (5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACVSGGGTATCTA AT-3') to target the 16S rRNA gene V4 region. The fungal ITS ribosomal region was targeted using the ITS1F+adapter ('5- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAG TAA-3') and ITS2R+adapter ('3- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCGAT GC-5') primers (Caporaso et al, 2010). The reaction mix contained 3.5 μ l of the 5 ng/ μ l DNA, 7 μ l of each primer (1 μ M), and 17.5 μ l of ThermoScientific Phusion Flash High-Fidelity PCR Mastermix (Thermo Fisher Scientific) for a total of 35 μ l in each well. Run conditions were: 95°C for 3 minutes, 25 cycles of [95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds], and 72°C for 30 seconds. PCR product was run on a 1.25% agarose gel to check for bands.

Amplified DNA was then cleaned using AmpURE beads (Beckman Coulter, Pasadena, CA) following the Illumina MiSeq denature protocol (Illumina, 2019). Samples were then Indexed with the NexteraXT index kit V2 set C and a reaction mix of 5 μ l DNA, 5 μ l each of Index primers 1 and 2, 25 μ l of Thermo Scientific Phusion Flash High-Fidelity PCR Mastermix (Thermo Fisher Scientific, Waltham, MA), and 10 μ l of PCR grade water was made for a total of 50 μ l in each well. Samples were then amplified under the following conditions: 95°C for 3 minutes, 8 cycles of [95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds] and 72°C for 5 minutes. Samples were cleaned again with AMPure beads following the denature protocol. Samples were pooled into one library using 2 μ l aliquots and run on a Bioanalyzer 2100 (Agilent Technologies) to confirm the product size. The library was then amplified using qPCR with KAPA qPCR mastermix and primers to determine the concentration. The library was then diluted to 12 pM with a 30% phiX (Illumina) spike-in and run on an Illumina MiSeq at the USDA using a 600-cycle V3 cartridge (FC-420-1003; Illumina, San Diego, CA).

Data analysis

The gene copy numbers were determined for each sample after running the functional gene qPCR. The copy numbers were calculated using the equation:

$$660 \text{ g} * \text{bp of template} = \text{g DNA}$$

$$\frac{\text{ng of DNA} * 6.02 \times 10^{23}}{1 \times 10^9 * \text{g DNA}} = \# \text{ of gene copies}$$

The average gene copy number per gram of wet soil was calculated for each restoration class along with the standard error of the mean. R version 3.6.3 was used for all data analyses (R Core team, 2020). Nested ANOVAs were run to determine significant ($p < 0.05$) differences in the functional gene abundances. The qPCR copy numbers were reported by taking the averages from each stream watershed and then taking those means and averaging them for each restoration treatment. The post-10 restoration site was not included in the univariate correlation calculations because the samples were only taken from one stream watershed ($n=1$).

The Illumina sequence outputs for the 16S rRNA gene amplicon reads and ITS ribosomal reads were assembled using the Divisive Amplicon Denoising Algorithm 2 (DADA2 v1.16.0) package (Callahan et al, 2016) in R (v1.16.0). The DADA2 pipeline (v1.16) and DADA2 ITS pipeline (v1.8), respectively were followed in order to filter and trim the reads, dereplicate, perform sample interference, merge paired reads, remove any chimeras, and assign taxonomy using the Silva reference database (SILVA v132) and UNITE ITS database. The packages phyloseq (v1.30.0) (McMurdie and Holmes, 2013) and vegan (v2.5-7) (Okansen et al, 2019), were used to analyze the output amplicon sequence variant (ASV) table. ITS samples were rarefied to 300 sequences. Rarefaction is usually accomplished using the minimum sequence number, however, this number was 23 for the ITS dataset. In order to maintain the most sample coverage, 500 sequences for each sample were analyzed, although 7 samples were still too low to meet this amount of sequences and were ultimately lost during the rarefaction process. Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity matrices were run with the samples in order to understand the beta diversity between the restoration classes. The ordinations were organized by averaging the community data from each stream branch, and then averaging those values for each restoration type. Homogeneity of group dispersion was checked using the Vegan functions betadisper and permutest. The envfit function was used to test for correlations between the community composition of the

samples and various soil properties and identify the significant ($p < 0.05$) soil factors. Vectors with the significant correlations were fitted to the NMDS ordination plots. Nested permutational multivariate analysis of variance (perMANOVA) was used to test for statistical significance ($p < 0.05$) of stream watershed nested in restoration type with the adonis function. Significant factors were further tested through pairwise comparisons using permutation MANOVAs, examining differences between group levels on the Bray-Curtis dissimilarity matrices. This was done by running 999 permutations with the pairwise.perm.manova function from the RvadeMemoire package (v0.9.81) (Hervé, 2021) and Bonferroni corrections to adjust p values after multiple testing. Lastly, the R package Tax4Fun2 (v1.1.5) (Wemhuer et al, 2020) was used to obtain and match the relative abundances of functional genes within the 16S rRNA gene sequences to the KEGG Orthology database in order to investigate the mean relative abundances of denitrification genes across restoration treatments. Significance was tested by running ANOVAs with the mean relative abundances of the denitrification genes with restoration treatment.

Results

Soil properties

The post-restoration soils were significantly higher in organic matter content than the pre and reference sites (5.9 ± 0.6 , p-values = 1.0×10^{-7} , 0.03), soil pH (5.9 ± 0.1 , $p = 0.002$, 0.00006), and the percentage of clay (17 ± 1 , $p = 1.0 \times 10^{-7}$, 1.2×10^{-6}) (Table 2.1). Post-restoration sites also had more phosphorus than the pre and reference sites ($p = 1.0 \times 10^{-7}$, 1.0×10^{-6}), more potassium than the pre sites ($p = 0.00001$), and more levels of magnesium ($p = 0$, 0), and calcium ($p = 0$, 0) than the pre and reference sites. The only

post-10 site had lower concentrations of phosphorus, potassium, magnesium, and calcium compared to the pre and post-restoration sites, however, was excluded from univariate comparisons because $n=1$. Reference soils were the most acidic (5.2 ± 0.2) and had less clay than post restoration soils. Reference soils also contained the lowest levels of magnesium and calcium (ppm) compared to the other restoration treatments.

Quantification of Microbial Groups

The bacterial 16S rRNA gene copy numbers averaged 2.7×10^6 in the pre-restoration sites, 2.3×10^6 in the post-restoration sites, and 2.1×10^6 in the post-10 restoration site (Figure 2.1). The reference site had significantly more 16S rRNA gene copies (3.8×10^6 copies g^{-1} soil) than the post restoration sites ($p = 0.005$) as revealed by a nested ANOVA. The fungal ITS copy numbers were two orders of magnitude lower than the bacterial 16S rRNA gene copies (Figure 2.1). On average the restoration treatment ranged from 2.2×10^4 to 6.6×10^4 gene copies. Nested ANOVA revealed no significant ($p < 0.05$) differences between the fungal ITS copy number averages. The nitrite reductase genes (*nirS* and *nirK*) revealed opposite patterns within the restoration treatments (Figure 2.1). The reference sites averaged 1.3×10^6 *nirK* gene copies, which was several magnitudes higher than the averages for the pre, post, and post-10 restorations. Conversely, the reference sites averaged 7.2×10^6 *nirS* gene copy numbers, which was an order of magnitude lower than the pre, post, and post-10 restoration averages for the *nirS* gene. The pre, post, and post-10 restoration sites for both the nitrite reductase genes (*nirK* and *nirS*) averaged between 1.3 and 2.3×10^6 gene copies. Nested ANOVAs comparing treatments for the *nirK* and *nirS* genes revealed that the average copy numbers were significantly different between the reference and pre and post treatments for only the *nirK* gene ($p = 0.00, 0.00$).

Microbial community analyses

A total of 8.4×10^7 bacterial 16S rRNA gene sequences remained after the DADA2 processing. The sequences were rarefied to 25,000 reads per samples and two samples were excluded due to low sequence numbers. The top five phyla from the rarefied reads taken from the mean relative abundances were: *Thaumarchaeota*, *Verrucomicrobia*, *Acidobacteria*, *Nitrospirae*, and *Proteobacteria* (Figure 2.2). A large majority of denitrifying bacteria are found within the *Proteobacteria* (Shapleigh, 2013). The three classes of proteobacteria found within a subset of the relative abundances of *Proteobacteria* in the 16S rRNA gene dataset were *Alphaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria*. Investigating further, we found the presence of known denitrifying genera within these classes; *Pseudomonas*, *Geobacter*, *Burkholderia*, *Sphingomonas*, and *Mesorhizobium*. Analyses of the 16S rRNA gene sequences with the R package, Tax4Fun2, revealed no significance in the mean relative abundances of denitrification genes (matched to the KEGG Orthology database) between restoration treatments (Table 2.3). Neither the Shannon's diversity index nor the Simpson's diversity index differed between the treatments in the bacterial 16S rRNA gene communities (Table 2.2).

A total of 1.1×10^7 fungal ITS sequences passed the DADA2 quality processing. These reads were rarefied to 500, which was the highest read count that retained the most samples. Seven samples were excluded due to low sequence counts. The top five phyla from the rarefied reads taken from the mean relative abundances were: *Basidiomycota*, *Ascomycota*, *Mortierellomycota*, *Rozellomycota*, and *Glomeromycota* (Figure 2.2). There are around 66 ECM fungal lineages (Tedersoo and Smith, 2013) and most are found within the *Basidiomycota* and the *Ascomycota*, the top phyla found within this dataset. The *Glomeromycota* are the only phylum in which AMF belong to, and the 4 orders found in this dataset; *Archaeosporales*, *Diversisporales*, *Glomerales*, and *Paraglomerales*, consist of around 25 genera of known AMF (Begum et al, 2019). The

average number of ASVs within each restoration treatment, per the mean of the stream watersheds can be found in Table 2.2, along with the average Shannon's diversity index and Simpson's diversity index. The 10-year-old post restoration group on average had the highest Shannon's and Simpson's diversity indices but was excluded from variance testing because $n=1$ (Table 2.2). One-way ANOVAs on Shannon's and Simpson's diversity revealed no significant differences between the indices of restoration type (Shannon $p = 0.105$, Simpson $p = 0.338$).

Bacterial 16S rRNA gene composition significantly differed between each restoration treatment (perMANOVA $p = 0.001$) and a nested perMANOVA with stream watershed and restoration treatment also revealed that the bacterial 16S rRNA gene composition was different across stream watersheds as well ($p = 0.001$) (Figure 2.3a). Pairwise comparisons using perMANOVAs on the NMDS revealed that the post and pre restoration treatments were significantly different ($p = 0.006$), as well as the post and reference restoration treatments ($p=0.006$). P values were adjusted using the Bonferroni correction method. Group dispersion homogeneity was significant at $p = 0.001$. The organic matter content ($p=0.001$), clay content ($p=0.001$), magnesium content ($p=0.001$), calcium content ($p=0.001$), potassium content ($p=0.011$), cation exchange capacity (CEC) ($p=0.001$), mehlisch phosphorus ($p=0.001$), and pH ($p=0.001$) correlated to bacterial composition.

Fungal ITS composition was also significantly different between each restoration treatment (perMANOVA $p=0.001$) and nested perMANOVA with stream watershed nested in restoration treatment revealed the fungal ITS composition was significantly different across the stream watersheds ($p = 0.001$) (Figure 2.3b). Pairwise comparisons on the NMDS showed significant differences ($p < 0.05$) between the reference and pre sites ($p = 0.006$), the reference and post sites ($p = 0.006$), the reference and post-10 sites ($p = 0.018$), as well as the pre and post sites ($p = 0.048$) and the post and post-10 sites ($p = 0.018$). Group dispersion homogeneity was significant at $p = 0.001$. Organic matter

content ($p=0.002$), pH ($p=0.001$), clay content ($p=0.003$), magnesium content ($p=0.001$), calcium content ($p=0.001$), and CEC ($p=0.001$) correlated to fungal composition.

Discussion

The aim of this study was to pair our soil microbial work with Fairfax County's existing soil property measures with the goal of understanding how the differences in the soil environment of stream restorations affect microbial communities. The data from the 17 stream sites in this study supported our hypothesis that the composition of bacterial and fungal communities based on 16S rRNA gene and ITS sequences would differ between pre- and post- restored sites, however the mean relative abundance of denitrifying genes did not significantly differ between restoration treatments for either *nirK* nor *nirS*. We also expected to see the bacterial 16S rRNA and fungal ITS gene abundances would increase post restoration, but we did not detect differences in the quantity of genes between treatments. We did observe more *nirK* in reference sites, but did not observe difference between the quantity of the two denitrification genes between pre- and post- restoration.

Soil properties

The post restoration sites (aged 3-4 years) had the highest average concentration of organic matter (OM), a result that typically indicates higher microbial biomass (Bossio et al, 2006), although microbial biomass was not measured for this study. More microbial biomass in the soil can correlate to higher soil C and N concentrations (Rice et al, 1997), suggesting more microbial cycling is occurring (Bosatta and Ågren, 1994 and Bauhus and Khanna, 1999). The post restoration sites having the highest levels of OM could therefore suggest that the soil conditions after stream restoration support microbial nutrient cycling. The post restoration sites also had the highest clay content, which also

supports the idea that the post restoration sites could have higher C and N concentrations, since soils high in clay (or fine textured) impede mineralization of OM leading to higher C and N (Wang et al, 2003). Higher clay content also usually drives the pH lower in soil systems, however, the pH values on average were the highest compared to the other treatments. pH can have a big influence in driving soil microbial communities (Arroyo et al, 2015 and Kang et al, 2021), and while clay content does drive the pH lower, concrete in urban environments tends to increase the pH (Brown et al, 2011). It is possible the post restoration sites have more impactation, especially since stream restoration involves bulldozing and other construction tools (Yochum, 2018). The post restoration sites also had the highest concentrations of phosphorus, potassium, magnesium, and calcium, suggesting they are more prone to runoff from surround impervious surfaces and/or nearby lawns. The reference sites that were in a protected forested area, had the lowest magnesium and calcium concentrations, which would suggest they were less impacted by the urban building environment, as calcium and magnesium can be input into the soil from the weathering of building material (Craul, 1985 and Plyaskina and Ladonin, 2009).

Quantification of Microbial Groups

Quantitative PCR was used to estimate the total bacteria and fungi within the riparian soils, as well as to explore the number of nitrite reductase genes (*nirK* and *nirS*) corresponding to denitrification genes in the soils. The reference sites had the most 16S rRNA gene copies per gram of soil per watershed average, compared to the other restoration treatments, though not significant. This result suggests that stream degradation and the restoration process does impact the bacterial communities, possibly lowering bacterial populations per gram of soil. Quantitative PCR does not differentiate between living and dead cells (Nocker and Camper, 2006), however, and this result only indicates the presence of more bacterial genes in the reference sites. There could also be extracellular DNA that is stabilized in the environment after the organism dies and is

often soil texture dependent adhering more to clays (Anderson et al, 2012 and Ruppert et al, 2019). The fungal ITS gene copy data revealed a similar pattern, however, the post restoration sites had slightly more ITS gene copies than the reference sites per watershed average. The differences in copy numbers were not significant, however, the post-10 restoration site was excluded from the analysis of variance as the samples were only from one stream watershed (n=1). The post-10 site did have less ITS copy numbers per watershed average than the other treatments, but we do not know if this difference is significant. Stream degradation and restoration processes did not appear to have an effect on fungal ITS gene copy numbers in the soils.

Denitrification is a microbially mediated process that involves a myriad of diverse bacteria from across over 60 different genera and make up around 5% of total soil microbial communities (Philippot et al, 2007). Some common denitrifiers are members of the phyla *Firmicutes*, *Actinobacteria*, *Bacteriodes*, and *Proteobacteria* (Bowen et al, 2016). Because denitrification occurs anaerobically, when the floodplain is saturated, there may be peaks of denitrification activity (Fellows et al, 2011), which would suggest a channel connection to the floodplain (McMillan and Noe, 2017). Establishing and maintaining this floodplain connection is often the goals of stream restoration. Targeting functional genes, with primers specific to genes in the denitrification pathway, is typically how denitrification ecology is studied (Bowen et al, 2016). We investigated two gene homologs involved in the nitrite reductase step of denitrification: *nirK* and *nirS*. While these two genes code for the same enzyme, *nirK* contains copper, while *nirS* contains cytochrome cd1 (Heylen et al, 2006). Bowen et al (2016) found that the two genes were not ecologically redundant. In that study, *nirK* was correlated to nitrous oxide production and better predicted denitrification enzyme activity (DEA) in acidic soils, while *nirS* better predicted DEA in alkaline soils and ratios of $N_2O:N_2$ ratios (Bowen et al, 2016). Our reference sites had the most acidic soils, as well as the most *nirK* abundance. Most denitrifying organisms will have one version of the gene, but not the other (Coyne

et al, 1989). Denitrifiers have been found within many bacterial lineages. In soils they most commonly belong to alpha-, beta-, and gamma- *Proteobacteria*, as well as *Aquificae*, *Bacterioidetes*, and *Firmicutes* (Philippot et al, 2007). Our results showed that per watershed average, the reference sites had magnitudes higher gene copies of *nirK* than the pre and post restoration sites ($p = 0.005$), while the opposite was true for the *nirS* gene. This finding is not entirely consistent with our 16S rRNA gene taxonomy findings; the restoration sites per stream branch average had less relative abundance of the *nirK* gene, however the differences in mean abundance were not significant. While this inconsistency should be reported, it is not very telling of the denitrifying community. Jones et al (2008) found that taxonomy is not a good indicator of *nirK* or *nirS* given that the phylogenies of the functional genes are inconsistent with 16S rRNA genes. The pre and post restoration sites had more gene copy numbers of *nirS* per watershed than the reference sites, which had one magnitude lower copy numbers. This suggests that the reference sites' soil conditions select for organisms that contain the *nirK* gene. Previous studies have observed that *nirK* organisms may produce more nitrous oxide (Graf et al. 2014) due to performing incomplete denitrification as they less often carry the *nosZI* gene (nitrous oxide reductase). This finding could be important for restoration success in that denitrifiers that carry *nirS* may mediate total denitrification and emitting less greenhouse gas (Bowen et al. 2020).

Microbial community analyses

The most abundant phyla of the bacterial 16S rRNA gene communities were the *Proteobacteria*, *Acidobacteria*, and *Verrucomicrobia*. These are all commonly found in soil and freshwater environments (Staley et al, 2013) however, the *Verrucomicrobia* can persist in environments that may be stressed due to human impact from urbanization

(Zhang et al, 2020). While denitrifying organisms are diverse and found across many taxa, the *Proteobacteria* contains a high abundance of denitrifying organisms in soils (Shapleigh, 2013). The classes within this dataset that were found to have known denitrifying genera were the *Alphaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*, including the genera; *Pseudomonas*, *Geobacter*, *Burkholderia*, *Sphingomonas*, and *Mesorhizobium*. The genera *Burkholderia* and *Mesorhizobium* are known to contain the *nirK* gene (Shapleigh, 2013). Different strains of *Pseudomonas* are known to have the *nirK* or *nirS* genes (Shapleigh, 2013). The most abundant fungal phyla were the *Basidiomycota* and *Ascomycota*. The *Ascomycota* are the most dominant soil fungal phylum, more so than the *Basidiomycota*, and exhibit more stress tolerance traits than the *Basidiomycota* (Egidi et al, 2019), which could explain their prevalence in our riparian systems. The *Basidiomycota* and the *Ascomycota* also hold the majority of ECM fungi genera, which consists of about 66 lineages overall (Tedersoo and Smith, 2013). The *Glomeromycota* was also a dominant fungal phylum within the fungal sequences. Further analysis of the orders within the *Glomeromycota* revealed the 4 orders that hold 25 genera of AMF between them.

The alpha diversity measures performed on both the bacterial 16S rRNA gene and fungal ITS communities, revealed no significant differences in either the species richness or evenness between the restoration groups. These results suggest that restoration and stream degradation do not significantly impact the diversity of soil bacterial and fungal communities. Conversely, a study in California found that stream restoration along the Trinity River increased the heterogeneity of microbial communities and the taxonomic and function diversities (Serrana et al, 2021). In order to better understand how the phylogeny impacted diversity, we tried also measured UniFrac distance for the 16S rRNA gene dataset. There were no significant groupings with this method and UniFrac distances cannot be used on fungal ITS datasets.

The beta diversity NMDS ordinations were a composite of the community data for each stream branch, i.e. the samples from each site, grouped by restoration treatment. The ordinations revealed that the bacterial community composition significantly differed between the post restoration sites and the 10-year-old post restoration sites when tested by perMANOVA. It is possible that the age of the restoration was the cause of this difference, however, it is interesting that the bacterial community compositions were not significantly different between the pre and post restoration sites, nor the post restoration sites and reference sites. The soil chemical factors that correlated to the bacterial composition and dispersion on the NMDS plot were OM content, clay content, Mg content, and pH. The post restoration sites had the highest organic matter and magnesium levels when averaged per mean of watershed per restoration treatment. OM content in the soil directly influences the microbial communities because it is a storehouse of nutrients (Mohammadi et al, 2011). Exchangeable Mg^{2+} cations in the soil have been found to correlate to bacterial gene copy numbers (Teste et al, 2021), as Mg promotes cell division (Webb, 1953).

Nested perMANOVAs of the NMDS ordinations of the fungal ITS community compositions revealed significant differences between the reference and pre, post, and post-10 sites, as well as the pre and post restoration sites. These results indicate that restoration processes and stream degradation have significant impacts on fungal community composition between groups. The significant soil chemical factors that correlated to the fungal composition were OM content, clay content, Mg content, and P content. Soil phosphorus (P) availability can limit microbial productivity (Vitousek et al, 2010), often encouraging symbiosis with ectomycorrhizal fungi (Meeds et al, 2021), in which nutrients like P are exchanged for carbon (Sellesse et al, 2006).

Conclusions

The stream restoration site in Fairfax County, VA selected for denitrifying organisms in the riparian floodplain soils that express the *nirS* homolog of the nitrite reductase enzyme, in which the product contains cytochrome cd1. The reference sites selected for the other nitrite reductase gene homolog, *nirK*. The soil pH could have contributed to environmental selection of one denitrifying organism over the other, as more acidic soils select for *nirK* organisms (Bowen et al, 2016) and our reference site soils were the most acidic. If restorations favor denitrifiers that carry *nirS*, this is positive from a greenhouse gas perspective because more complete denitrification may be occurring, as *nirK* organisms are less likely to contain the nitrous oxide reductase (*nosZI*) genes required for complete denitrification. The restoration process impacted the beta diversity of the fungal ITS community composition, but not the alpha diversity of either the bacterial or fungal communities. Restoration techniques that alter OM content, soil texture, cations like Mg, and P levels, may impact the fungal community composition, which could impact the vegetative communities that form symbiosis with mycorrhizal fungi. Future monitoring of stream restoration and evaluation of its success should include microbial methods. The more significant findings of this study suggest that taking additional soil samples in order to test for *nirK/nirS* gene abundances via qPCR would be valuable in understanding the scope of the denitrification cycle occurring in the floodplain soils. Additionally, focusing on mycorrhizal abundance through microscopy of root tips or qPCR of AMF and ECM fungal genes would aid in understanding the success of the floodplain vegetative functioning.

Table 2.1: Soil Properties across treatments. N denotes the number of watersheds per treatment category.

Treatments	N	Soil Texture			% Organic Matter	pH	Mehlich 3- P	K	Mg	Ca
		% Sand	% Silt	% Clay			ppm			
Pre	7.0	54 ± 5.0	37 ± 5.0	10.0 ± 1.0	3.8 ± 0.30	5.5 ± 0.10	9.5 ± 1.3	62 ± 8.0	130 ± 15	710 ± 130
Post	6.0	50.0 ± 3.0	33 ± 4.0	17.0 ± 1.0	5.9 ± 0.60	5.9 ± 0.10	20.0 ± 3.4	120 ± 23	200 ± 27	1200 ± 140
Post 10	1.0	74	14	12	3.6	5.8	6.2	53	109	648
Reference	3.0	55 ± 4.0	35 ± 6.0	10.0 ± 3.0	4.6 ± 0.60	5.2 ± 0.20	7.5 ± 2.4	101 ± 22	92 ± 15	520 ± 110

Table 2.2: Alpha diversity measures across treatments, including average ASV counts per treatment. The averages of each sample per stream branch, i.e. sampling site, were averaged together per restoration treatment. N denotes number of samples per treatment category.

	16S rRNA gene				ITS			
Treatments	N	ASVs	Shannon's	Simpson's	N	ASVs	Shannon's	Simpson's
Pre	7.0	2400 $\pm$ 130	7.1 $\pm$ 0.05	0.99 $\pm$ 0.00	7.0	70.2 $\pm$ 5.4	3.6 $\pm$ 0.05	0.94 $\pm$ 0.00
Post	6.0	2200 $\pm$ 180	7.0 $\pm$ 0.09	0.99 $\pm$ 0.00	6.0	82 $\pm$ 12	3.5 $\pm$ 0.19	0.92 $\pm$ 0.02
Post 10	1.0	2600	7.2	0.99	1.0	97	4.0	0.97
Reference	3.0	2100 $\pm$ 82	6.9 $\pm$ 0.02	0.99 $\pm$ 0.0	3.0	80.1 $\pm$ 3.0	3.6 $\pm$ 0.10	0.94 $\pm$ 0.02

Table 2.3: The mean relative abundances of denitrification genes from the 16S rRNA gene sequences calculated with the Tax4Fun2 R package and matched with the KEGG Orthology database for prokaryotes.

		<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	<i>narGZ, nxrA</i>
Treatments	N	K00368	K15864	K00376	K00370
Pre	7.0	9.3 ± 1.0E-05	0.34 ± 7.8E-07	2.4 ± 32E-05	9.8 ± 94E-05
Post	6.0	8.2 ± 8.2E-05	0.55 ± 9.4E-07	2.3 ± 34E-05	9.0 ± 74E-05
Post 10	1.0	6.7E-05	4.9E-06	1.6E-05	8.1E-05
Reference	3.0	7.1 ± 8.6E-06	0.25 ± 4.5E-07	2.0 ± 69E-05	7.8 ± 65E-05

Figure 2.1: Quantitative PCR gene copy numbers for bacterial 16S rRNA gene abundance, fungal ITS gene abundance, and bacterial nitrite reductase functional gene abundances (*nirS* and *nirK*). Average copy numbers for each sample in a distinct stream watershed were taken. The means of those watershed averages were then taken for each restoration treatment, and then standard error of the mean was calculated.

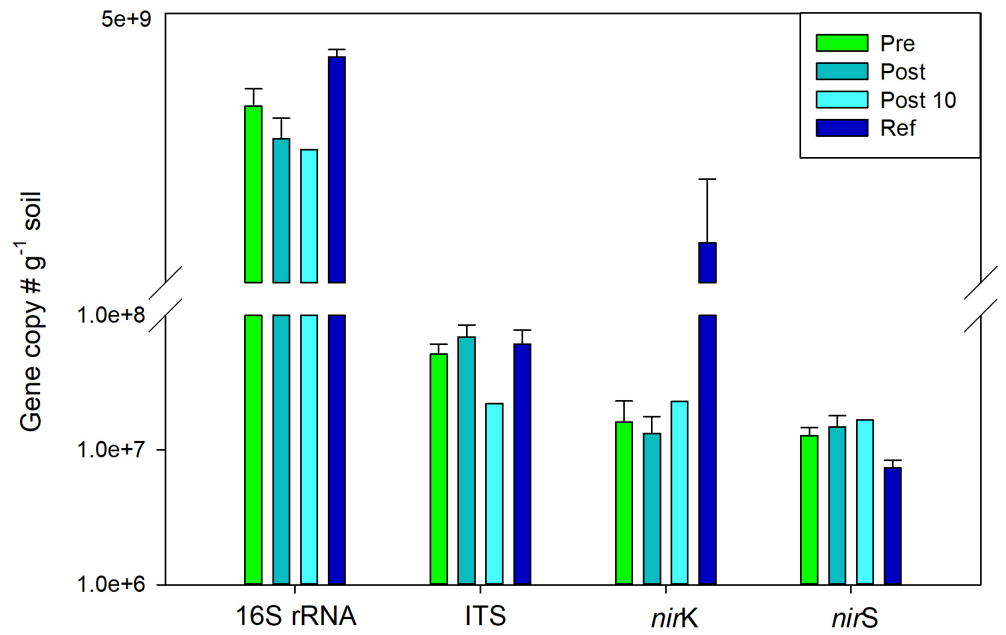


Figure 2.2: Relative abundance of the top 8 phyla from the bacteria 16S rRNA gene and fungal ITS communities. The figure is faceted by restoration treatment (post-, post-10-, pre-, and reference-).

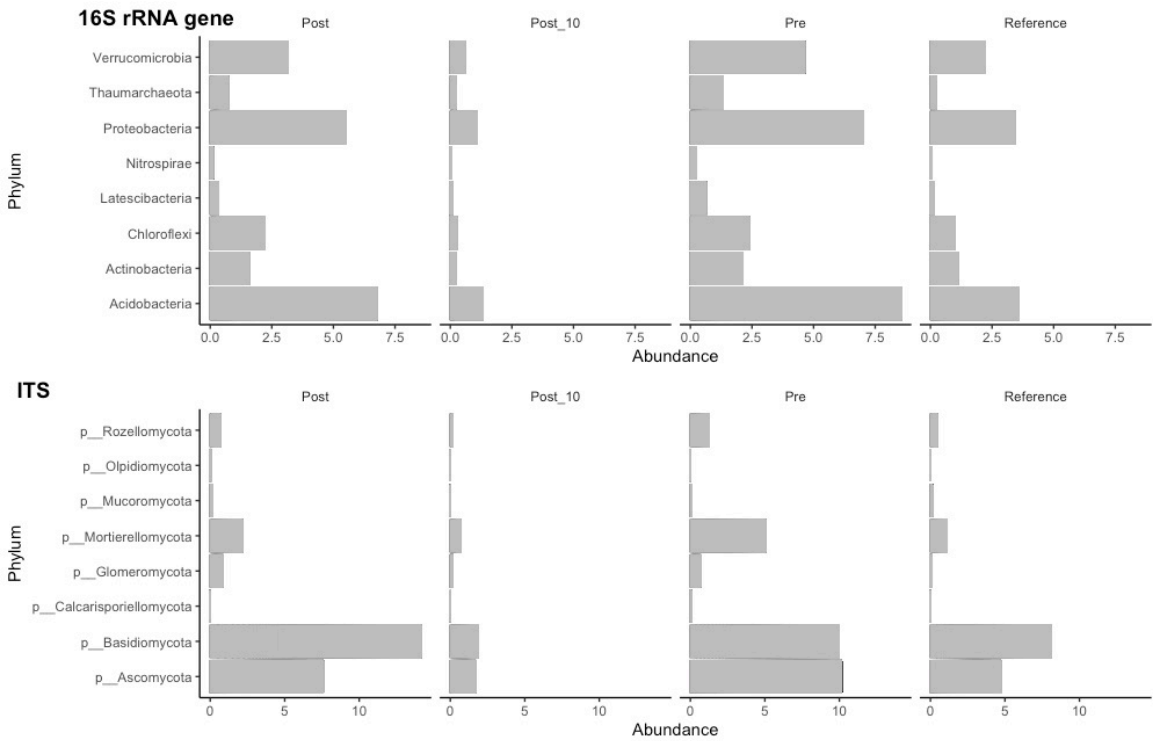
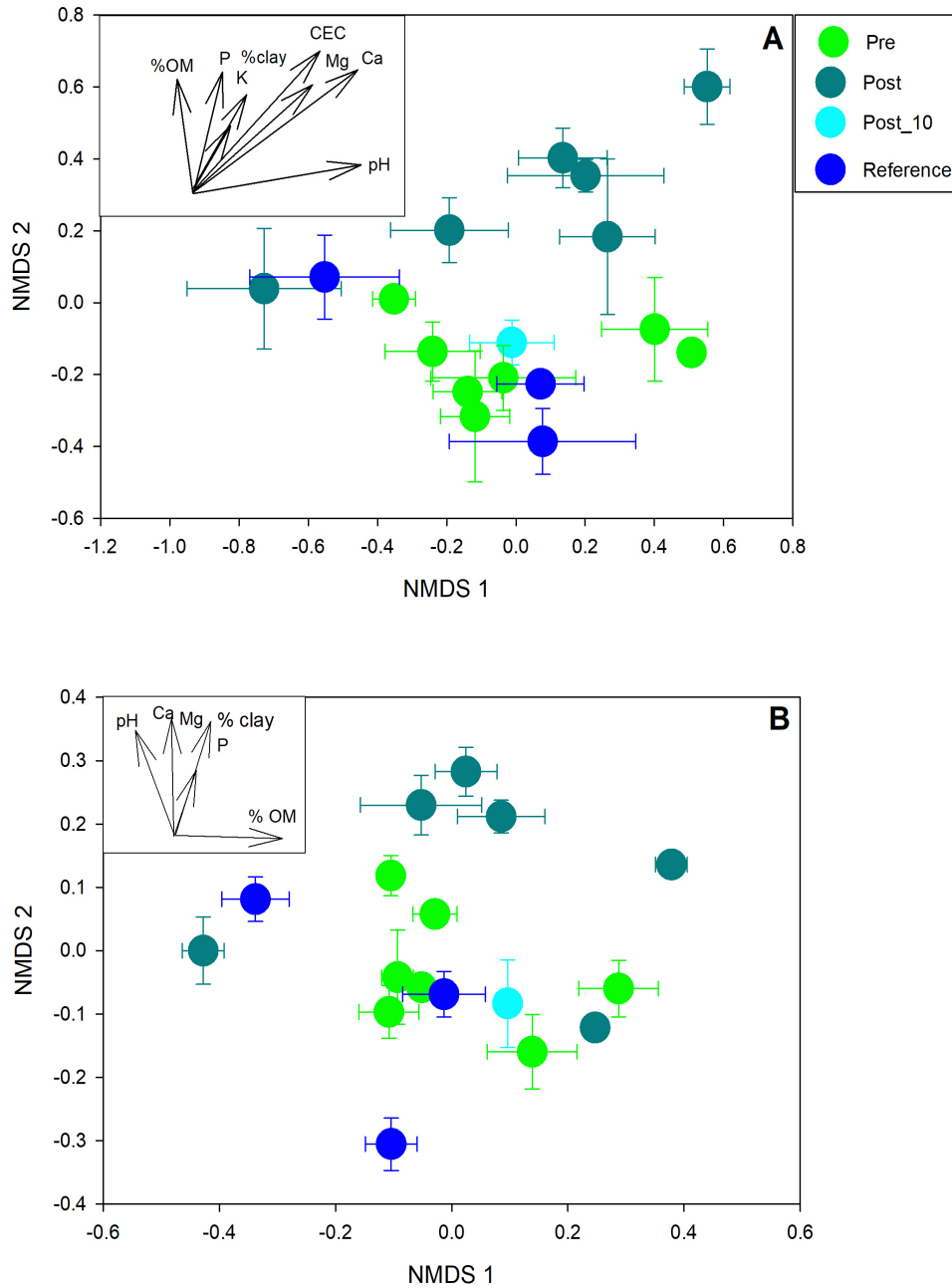


Figure 2.3: Non-metric multi-dimensional scaling (NMDS) based on a bray dissimilarity matrix showing divergence of **a**: bacterial 16S rRNA communities and **b**: fungal ITS communities. Biplot arrows in each panel show the correlations of significant ($p < 0.05$) soil properties to the NMDS distances. Points are the averages of each sample from a stream watershed.



References

- Andersen, K., Bird, K. L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjær, K. H., Orlando, L., Gilbert, M. T. P., & Willerslev, E. (2012). Meta-barcoding of ‘dirt’ DNA from soil reflects vertebrate biodiversity. *Molecular Ecology*, 21(8), 1966–1979. <https://doi.org/10.1111/j.1365-294X.2011.05261.x>
- Andersen, R., Chapman, S. J., & Artz, R. R. E. (2013). Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*, 57, 979–994. <https://doi.org/10.1016/j.soilbio.2012.10.003>
- Arroyo, P., Sáenz de Miera, L. E., & Ansola, G. (2015). Influence of environmental variables on the structure and composition of soil bacterial communities in natural and constructed wetlands. *Science of The Total Environment*, 506–507, 380–390. <https://doi.org/10.1016/j.scitotenv.2014.11.039>
- Barber, N. A., Lamagdeleine-Dent, K. A., Willand, J. E., Jones, H. P., & McCravy, K. W. (2017). Species and functional trait re-assembly of ground beetle communities in restored grasslands. *Biodiversity and Conservation*, 26(14), 3481–3498. <https://doi.org/10.1007/s10531-017-1417-6>
- Bauhus, J., & Khanna, P. (1999). *The significance of microbial biomass in forest soils* (pp. 77–110).
- Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Frontiers in Plant Science*, 10. <https://www.frontiersin.org/article/10.3389/fpls.2019.01068>
- Bergsten, A., Bodin, Ö., & Ecke, F. (2013). Protected areas in a landscape dominated by logging – A connectivity analysis that integrates varying protection levels with competition–colonization tradeoffs. *Biological Conservation*, 160, 279–288. <https://doi.org/10.1016/j.biocon.2013.01.016>
- Bernhardt, E. S., & Palmer, M. A. (2007). Restoring streams in an urbanizing world. *Freshwater Biology*, 52(4), 738–751. <https://doi.org/10.1111/j.1365-2427.2006.01718.x>
- Bernhardt, E. S., Palmer, M. A., Allen, J. D., Alexander, G., Barnas, K., Brooks, S., Carr, J., Clayton, S., Dahm, C., Follstad-Shah, J., Galat, D., Gloss, S., Goodwin, P., Hart, D., Hassett, B., Jenkinson R., Katz, S., Knodolf, G. M., Lake, P. S., ... Sudduth, E. (2005). Synthesizing U.S. River Restoration Efforts. *Science*, 308(5722), 636–637. <https://doi.org/10.1126/science.1109769>
- Bosatta, E., & Ågren, G. I. (1994). Theoretical analysis of microbial biomass dynamics in soils. *Soil Biology and Biochemistry*, 26(1), 143–148. [https://doi.org/10.1016/0038-0717\(94\)90206-2](https://doi.org/10.1016/0038-0717(94)90206-2)

Bossio, D. A., Fleck, J. A., Scow, K. M., & Fujii, R. (2006). Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biology and Biochemistry*, 38(6), 1223–1233. <https://doi.org/10.1016/j.soilbio.2005.09.027>

Bowen, H. (2016). *The Distribution and Function of Denitrification Genes: Exploring Agricultural Management and Soil Chemical Implications*. 138.

Bowen, H., Maul, J. E., Cavigelli, M. A., & Yarwood, S. (2020). Denitrifier abundance and community composition linked to denitrification activity in an agricultural and wetland soil. *Applied Soil Ecology*, 151, 103521. <https://doi.org/10.1016/j.apsoil.2020.103521>

Brown, S., Miltner, E., & Cogger, C. (2012). Carbon Sequestration Potential in Urban Soils. In R. Lal & B. Augustin (Eds.), *Carbon Sequestration in Urban Ecosystems* (pp. 173–196). Springer Netherlands. https://doi.org/10.1007/978-94-007-2366-5_9

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., ... Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335–336. <https://doi.org/10.1038/nmeth.f.303>

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, 19(4), 988–995. <https://doi.org/10.1111/gcb.12113>

Coyne, M. S., Arunakumari, A., Averill, B. A., & Tiedje, J. M. (1989). Immunological identification and distribution of dissimilatory heme cd1 and nonheme copper nitrite reductases in denitrifying bacteria. *Applied and Environmental Microbiology*, 55(11), 2924–2931. <https://doi.org/10.1128/aem.55.11.2924-2931.1989>

Craul, P. J. (1985). A Description of Urban Soils and Their Desired Characteristics. *Journal of Arboriculture*, 11(11), 330–339.

Dosskey, M. G., Vidon, P., Gurwick, N. P., Allan, C. J., Duval, T. P., & Lowrance, R. (2010). The Role of Riparian Vegetation in Protecting and Improving Chemical Water Quality in Streams¹: THE ROLE OF RIPARIAN VEGETATION IN PROTECTING AND IMPROVING CHEMICAL WATER QUALITY IN STREAMS. *JAWRA Journal of the American*

Water Resources Association, 46(2), 261–277. <https://doi.org/10.1111/j.1752-1688.2010.00419.x>

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., & Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature Communications*, 10(1), 2369. <https://doi.org/10.1038/s41467-019-10373-z>

Epp Schmidt, D. J., Pouyat, R., Szlavecz, K., Setälä, H., Kotze, D. J., Yesilonis, I., Cilliers, S., Hornung, E., Dombos, M., & Yarwood, S. A. (2017). Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nature Ecology & Evolution*, 1(5), 0123. <https://doi.org/10.1038/s41559-017-0123>

Fellows, C. S., Hunter, H. M., Eccleston, C. E. A., De Hayr, R. W., Rassam, D. W., Beard, N. J., & Bloesch, P. M. (2011). Denitrification potential of intermittently saturated floodplain soils from a subtropical perennial stream and an ephemeral tributary. *Soil Biology and Biochemistry*, 43(2), 324–332. <https://doi.org/10.1016/j.soilbio.2010.10.019>

Fierer, N., Jackson, J. A., Vilgalys, R., & Jackson, R. B. (2005). Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays. *Applied and Environmental Microbiology*, 71(7), 4117–4120. <https://doi.org/10.1128/AEM.71.7.4117-4120.2005>

Gift, D. M., Groffman, P. M., Kaushal, S. S., & Mayer, P. M. (2010). Denitrification Potential, Root Biomass, and Organic Matter in Degraded and Restored Urban Riparian Zones. *Restoration Ecology*, 18(1), 113–120. <https://doi.org/10.1111/j.1526-100X.2008.00438.x>

Graf, D. R. H., Jones, C. M., & Hallin, S. (2014). Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N₂O Emissions. *PLOS ONE*, 9(12), e114118. <https://doi.org/10.1371/journal.pone.0114118>

Griffith, M. B., & McManus, M. G. (2020). Consideration of spatial and temporal scales in stream restorations and biotic monitoring to assess restoration outcomes: A literature review, part 1. *River Research and Applications*, 36(8), 1385–1397. <https://doi.org/10.1002/rra.3692>

Groffman, P. M., & Crawford, M. K. (2003). Denitrification Potential in Urban Riparian Zones. *Journal of Environmental Quality*, 32(3), 1144–1149. <https://doi.org/10.2134/jeq2003.1144>

Harris, J. A. (2003). Measurements of the soil microbial community for estimating the success of restoration. *European Journal of Soil Science*, 54(4), 801–808. <https://doi.org/10.1046/j.1351-0754.2003.05559.x>

- Heylen, K., Gevers, D., Vanparys, B., Wittebolle, L., Geets, J., Boon, N., & De Vos, P. (2006). The incidence of nirS and nirK and their genetic heterogeneity in cultivated denitrifiers. *Environmental Microbiology*, 8(11), 2012–2021. <https://doi.org/10.1111/j.1462-2920.2006.01081.x>
- Kang, E., Li, Y., Zhang, X., Yan, Z., Wu, H., Li, M., Yan, L., Zhang, K., Wang, J., & Kang, X. (2021). Soil pH and nutrients shape the vertical distribution of microbial communities in an alpine wetland. *Science of The Total Environment*, 774, 145780. <https://doi.org/10.1016/j.scitotenv.2021.145780>
- Kaushal, S. S., Groffman, P. M., Mayer, P. M., Striz, E., & Gold, A. J. (2008). Effects of Stream Restoration on Denitrification in an Urbanizing Watershed. *Ecological Applications*, 18(3), 789–804. <https://doi.org/10.1890/07-1159.1>
- Mavimbela, L. Z., Sieben, E. J. J., & Procheş, Ş. (2018). Invasive alien plant species, fragmentation and scale effects on urban forest community composition in Durban, South Africa. *New Zealand Journal of Forestry Science*, 48(1), 19. <https://doi.org/10.1186/s40490-018-0124-8>
- McMillan, S. K., & Noe, G. B. (2017). Increasing floodplain connectivity through urban stream restoration increases nutrient and sediment retention. *Ecological Engineering*, 108, 284–295. <https://doi.org/10.1016/j.ecoleng.2017.08.006>
- McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, 8(4), e61217. <https://doi.org/10.1371/journal.pone.0061217>
- McNeish, R. E., Benbow, M. E., & McEwan, R. W. (2017). Removal of the Invasive Shrub, *Lonicera maackii* (Amur Honeysuckle), from a Headwater Stream Riparian Zone Shifts Taxonomic and Functional Composition of the Aquatic Biota. *Invasive Plant Science and Management*, 10(3), 232–246. <https://doi.org/10.1017/inp.2017.22>
- Meeds, J. A., Marty Kranabetter, J., Zigg, I., Dunn, D., Miros, F., Shipley, P., & Jones, M. D. (2021). Phosphorus deficiencies invoke optimal allocation of exoenzymes by ectomycorrhizas. *The ISME Journal*, 15(5), 1478–1489. <https://doi.org/10.1038/s41396-020-00864-z>
- Mehlich, A. (1984b). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, 15(12), 1409–1416. <https://doi.org/10.1080/00103628409367568>
- MiSeq System Denature and Dilute Libraries Guide (15039740)*. (n.d.).
- Mohammadi, K., Heidari, G., Khalesro, S., & Sohrabi, Y. (2011). Soil management, microorganisms and organic matter interactions: A review. *AFRICAN JOURNAL OF BIOTECHNOLOGY*, 10, 19840–19849. <https://doi.org/10.5897/AJBX11.006>

- Moreno-Mateos, D., Barbier, E. B., Jones, P. C., Jones, H. P., Aronson, J., López-López, J. A., McCrackin, M. L., Meli, P., Montoya, D., & Rey Benayas, J. M. (2017). Anthropogenic ecosystem disturbance and the recovery debt. *Nature Communications*, 8(1), 1–6. <https://doi.org/10.1038/ncomms14163>
- Nocker, A., & Camper, A. K. (2006). Selective Removal of DNA from Dead Cells of Mixed Bacterial Communities by Use of Ethidium Monoazide. *Applied and Environmental Microbiology*, 72(3), 1997–2004. <https://doi.org/10.1128/AEM.72.3.1997-2004.2006>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M., Szöcs, E., & Wagner, H. (2020). *Vegan community ecology package version 2.5-7 November 2020*.
- Peay, K. G., Kennedy, P. G., & Bruns, T. D. (2011). Rethinking ectomycorrhizal succession: Are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecology*, 4(3), 233–240. <https://doi.org/10.1016/j.funeco.2010.09.010>
- Philippot, L., Hallin, S., & Schlöter, M. (2007). Ecology of Denitrifying Prokaryotes in Agricultural Soil. *Advances in Agronomy*, 96, 249–305. [https://doi.org/10.1016/S0065-2113\(07\)96003-4](https://doi.org/10.1016/S0065-2113(07)96003-4)
- Phillips, R. P., Brzostek, E., & Midgley, M. G. (2013). The mycorrhizal-associated nutrient economy: A new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist*, 199(1), 41–51. <https://doi.org/10.1111/nph.12221>
- Plyaskina, O. V., & Ladonin, D. V. (2009). Heavy metal pollution of urban soils. *Eurasian Soil Science*, 42(7), 816–823. <https://doi.org/10.1134/S1064229309070138>
- Rice, C. W., Moorman, T. B., & Beare, M. (1997). Role of Microbial Biomass Carbon and Nitrogen in Soil Quality. In *Methods for Assessing Soil Quality* (pp. 203–215). John Wiley & Sons, Ltd. <https://doi.org/10.2136/sssaspecpub49.c12>
- Roley, S. S., Tank, J. L., Stephen, M. L., Johnson, L. T., Beaulieu, J. J., & Witter, J. D. (2012). Floodplain restoration enhances denitrification and reach-scale nitrogen removal in an agricultural stream. *Ecological Applications*, 22(1), 281–297. <https://doi.org/10.1890/11-0381.1>
- Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17, e00547. <https://doi.org/10.1016/j.gecco.2019.e00547>

Selosse, M.-A., Richard, F., He, X., & Simard, S. W. (2006). Mycorrhizal networks: Des liaisons dangereuses? *Trends in Ecology & Evolution*, 21(11), 621–628.
<https://doi.org/10.1016/j.tree.2006.07.003>

Serrana, J. M., Li, B., Sumi, T., Takemon, Y., & Watanabe, K. (2021). Profiling the microbial community structure and functional diversity of a dam-regulated river undergoing gravel bar restoration. *Freshwater Biology*, 66(11), 2170–2184.
<https://doi.org/10.1111/fwb.13824>

Shapleigh, J. P. (2013). Denitrifying Prokaryotes. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The Prokaryotes: Prokaryotic Physiology and Biochemistry* (pp. 405–425). Springer. https://doi.org/10.1007/978-3-642-30141-4_71

Soils_map_guide.pdf. (n.d.). Retrieved October 28, 2021, from
https://www.fairfaxcounty.gov/landdevelopment/sites/landdevelopment/files/assets/documents/pdf/publications/soils_map_guide.pdf

Sparks, D. L., Soil Science Society of America, & American Society of Agronomy. (1996). *Methods of soil analysis. Part 3, Part 3*. Soil Science Society of America : American Society of Agronomy.

Staley, C., Unno, T., Gould, T. j., Jarvis, B., Phillips, J., Cotner, J. b., & Sadowsky, M. j. (2013). Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. *Journal of Applied Microbiology*, 115(5), 1147–1158. <https://doi.org/10.1111/jam.12323>

Tedersoo, L., & Smith, M. E. (2013). Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, 27(3), 83–99. <https://doi.org/10.1016/j.fbr.2013.09.001>

Teste, F. P., Lambers, H., Enowashu, E. E., Laliberté, E., Marhan, S., & Kandeler, E. (2021). Soil microbial communities are driven by the declining availability of cations and phosphorus during ecosystem retrogression. *Soil Biology and Biochemistry*, 163, 108430. <https://doi.org/10.1016/j.soilbio.2021.108430>

Thompson, J., Pelc, C. E., Brogan, W. R., & Jordan, T. E. (2018). The multiscale effects of stream restoration on water quality. *Ecological Engineering*, 124, 7–18.
<https://doi.org/10.1016/j.ecoleng.2018.09.016>

Tuttle, A. K., McMillan, S. K., Gardner, A., & Jennings, G. D. (2014). Channel complexity and nitrate concentrations drive denitrification rates in urban restored and unrestored streams. *Ecological Engineering*, 73, 770–777.
<https://doi.org/10.1016/j.ecoleng.2014.09.066>

- Violin, C. R., Cada, P., Sudduth, E. B., Hassett, B. A., Penrose, D. L., & Bernhardt, E. S. (2011). Effects of urbanization and urban stream restoration on the physical and biological structure of stream ecosystems. *Ecological Applications*, 21(6), 1932–1949. <https://doi.org/10.1890/10-1551.1>
- Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., & Tilman, D. G. (1997). Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications*, 7(3), 737–750. [https://doi.org/10.1890/1051-0761\(1997\)007\[0737:HAOTGN\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2)
- Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20(1), 5–15. <https://doi.org/10.1890/08-0127.1>
- Wang, W. J., Dalal, R. C., Moody, P. W., & Smith, C. J. (2003). Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biology and Biochemistry*, 35(2), 273–284. [https://doi.org/10.1016/S0038-0717\(02\)00274-2](https://doi.org/10.1016/S0038-0717(02)00274-2)
- Webb, M. (1953). Effects of Magnesium on Cellular Division in Bacteria. *Science*, 118(3073), 607–611. <https://doi.org/10.1126/science.118.3073.607>
- Wemheuer, F., Taylor, J. A., Daniel, R., Johnston, E., Meinicke, P., Thomas, T., & Wemheuer, B. (2020). Tax4Fun2: Prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. *Environmental Microbiome*, 15(1), 11. <https://doi.org/10.1186/s40793-020-00358-7>
- Wiseman, P. E., & Wells, C. (2005). Soil Inoculum Potential and Arbuscular Mycorrhizal Colonization of *Acer Rubrum* in Forested and Developed Landscapes 7.
- Wortley, L., Hero, J.-M., & Howes, M. (2013). Evaluating Ecological Restoration Success: A Review of the Literature. *Restoration Ecology*, 21(5), 537–543. <https://doi.org/10.1111/rec.12028>
- Yan, L., Xie, C., Xu, X., & Che, S. (2019). The influence of revetment types on soil denitrification in the adjacent tidal urban riparian zones. *Journal of Hydrology*, 574, 398–407. <https://doi.org/10.1016/j.jhydrol.2019.04.071>
- Yochum, S. (2018). Guidance for Stream Restoration. *United States Department of Agriculture*, 112.
- Zhang, Y., Ji, G., Wu, T., & Qiu, J. (2020). Urbanization significantly impacts the connectivity of soil microbes involved in nitrogen dynamics at a watershed scale. *Environmental Pollution*, 258, 113708. <https://doi.org/10.1016/j.envpol.2019.113708>

Chapter 3: Soil Microbial communities within urban forest of different qualities in Fairfax County, VA

Abstract

Urban forests are increasingly important in Fairfax County, VA, as population and development grow at a steady rate. Providing climate change resilience and a variety of ecosystem services, such as pollution removal and cooling, urban forests are being widely studied in cities around the world, in order to understand the benefits of urban forest habitat to the community, as well as the risks they face from urbanization. The Fairfax County Forestry Department used the U.S. Forest Service's software tool, i-Tree Eco based on the Urban Effects (UFORE) (Nowak and Crane, 2000) model to survey forests across the county. We selected a subsample of their surveyed sites and divided them into three quality groups based on forest quality: high, mid, and low. We aimed to investigate the effects of urbanization on the soil characteristics, the bacterial and fungal abundance and community composition, and the colonization of tree roots by arbuscular mycorrhizal fungi (AMF). The most significant impacts that urbanization had on the forest quality types were increases in soil constituents like phosphorus (P), magnesium (Mg), and calcium (Ca), as well as decreases in AMF root colonization and decreased abundance of ectomycorrhizal (ECM) fungal sequences.

Introduction

Urban forests can provide climate change resilience. For example, they can mitigate the urban heat island effect through evapotransporative cooling (Rahman et al. 2018), improve air quality (Nowak et al. 2018), and may increase (C) sequestration (Livesley et al, 2016; Moss et al. 2019). Urban forests removed 16,500 tons of air pollution across 83 Canadian cities in 2010 (Nowak et al. 2018). Groffman et al. (1995) observed that urban forest soils had lower labile C and higher passive C than rural forests and a greater potential to sequester C. Recognizing the benefits of these systems, urban foresters seek to maintain and manage forest biodiversity and improve tree health. Using the U.S. Forest Service's i-Tree software, urban managers can collect information like tree species, leaf area, diameter at breast height (DBH), and canopy cover. These data can be synthesized to determine overall forest health (i-Treetools.org) and assign a score (1-82.5).

Urban forests face a host of challenges including pests, impervious surfaces, nutrient loading, and non-native species. They typically have less biodiversity and less native species than their rural counterparts (Vitousek et al, 1997). They also have higher soil nutrients, including nitrogen (N) (Pouyat and Trammell, 2019). Lower soil C and N ratios can increase litter decomposition and denitrification rates (Van der Heijden et al, 2008). Soil C is often a limiting factor of denitrification in urban forests due to the variable soil and vegetation conditions in the patchy forest ecosystems (Rosenblatt et al, 2001; Ullah and Faulkner, 2006; Gift et al, 2010), leading to less N removal. Urban forests typically have more edge habitat due to the volume of impervious surfaces. The edges are exposed to more sunlight, runoff, and fluctuations in humidity. These factors

can increase soil temperature and decrease moisture (Vitousek et al, 1994; McDonnell et al, 1997; Sun and Zhau, 2016). Soil compaction in urban environments, resulting from construction or increased traffic, can lead to changes in soil structure, and limits water and nutrient infiltration (Nawaz et al, 2013). Changes in above-ground vegetation composition due to urbanization can affect the below-ground microbial communities (Mitchell et al, 2010), but i-Tree and other quality tools do not assess root systems, soil properties, or the soil microbial community (Wortley et al, 2013).

Mycorrhizal fungal communities can impact forest communality structure and function (Karpati et al, 2010), yet urban fungal community dynamics are often overlooked in urban ecology studies (Newbound et al, 2010). Mycorrhizal fungi form symbiotic relationships with plant species, in which C is obtained by the fungi in exchange for nutrients (Newbound et al, 2010). AMF form symbiosis with over 75% of extant plant species (Kivlin et al, 2011). They can contribute to the nutrient acquisition, stress tolerance, and pathogen resistance (Newsham et al, 1995 and Lenoir et al, 2016). Forests with AMF symbionts maintain higher diversity through a network of mycorrhizae that redistributes symbiotic costs and benefits between trees of the same or different species (Fellbaum et al, 2014; Babikova et al, 2014). The hyphae of AMF infect the cortical cells within the fine roots of the symbiotic tree, forming “arbuscules” that exchange nutrients from the soil with the tree, in exchange for reduced C (Wang et al, 2017). Identifying and quantifying AMF symbiosis within urban forests could provide an additional variable in determining forest health.

Conversely to AMF, ectomycorrhizal (ECM) fungi create a mycelial mantle around the fine plant roots, forming a hartig net (Anderson and Cairney, 2007).

Ectomycorrhizal fungi are drivers of forest ecosystem processes (Read et al, 2004 and Nehls, 2008). They distribute C, water, and other nutrients to the surrounding soil (Hogberg and Hogberg, 2002; Hobbie et al, 2006; Anderson and Cairney, 2007). In urban forests, when trees are removed for development or soils are disturbed, ECM fungal populations decline (Epp Schmidt et al, 2017). Investigating the mycorrhizal and other soils microbial communities through high throughput sequencing can give an idea of the microbial mediated processes occurring in the urban forest.

Fairfax County, VA, an urban center in the greater DC metropolitan area, has experienced rapid population growth over the last century (fairfaxcounty.gov). From 1940 to 1990, the population increased nearly twenty-fold from 40,929 to 818,584 residents. Since the 90s, the county has experienced a stable 13% growth rate reaching 1.2 million residents in 2021 (fairfaxcounty.gov). The Fairfax County Urban Forest Management Division used the U.S. Forest Service's software tool, i-Tree Eco based on the Urban Effects (UFORE) (Nowak and Crane, 2000) model to survey forests across the county. A county-wide ecosystem services valuation, completed in 2017, measured 200 1/10-acre plots. Parameters such as cover, diversity and biomass were included. Using a subset of the county's i-Tree plots, we selected plots that had different i-Tree scores to test two hypotheses. First, we hypothesized that the soil bacterial and fungal communities would differ in quantity and composition between low, mid, and high-quality plots. Secondly, we predicted that there would be an increase in mycorrhizal fungi, detectable in sequencing libraires matched to fungal functional guilds and by the percentage of roots colonized by AMF.

Materials and Methods

Site Selection and Sampling

Fairfax County surveyed 200 i-Tree plots which ranged in quality from 1-82.5 points based on factors such as impervious surface, human impact, invasive plants, signs of pests, and tree health (Figure S1). In this chapter, a total of 26 i-Tree plots (0.04 hectares) were selected and categorized as high, mid, or low quality. We excluded plots with scores lower than 35 because they were not forested. Plot selection was designed to capture geographic variation across the county, but we were additionally limited to plots that had not seen substantial disturbance from 2017 to 2019 and by landowner permission. A pilot study was conducted in 2017 on 18 forested riparian plots in the Difficult Run watershed. The results from this study were used to conduct a power analysis to determine how many samples would be needed in each parameter (high, mid, or low quality) to report significance at a power of 0.8. The value was determined to be 8 plots. Ten plots were considered high quality (scores ranged from 69.5-73), eight plots were characterized as mid quality (scores ranging from 50-68), and eight plots were categorized as low quality (scores ranging from 38-49) (Figure 3.1).

In the Fall of 2019, the selected i-Tree plots were located based on GPS coordinates. Five soil cores (2 cm diameter x 15 cm depth) were taken aseptically at the center of each plot within a 1m x 1m zone. Individual cores were combined into plastic sampling bags, totaling 1 soil sample per plot, and any invertebrates or large seeds (e.g. acorns) were removed. Each plot sample was collected and returned to the lab the same day, where they were stored at -40°C for further analysis. Using a spade, fine root samples were collected at the drip line of two trees within each plot. One tree was an

ECM-associated tree (*Quercus* spp.) and the second was an AMF-associated tree (*Acer* spp., *Carya*, and *Liriodendron*). A full list of sampled tree species can be found in Table 3.1. Roots were kept moist in the field and washed in the lab with distilled water and then stored in 50% ethanol.

Soil chemical and textural analyses

Fifty grams of soil per sample was sent out for chemical and textural analyses (Waypoint Analytical Laboratories, Richmond, VA, Large, 2017). Organic matter (OM) content was determined based on loss on ignition and reported as a percent (Sparks, 1996). Phosphorus was determined by Mehlich-3 extractions (ppm) (Mehlich, 2008).

Microbial biomass

Microbial biomass C was determined using 2 g soil (dry wt.) in each fumigated and unfumigated sample container (Howorth and Paul, 1994). Fumigated samples were incubated under a chloroform vacuum for 48 hours. Both fumigated and unfumigated samples were extracted with 1 M K_2SO_4 for 1 hour at 30 rpm. Solutions were filtered through 42 Whatman filter paper pre-wetted with 1 M K_2SO_4 . Samples were analyzed for total organic carbon (TOC) on a Shimadzu analyzer. Microbial biomass C (MBC) was calculated using the formula:

- (1) $TOC \text{ (mg/L)} * \text{extractant volume (L)} = TOC \text{ (mg C)}$
- (2) $TOC \text{ (mg C)} / \text{g dry soil equivalents} = \text{mg C per g dry soil}$
- (3) $TOC_{\text{fumigated}} - TOC_{\text{unfumigated}} = \text{Total Extractable C (TEC, mg C / g dry soil)}$

$$(4) \quad \text{TEC} * \text{Carbon Efficiency Factor (CEF - 0.37)} = \text{MBC (mg C / g dry)}$$

Quantification of Microbial Groups

Soil DNA was extracted using a DNeasy PowerLyzer PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) with the recommended 0.25 g of soil. Lysis was performed using a FastPrep-24 Instrument (MP Biomedical, Solon, OH) set at 5.5 m/s for 45 seconds. Extractions were eluted to a final volume of 100 μ l and quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA or Grand Island, NY).

DNA samples were diluted to 2.5 ng/ μ l for qPCR analyses. Two marker genes were quantified for each sample. The two genes of interest were 16S eubacterial rRNA (used to compare total bacterial biomass) and the fungal ribosomal ITS region (used to compare total fungal biomass). (Table S2). A 2 μ l aliquot of each dilution was added to a reaction mixture of SYBR Green qPCR ReadyMix with Rox (Sigma, St. Louis, MO), 1 μ M forward and reverse primers, and sterile water for a total reaction volume of 20 μ l. Each sample was run in triplicate on a StepOnePlus Real-Time PCR thermocycler (Applied Biosystems, Foster City, CA) with a 10-fold diluted plasmid standard set per plate for each gene. Run conditions and primer sets can be found in table S2.

Illumina sequencing

For sequencing, DNA samples were diluted to 5 ng/ μ l and amplified with the F515+adapter primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTA A-3') and the R806+adapter primer (5'-

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACVSGGGTATCTA
 AT-3') to target the 16S rRNA gene V4 region. The fungal ITS ribosomal region was
 targeted using the ITS1F+adapter ('5-
 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAG
 TAA-3') and ITS2R+adapter ('3-
 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCGAT
 GC-5') primers (Caporaso et al, 2010). The reaction mix contained 3.5 μ l of the 5 ng/ μ l
 DNA, 7 μ l of each primer (1 μ M), and 17.5 μ l of ThermoScientific Phusion Flash High-
 Fidelity PCR Mastermix (Thermo Fisher Scientific, Waltham, MA) for a total of 35 μ l in
 each well. Run conditions were: 95°C for 3 minutes, 25 cycles of [95°C for 30 seconds,
 55°C for 30 seconds, 72°C for 30 seconds], and 72°C for 30 seconds. PCR product was run
 on a 1.25% agarose gel to check for bands.

Amplified DNA was then cleaned using AmpURE beads (Beckman Coulter,
 Pasadena, CA) following the Illumina MiSeq denature protocol (Illumina, 2019).
 Samples were then Indexed with the NexteraXT index kit V2 set C and a reaction mix of
 5 μ l DNA, 5 μ l each of Index primers 1 and 2, 25 μ l of ThermoScientific Phusion Flash
 High-Fidelity PCR Mastermix (Thermo Fisher Scientific, Waltham, MA), and 10 μ l of
 PCR grade water was made for a total of 50 μ l in each well. Samples were then amplified
 under the following conditions: 95°C for 3 minutes, 8 cycles of [95°C for 30 seconds, 55°C
 for 30 seconds, 72°C for 30 seconds] and 72°C for 5 minutes. Samples were cleaned again
 with AMPure beads following the denature protocol. Samples were pooled into one
 library using 2 μ l aliquots and run on a Bioanalyzer 2100 (Agilent Technologies) to
 confirm the product size. The library was then amplified using qPCR with KAPA qPCR

kit (KAPA Biosystems, Roche, Basel, Switzerland). The library was then diluted to 12 pM with a 30% PhiX (Illumina) spike-in and run on an Illumina MiSeq using a 600-cycle V3 cartridge (FC-420-1003; Illumina, San Diego, CA).

AM fungal quantification

Preserved roots were cut in 1 cm segments and cleared and dyed using a modified method by Brundrett (1984), due to the heavy pigmentation of tree roots. Firstly, roots were cleared by soaking in 10% KOH and autoclaved at 121°C for 45 minutes. After rinsing in tap water, roots were soaked in 5% bleach solution for 15 minutes. They were then rinsed in tap water and acidified in 1% HCl for 20 minutes. Roots were placed directly in 0.05% trypan blue in lactoglycerol solution for 2 hours (Burke, 2008). They were then transferred to 50% glycerol to de-stain overnight (Burke, 2008). Roots were mounted on slides in sets of 12 root segments with a drop of lactoglycerol. A glass coverslip was placed overtop the roots and the slide was sealed using clear nail polish along the borders of the coverslip. Slides were examined at 40x magnification using a brightfield microscope and the method outlined by McGonigle et al. (1990) in order to obtain a percentage of AM colonization. A mark of AM colonization included hyphae, arbuscules, hyphal coils, and/or vesicles. A mark of other fungal colonization included dark septate endophytes (DSE). No presence of fungi was recorded as well. Note: samples were collected to also quantify ECM fungi, but the ethanol preservation technique left root tips opaque and not usable for counts of ECM root tip colonization.

Data analyses

The bacterial 16S rRNA and fungal ITS gene copy numbers were analyzed for variance between quality types using a one-way ANOVA test in R. Tukey honest significant differences were calculated by performing multiple pairwise-comparisons between the means of the quality types. The one-way ANOVA and Tukey honest significant differences were also performed on the microbial biomass C data.

The Illumina sequence outputs for the 16S rRNA gene amplicon reads and ITS ribosomal reads were assembled and processed using the Divisive Amplicon Denoising Algorithm 2 (DADA2 v1.16.0) package (Callahan et al, 2016) in R (v1.16.0). The DADA2 pipeline (v1.16) and DADA2 ITS pipeline (v1.8), respectively were followed in order to filter and trim the reads, dereplicate, perform sample interference, merge paired reads, remove any chimeras, and assign taxonomy using the Silva reference database (SILVA v132) and UNITE ITS database. The packages phyloseq (v1.30.0) (McMurdie and Holmes, 2013) and vegan (v2.5-7) (Okansen et al, 2020), were used to analyze the output amplicon sequence variant (ASV) table. ITS samples were rarefied to 500 sequences. Rarefaction is usually accomplished using the minimum sequence number, however, this number was 23 for the ITS dataset. In order to maintain the most sample coverage, 500 sequences for each sample were analyzed, although 7 samples were still too low to meet this threshold and were excluded by the rarefaction step. Nonmetric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity matrices were run with the samples in order to understand the beta diversity between the quality types. Homogeneity of group dispersion was checked using the Vegan functions betadisper and permutest. The envfit function was used to test for correlations between the community

composition of the samples and various soil properties and identify the significant ($p < 0.05$) soil factors. Vectors with the significant correlations were fitted to the NMDS ordination plots. Permutational multivariate analysis of variance (perMANOVA) was used to test for statistical significance ($p < 0.05$) with the `adonis` function. Significant factors were further tested through pairwise comparisons using permutation MANOVAs, examining differences between group levels on the Bray-Curtis dissimilarity matrices. This was done by running 999 permutations with the `pairwise.perm.manova` function from the `RvaideMemoire` package (v0.9.81) (Hervé, 2021) and Bonferroni corrections to adjust p values after multiple testing. Fungal functional guilds were determined using the FungalTraits database (Pöhlme et al, 2021). The ITS sequence taxonomy object was fastmelted with the FungalTraits2021 csv file containing the primary lifestyle information and the `dplyr` package (v1.0.7) (Wickham et al, 2018) was used to filter amplicon sequences that belonged to the ECM fungi.

Results

Soil Properties and i-Tree Eco Metrics

The average quality score of the low-quality sites was 41.9 ± 0.9 , while the mid quality sites averaged 60.5 ± 1.8 , and the high quality sites averaged 71.3 ± 0.5 . The mid quality sites had the widest range of scores. The three-forest quality types all had similar soil textures. There was no discernable difference between the percentages of sand ($p = 0.96$), silt ($p = 0.94$), and clay ($p = 0.90$). The high-quality forest soils had the highest organic matter content ($4.9\% \pm 0.1$, $p = 0.47$) and were the most acidic (4.9 ± 0.1 , $p =$

0.13), but neither difference was significant. The low-quality sites had the highest mean levels of phosphorus and were significantly different compared to the high quality soils ($p = 0.02$). Potassium levels were not significantly different between the quality types, however the magnesium and calcium levels were both significantly different between the low and high quality sites (Mg $p = 0.05$, Ca $p = 0.01$) (Table 3.2).

Microbial biomass and Quantification of Microbial Groups

The average microbial biomass carbon (MBC) per forest quality type ranged from 59 to 74 μg of C per gram of soil (Figure 3.3c), with the high-quality sites averaging the most (74 ± 21). A one-way ANOVA testing for significance between treatments revealed no significant differences between the MBC per quality types ($p=0.51$).

The high-quality sites averaged more bacterial 16S rRNA gene copies per g soil ($7.1 \times 10^{10} \pm 2.3 \times 10^{10}$) than the mid and low quality sites ($3.7 \times 10^{10} \pm 2.7 \times 10^9$ and $3.7 \times 10^{10} \pm 8.6 \times 10^9$, respectively). However none of the average bacterial gene copy abundances were significantly different from each other ($p=0.256$) as tested by a one-way ANOVA along with Tukey pairwise comparisons between groups (Figure 3.3a).

The fungal ITS copy numbers were two orders of magnitude lower than the bacterial 16S rRNA gene copies. They averaged $1.6 \times 10^8 \pm 2.6 \times 10^7$ per g soil in the high quality sites, $7.0 \times 10^7 \pm 2.6 \times 10^7$ per g soil in the mid quality sites, and $9.2 \times 10^7 \pm 3.3 \times 10^7$ per g soil in the low quality sites. The high quality sites averaged more fungal ITS copy numbers than the mid and low quality sites, however, this difference was not significant ($p=0.0724$) based on a one-way ANOVA and Tukey pairwise comparisons (Figure 3.3b).

Microbial Community Analyses

16S rRNA gene

An average of 136,041 16S rRNA gene sequences were generated per sample. Samples were rarefied to the minimum sample count of 64,033 reads for downstream processing. The average number of ASVs per quality type can be found in Table 3.3 along with the average Shannon's diversity index and Simpson's diversity index per restoration treatment. The most abundant bacterial and fungal phyla, measured by mean relative abundance, can be found in Figure 3.2 A one-way ANOVA on Shannon's and Simpson's diversity indices revealed no significance between any of the quality types ($p=0.057$ and $p = 0.31$).

Significant ($p < 0.05$) vegetative and soil chemistry factors that affected the distances between samples amongst the Bray-Curtis dissimilarity matrix were: quality score ($p=0.012$), nitrogen release (ENR) ($p=0.044$), pH ($p=0.001$), cation exchange capacity (CEC) ($p=0.001$), magnesium content ($p=0.001$), and calcium content ($p=0.001$). (Fig 3.4a). The Bray-Curtis dissimilarity matrix revealed homogeneity of group dispersion ($p = 0.021$) and PERMANOVA tests revealed significant differences between the quality groups ($p = 0.003$). The quality groups were further investigated using pairwise comparisons, which revealed no significant differences between the high and low quality groups ($p=0.003$), but no significance between high and mid quality groups ($p=0.147$) and the mid and low quality groups ($p = 0.246$).

ITS

An average of 29,422 fungal ITS sequences were generated per sample. Samples were rarefied to the minimum sample count, 1733 reads for downstream processing. The

average number of ASVs per quality type can be found in Table 3.3, along with the average Shannon's diversity index and Simpson's diversity index per restoration treatment. There was no significant difference ($p < 0.05$) in alpha diversity between the quality types within the fungal sequences for either Shannon or Simpson diversity measures. ANOVA on Shannon diversity grouped by quality had a p-value = 0.412 and Shannon diversity had a p-value = 0.197.

The significant ($p < 0.05$) soil chemistry factors that affected the distances between samples amongst the Bray-Curtis dissimilarity matrix were quality score ($p=0.001$), calcium content ($p=0.001$), magnesium content ($p=0.001$), potassium content ($p=0.008$), pH ($p=0.001$), and CEC ($p=0.001$). Unlike with the 16S rRNA gene sequences, there was no homogeneity of group dispersion ($p=0.072$) within the Bray-Curtis dissimilarity matrix. PERMANOVA tests revealed significant differences between quality groups ($p=0.02$) and pairwise comparisons of these groups also revealed significant differences between the high and low quality groups ($p=0.009$), but not the high and mid ($p=0.201$) or mid and low ($p=0.405$) (Figure 3.4b).

In order to understand the ECM fungal abundance, the fungal ITS sequences are matched to the FungalTraits2021 database, containing the primary and secondary lifestyles of known fungi, as well as metabolic data. This step was necessary as we were not able to quantify the ECM fungi microscopically as the roots collected for this method were compromised. ASVs that were identified as being ECM as their primary lifestyle were analyzed for mean relative abundance by quality type. ASVs identified as AMF, soil saprotrophs, and plant pathogens were also analyzed for mean relative abundance by quality type. The relative abundance of ECM fungal associated sequences trended

toward increasing with quality of forest. The high-quality plots had the most ectomycorrhizal associated sequences compared to the mid and low quality plots, with low quality plots having the least amount of associated sequences (Figure 3.5). Forty-three different genera of ECM fungi from the Agaricomycetes, Pezizomycetes, Dothideomycetes, and the Eurotiomycetes classes were found within the ITS amplicon dataset. The saprotrophs also trended toward increasing with quality of forest and the AMF sequences trended toward decreasing with forest quality (Figure 3.5).

AM Colonization

Percent AMF colonization significantly increased with i-Tree quality score ($p=0.0038$). Further testing with Tukey honest significant different comparisons revealed that the high and low quality groups accounted for the significant difference ($p=0.003$, Figure 3.6). The high-quality sites (scores averaging 71.3 ± 0.5) clustered together between a range of AMF colonization of 40-80%. The “mid” quality sites (with scores averaging 60.5 ± 1.8) split with half below 40% AMF colonization and half above 40% AMF colonization. The low-quality sites (scores averaging 41.9 ± 0.9) trend below 40% AMF colonization, more so at around 30% colonization with a few outliers trending more like the mid and high-quality sites.

Discussion

As a continuously growing urban center in the greater Washington D.C. metropolitan area, Fairfax County, VA serves as an ideal case study on how the microbial communities are impacted by urban development. The goal of this study was to

understand how the presence/absence of various factors associated with the urban forest, such as invasive plant prevalence, pests/disease, edge impacts, and anthropogenic development might alter the soil bacterial and fungal communities. We predicted that the bacterial and fungal communities would differ in number and composition between the different forest quality levels. In addition, we hypothesized that both ECM and AMF fungi populations would increase with forest quality, as well as the root colonization by AMF.

Soil Properties

The low-quality sites had the highest levels of soil phosphorus, magnesium, and calcium. These sites typically scored low because they were anthropogenically impacted, i.e. impervious surface or lawn cover, and/or building development or restoration activities, in addition to tree health and invasive species impacts. Being adjacent to neighborhoods, roads, or other developed areas can leave forests susceptible to nutrient runoff from lawn fertilizers or storm water (Groffman et al, 2003; Whitney, 2010; Livesley et al, 2016). This could explain the higher levels of phosphorus in the low quality soils. Building erosion and construction materials can leach magnesium and calcium into the surrounding environment (Craul, 1985 and Plyaskina and Ladonin, 2009), which would suggest that the low quality forested soils were more severely impacted by urbanization. The microbial biomass was not significantly different between the i-Tree qualities, however, the high-quality sites averaged more MBC than mid or low qualities. Microbial biomass is often correlated to higher levels of C in the soil and more microbial nutrient cycling (Bosatta and Agren, 1994 and Bauhus and Khanna, 1999). The high-quality sites typically were in more established forests, with healthier trees, and less

invasive plants. Urbanization over time can reshape the natural forests, reducing green space, creating man-made boundaries that disconnect patch habitats, resulting in more forest fragments and edge habitat (Urban et al, 1987, Bergsten et al, 2013). This combined with ongoing development can lead to less well-established forests that are vulnerable to deer browse and invasive species cover (Mavimbela et al, 2018). These factors could lead to less microbial cycling.

Quantification of Microbial Groups

We expected to see differences in the bacterial and fungal gene copy numbers of the different quality sites. While there was more bacterial 16S rRNA and fungal ITS gene copies in the high-quality sites, these differences were not significant. Quantitative PCR does not differentiate between living and dead cells, and therefore can overestimate the active population (Nocker and Camper, 2006). This result really only indicates the presence of more bacterial and fungal genes in the high-quality sites. There could also be the presence of extracellular DNA which is soil texture dependent, with more stabilization on clay surfaces (Anderson et al, 2012 and Ruppert et al, 2019). These results suggest that urbanization did not have a discernable impact on bacterial and fungal cell populations within the soil and that forest stand quality did have an impact on the bacterial and fungal quantities.

Microbial Community Analyses

The top 3 phyla in the bacterial 16S rRNA gene communities in each quality type were *Acidobacteria*, *Proteobacteria*, and *Verrucomicrobia*. These phyla are commonly found in soil environments, however *Verrucomicrobia* has been known to persist in urban

environments (Zhang et al, 2020), which could explain its abundance in these urban soils. One of the most abundant genera within the soils was Candidatus *Udaeobacter* of the *Verrucomicrobia*. This genus is ubiquitous in many soils, and has been discovered to increase in abundance upon the addition of antibiotics to the soil (Willms et al, 2020). This finding has implications about the ability of this genus to not only resist antibiotics, but efficiently uptake leached nutrients from adjacent non-resistant bacteria within the soil. Having such a large proportion of this genus within the County's forested soils could therefore suggest the presence of antibiotics from the urban environment within the forested soils, furthering the urbanization impact. Another genus within the *Verrucomicrobia*, Candidatus *Xiphinematobacter*, was abundant across all forested soils, and has been observed to form endosymbiosis with the nematode species, *Xiphinema americanum* (Vandekerckhove et al, 2000). The *Acidobacteria* are abundant in soil environments and tend to be slow growing, but actively degrade plant carbon, and are tolerant to low nutrient levels, as well as fluctuation soil conditions (Männistö et al, 2012). The genus *Bryobacter*, belonging to the *Acidobacteria* was also found in nearly all of the soil samples. Only one species of *Bryobacter*, isolated from boreal *Sphagnum* peat bogs, has been described: *Bryobacter aggregatus*, inhabiting acidic wetlands and soils (Dedysh, 2019). The ability for this slow growing species to persist in the County's forested samples could suggest the presence of hydric soils. The nitrogen fixing genus, *Bradyrhizobium*, belonging to the *Proteobacteria*, was found in nearly all of the soil samples in each quality forest type, suggesting the plant communities within these sites have formed symbiotic relationships with the proteobacteria in order to fix nitrogen.

Shannon's and Simpson's diversity measures revealed no significant differences in the richness and evenness of bacterial or fungal species between quality types, however, further analysis using two-way ANOVAs to test correlation with the different plot health factors revealed that the quality score of the plots, as influenced by tree establishment/maintenance, and the dominant tree species in the plot, impacted the bacterial and fungal Shannon's diversity index. This result indicates that the dominant tree species in a forest, as well as the establishment of that forest, impacts the richness and evenness of the bacterial and fungal soil communities. The plots were most often dominated by *Quercus*, *Liriodendron*, or *Acer* species; the mid and low quality forests having several *Acer* dominated plots. Along the Eastern United States, many once dominated *Quercus* forested stands are now being dominated by shade-tolerant, fast growing species like *Acer rubrum* due to the lack of fire disturbance needed to maintain a *Quercus* overstory (Alexander and Arthur, 2014). As *Acer rubrum* presence increases, initial leaf litter decomposition increases, as well as N immobilization, but decreases with leaf litter contribution by the *Acer* species (Alexander and Arthur, 2014). The mid and low quality forested stands had several plots dominated by *Acer* species, while the high quality stands were only dominated by *Quercus* or *Liriodendron*. This could impact initial litter decomposition rates in the soil as the leaves fall. *Quercus* species are often associated with ECM fungal symbiosis (Cairney and Chambers, 1999). This relationship allows the roots of the tree to extend into the soil through the mycorrhizae network, possibly altering the soil nutrients available to the surrounding bacteria.

Both the 16S rRNA gene and fungal ITS NMDS ordination significantly separated by quality type, as revealed by perMANOVAs on the Bray distances,

specifically between the high and low quality groupings, suggesting that the effects of urbanization impact the bacterial and fungal community compositions in forested soils. These results do not indicate the effect of urbanization on microbial cycling or functioning though, and future studies should evaluate these processes. Soil chemical factors that were significantly correlated to Bray distance in both the bacterial and fungal sequences were soil pH, magnesium and calcium levels, and CEC. These soil factors, including OM, have been found to affect heavy metal toxicity and accumulation in urban soils in France and China (Waterlot et al, 2012 and Liu et al, 2016). Potassium levels significantly correlated to the differences in fungal community composition. Potassium levels have been shown to correlate to soil moisture (Luebs et al, 1956), which has a direct influence on the fungal community composition (Hawkes et al, 2010 and Deepika and Kothamasi, 2014).

When investigating the fungal ITS sequences based on primary lifestyle, we were able to determine how the ectomycorrhizal communities differed between forest quality types. These results showed that the higher quality sites had the highest mean relative abundance of ECM fungi, while the low quality sites had the lowest mean relative ECM fungal abundance. ECM fungi are active participants in tree-soil nutrient exchange (Selosse et al, 2006) and provide benefits to the vegetative community such as increased uptake of soil nutrients (Tibbett and Sanders, 2002). ECM fungi can be associated with N-limited forest soils, however, as they are opportunists that find a niche with vegetation lacking sufficient nutrient uptake (Kranabetter et al, 2008). Urbanization in the case of this study, in which lower quality forests were characterized by human development

impact, invasive species cover, and impacts to tree health and establishment, resulted in forests that had less abundance of ectomycorrhizal fungi.

Arbuscular mycorrhizal fungi can be an indicator of forest health (Gupta, 2020) in part because they supply mineral nutrient to the trees in exchange for C (Smith and Read, 2008) and form extensive mycorrhizal networks in the soil, connecting plant roots and serving as nutrient sinks for the surrounding soil microbial communities (Corradi and Bonfante, 2012). AMF can be sensitive to pollution and other anthropogenic stresses and are often used as an indicator of forest health (Leyval et al, 2002). We hypothesized that we would find higher percentages of AMF colonization in the higher quality forests, which were less impacted by urbanization. The AMF colonization was significantly higher in the roots of the high quality forests than the roots of the low quality forests, which were the most impacted by human development and influence. Davison et al (2011) found that AMF composition correlated to the age of the forest stands. The high quality forests in our study were typically more established with older trees. AMF populations can suffer from stresses in the host's environment (Bennett and Bever, 2008), which could explain why the low-quality sites, with more P, Mg, and Ca levels had lower percentages of AMF root colonization. Urbanization has a direct impact of AMF tree root colonization, which can lead to vulnerability of the trees in the form of loss of nutrient uptake and pathogen protection. Interestingly, we found that the mean relative abundance of AMF sequences decreased with forest quality, however, these sequences were only identified in two of the high quality sites. This could be because the AMF soil hyphal network was not as extensive as the ECM fungi network in these soils, though this metric was not measured in this study.

Conclusions

The most significant impacts that urbanization had on the forest quality types were increases in soil constituents like P, Mg, and Ca, as well as decreases in AMF root colonization and decreased abundance of ECM fungal sequences. The high-quality sites had significantly more root colonization of AMF, and ECM fungal sequences. The high quality sites also had greater OM content. Bacterial and fungal communities significantly differed between the high and low quality categories. These findings suggest that microbial metrics should be included in future efforts to evaluate urban forests using the i-Tree Eco metrics in order to fully understand the ecosystem services provided by the forest, both above and below ground. Microscopy or molecular methods in order to quantify mycorrhizal fungi should be utilized, as both the AMF and ECM fungi in this study were significantly different between the high and low quality forest types

Table 3.1: i-Tree Eco plot information, including the number of tree species found at each plot, the dominant tree species per plot, as well as the average tree height per plot. Fine root samples to quantify AMF were sampled mainly from *Acer* trees and roots to quantify ECM fungal quantities were taken mainly from *Quercus* trees.

Sample Number	Quality	Number of Tree Species	Dominant Tree Genus per plot	Average Tree Height (Ft)	Tree Sampled for AMF	Tree Sampled for ECM Fungi
1	High	13	<i>Quercus</i>	39.3	<i>Acer</i>	<i>Quercus</i>
2	High	7	<i>Quercus</i>	42.4	<i>Acer</i>	<i>Quercus</i>
3	High	6	<i>Liriodendron</i>	34.2	<i>Acer</i>	<i>Quercus</i>
4	High	6	<i>Quercus</i>	55.1	<i>Acer</i>	<i>Quercus</i>
5	High	8	<i>Liriodendron</i>	63.5	<i>Acer</i>	<i>Quercus</i>
6	High	8	<i>Quercus</i>	51.3	<i>Fagus</i>	<i>Quercus</i>
7	High	6	<i>Quercus</i>	50.5	<i>Acer</i>	<i>Quercus</i>
8	High	13	<i>Liriodendron</i>	50.1	<i>Acer</i>	<i>Quercus</i>
9	High	11	<i>Quercus</i>	29.4	<i>Acer</i>	<i>Quercus</i>
10	High	7	<i>Quercus</i>	46.8	<i>Acer</i>	<i>Quercus</i>
11	Mid	11	<i>Carya</i>	48.3	<i>Acer</i>	<i>Quercus</i>
12	Mid	19	<i>Quercus</i>	43.2	<i>Acer</i>	<i>Quercus</i>
13	Mid	11	<i>Quercus</i>	27.0	<i>Acer</i>	<i>Quercus</i>
14	Mid	5	<i>Quercus</i>	47.1	<i>Acer</i>	<i>Quercus</i>
15	Mid	8	<i>Acer</i>	49.9	<i>Acer</i>	<i>Quercus</i>
16	Mid	3	<i>Acer</i>	49.8	<i>Acer</i>	<i>Quercus</i>
17	Mid	5	<i>Platanus</i>	56.3	<i>Acer</i>	<i>Quercus</i>
18	Mid	9	<i>Quercus</i>	48.7	<i>Acer</i>	<i>Quercus</i>
19	Low	12	<i>Quercus</i>	25.5	<i>Juglans</i>	<i>Quercus</i>
20	Low	3	<i>Platanus</i>	38.5	<i>Platanus</i>	<i>Quercus</i>
21	Low	1	<i>Acer</i>	29.0	<i>Acer</i>	<i>Quercus</i>
22	Low	3	<i>Liriodendron</i>	68.0	<i>Acer</i>	<i>Pinus</i>
23	Low	9	<i>Liriodendron</i>	50.8	<i>Acer</i>	<i>Quercus</i>
24	Low	6	<i>Liriodendron</i>	49.0	<i>Acer</i>	<i>Quercus</i>

25	Low	6	<i>Acer</i>	24.2	<i>Acer</i>	<i>Quercus</i>
26	Low	9	<i>Acer</i>	45.2	<i>Acer</i>	<i>Quercus</i>

Table 3.2. Soil Properties for low, mid, and high quality forest stands.

Treatments	N	Quality Score	Soil Texture			% Organic Matter	pH	Mehlich 3-P (ppm)	K	Mg ppm	Ca
			% Sand	% Silt	% Clay						
Low	8	42 ± 0.9	52 ± 6.0	39 ± 5.0	8.0 ± 1.0	3.6 ± 0.4	5.4 ± 0.2	16 ± 2.3	74 ± 10	109 ± 25	540 ± 130
Mid	8	61 ± 1.8	53 ± 7.0	38 ± 5.0	9.0 ± 2.0	3.6 ± 0.4	5.0 ± 0.2	12 ± 2.5	45 ± 8.0	58 ± 7	290 ± 65
High	10	71 ± 0.5	51 ± 4.0	40 ± 4.0	9.0 ± 1.0	4.3 ± 0.5	4.9 ± 0.1	8.0 ± 1.4	53 ± 5.0	59 ± 9	190 ± 35

Table 3.3. Alpha diversity measures across quality forest types, including average ASV counts per treatment. N denotes number of samples per treatment category.

Treatments	16S rRNA gene				ITS			
	N	ASVs	Shannon's	Simpson's	N	ASVs	Shannon's	Simpson's
Low	8.0	2900 ±83	7.0 ±0.10	0.99 ±0.0	8.0	120 ±15	3.5±0.20	0.93±0.010
Mid	8.0	1900 ±120	6.8 ±0.10	0.99 ±0.0	8.0	150 ±17	3.8±0.10	0.95±0.010
High	10.0	2700 ±180	7.1 ±0.050	0.99 ±0.0	10.0	130 ±10.0	3.7±0.10	0.95±0.010

Figure 3.1. i-Tree Eco plots in Fairfax County, VA. Blue circles mark the high quality sites (10 sites), green circles mark the mid quality sites (8 sites), and red circles mark the low quality sites (8 sites). High quality scores ranged from 69.5-73, mid quality scores ranged from 50-68, and low quality scores ranged from 38-49.

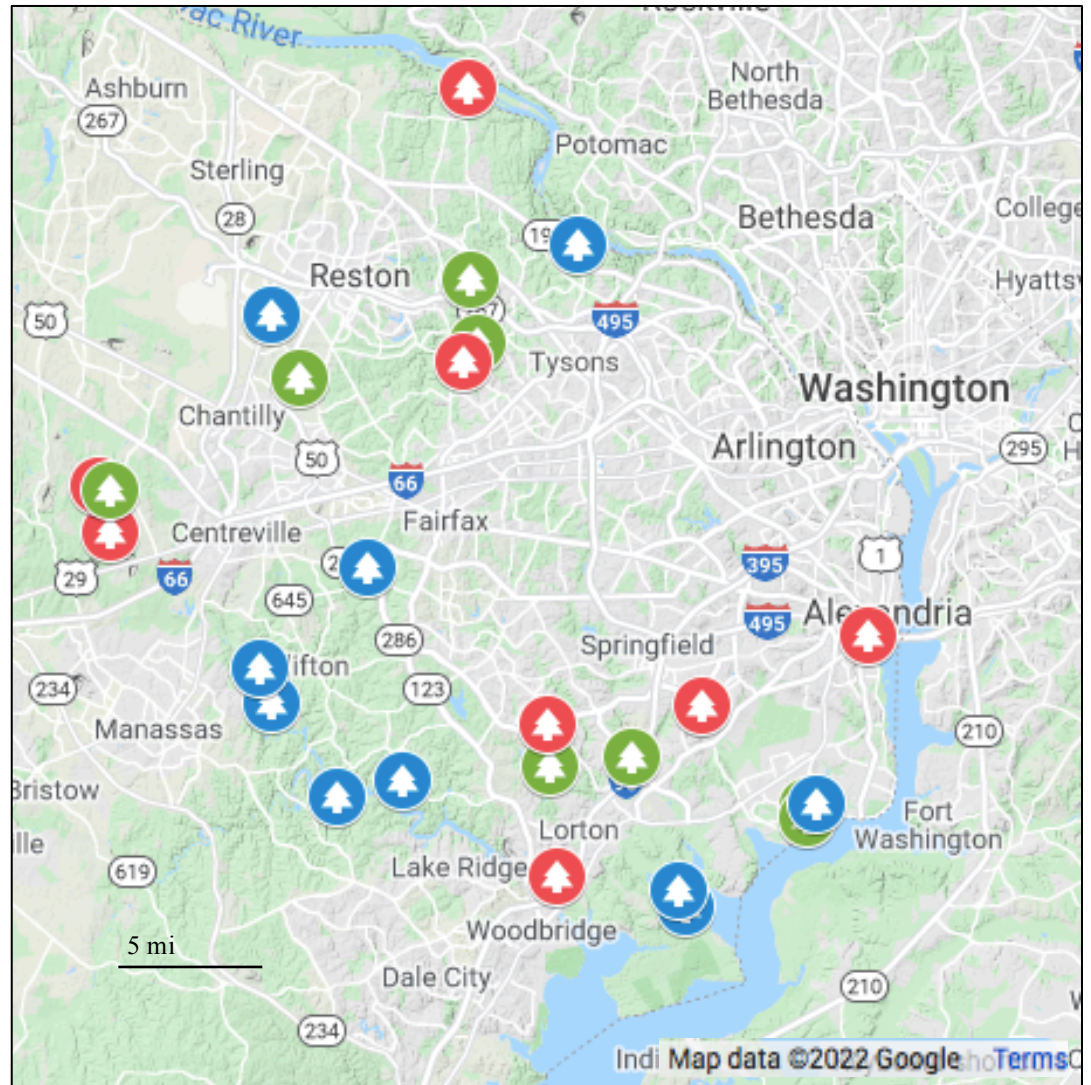


Figure 3.2. Relative abundance of the top 10 phyla from the bacteria (16S rRNA gene) and fungal (ITS) communities.

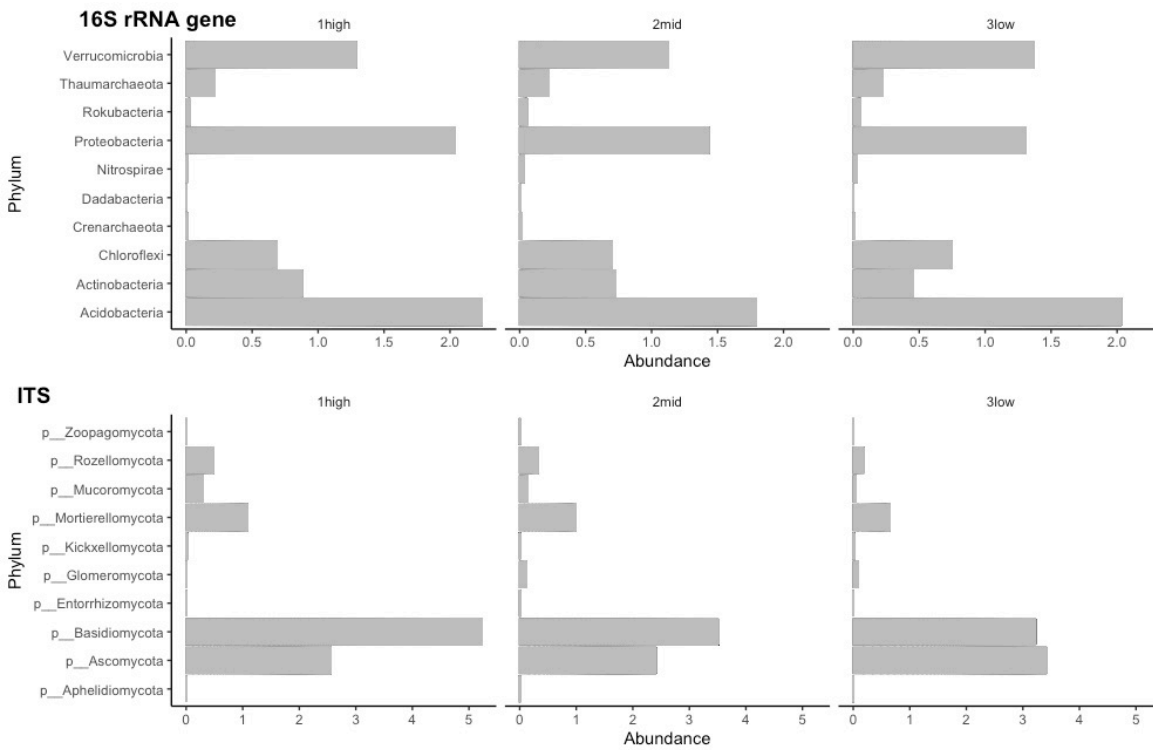


Figure 3.3. The quantity of soil microorganisms as determined by A) 16S rRNA gene qPCR, B) ITS qPCR, and C) microbial biomass C determined by fumigation extraction. Means and SE bars are shown.

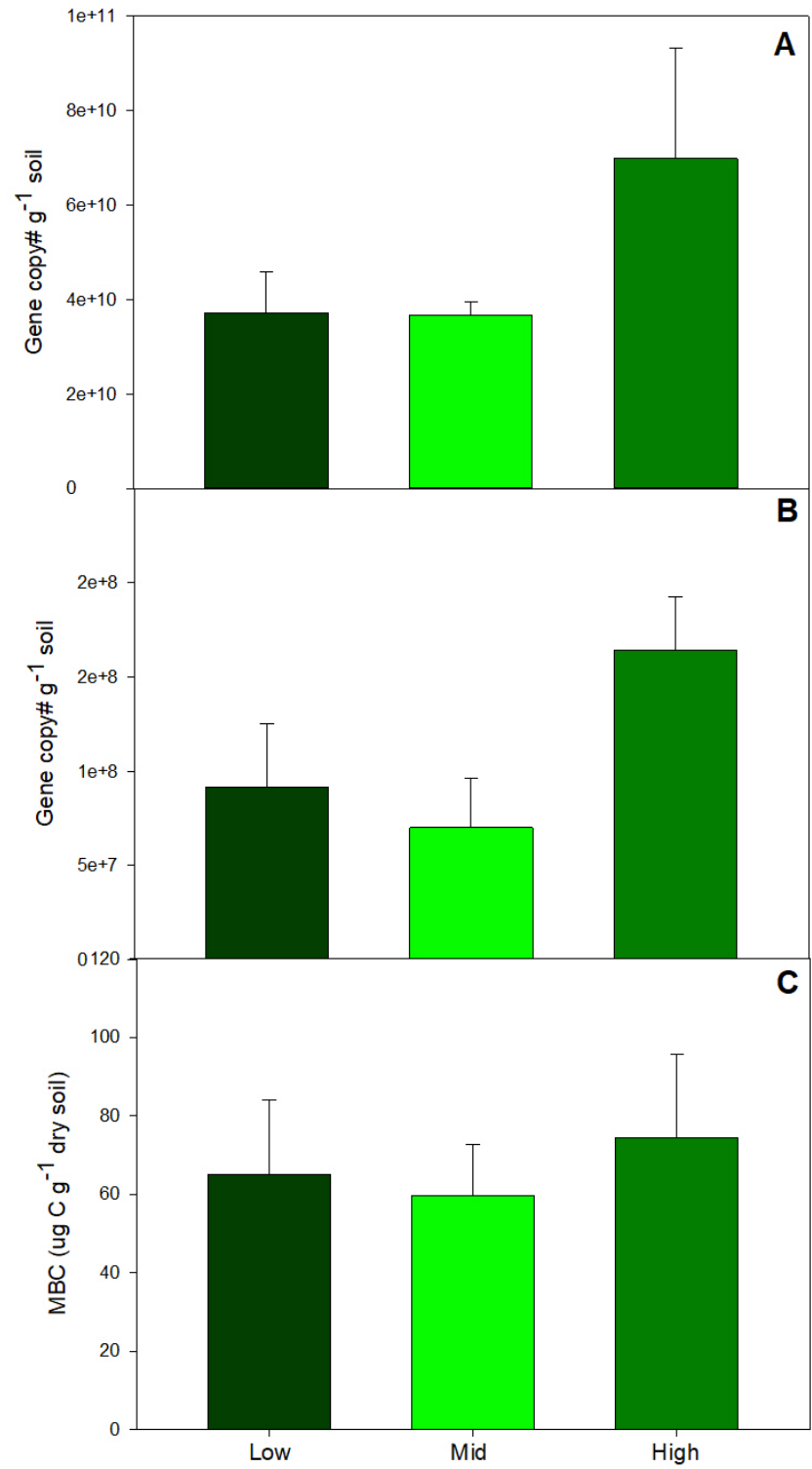


Figure 3.4. Non-metric multi-dimensional scaling (NMDS) based on a bray dissimilarity matrix showing divergence of **a**: bacterial 16S rRNA communities and **b**: fungal ITS communities Stars show the mean of treatments with SE bars and significant environmental variables are shown as vectors in the upper left corner of panel A and lower left corner of panel B. i-Tree Eco quality scores are labels on points. Note: NMDS1 is on the y-axis.

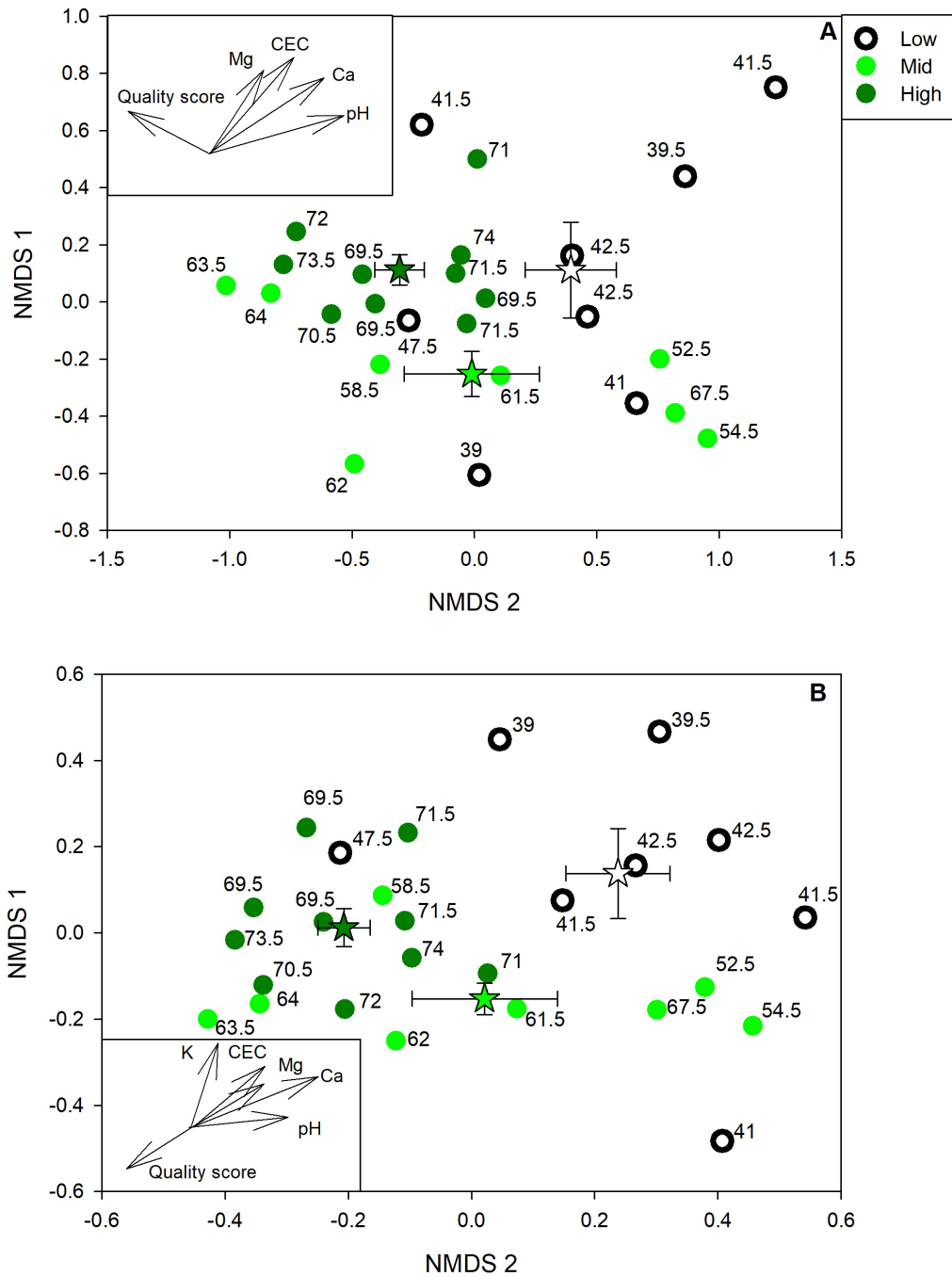


Figure 3.5: Mean relative abundance of the primary lifestyle sequences, including ECM fungi, AMF, soil saprotrophs, and plant pathogens, subsetting from the fungal ITS sequence dataset and organized by quality type.

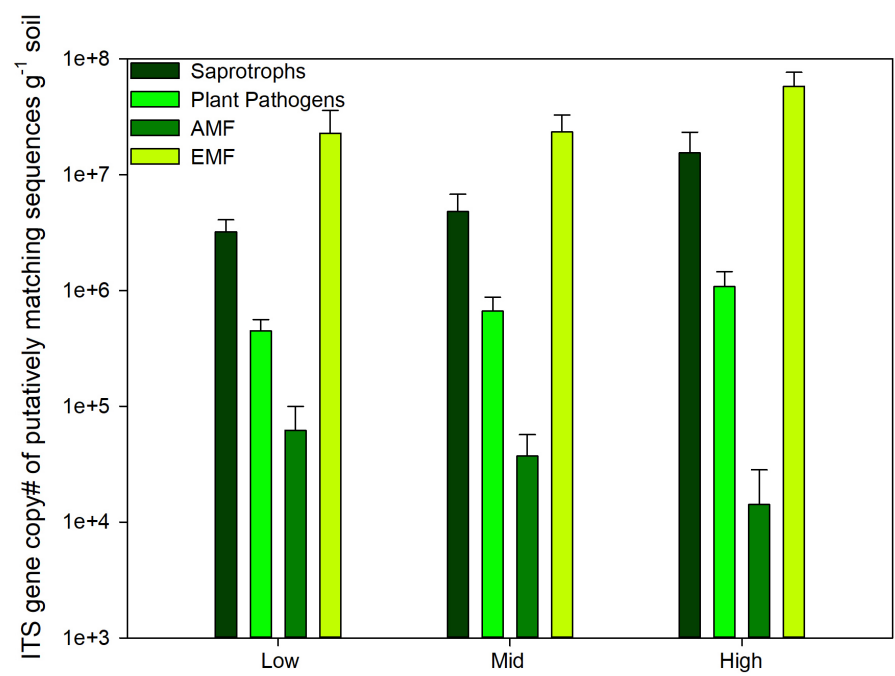
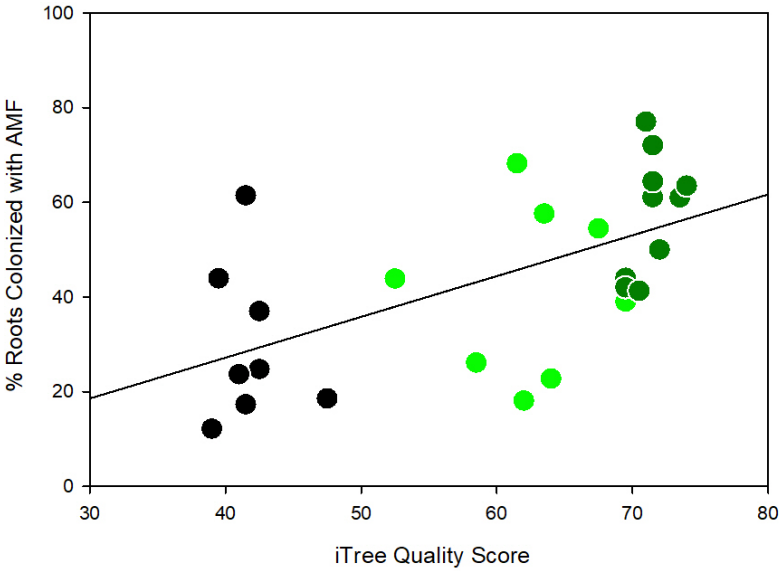


Figure 3.6: Percent AMF root colonization presented per i-Tree Eco plot quality score. Black points represent low-quality sites, light green points represent mid-quality sites, and dark green points represent the high-quality sites.



References

- Alexander, H. D., & Arthur, M. A. (2014). Increasing Red Maple Leaf Litter Alters Decomposition Rates and Nitrogen Cycling in Historically Oak-Dominated Forests of the Eastern U.S. *Ecosystems*, 17(8), 1371–1383. <https://doi.org/10.1007/s10021-014-9802-4>
- Alvey, A. A. (2006). Promoting and preserving biodiversity in the urban forest. *Urban Forestry & Urban Greening*, 5(4), 195–201. <https://doi.org/10.1016/j.ufug.2006.09.003>
- Andersen, K., Bird, K. L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjær, K. H., Orlando, L., Gilbert, M. T. P., & Willerslev, E. (2012). Meta-barcoding of ‘dirt’ DNA from soil reflects vertebrate biodiversity. *Molecular Ecology*, 21(8), 1966–1979. <https://doi.org/10.1111/j.1365-294X.2011.05261.x>
- Anderson, I. C., & Cairney, J. W. G. (2007). Ectomycorrhizal fungi: Exploring the mycelial frontier. *FEMS Microbiology Reviews*, 31(4), 388–406. <https://doi.org/10.1111/j.1574-6976.2007.00073.x>
- Andrew F. Smith & Sally E. Smith. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? | SpringerLink. *Plant and Soil*, 348. <https://link.springer.com/article/10.1007%2Fs11104-011-0865-0>
- Ao, L., Zhao, M., Li, X., & Sun, G. (2021). Different Urban Forest Tree Species Affect the Assembly of the Soil Bacterial and Fungal Community. *Microbial Ecology*. <https://doi.org/10.1007/s00248-021-01754-3>
- Babikova, Z., Johnson, D., Bruce, T., Pickett, J., & Gilbert, L. (2014). Underground allies: How and why do mycelial networks help plants defend themselves? *BioEssays*, 36(1), 21–26. <https://doi.org/10.1002/bies.201300092>
- Bauhus, J., & Khanna, P. (1999). *The significance of microbial biomass in forest soils* (pp. 77–110).
- Bennett, A. E., & Bever, J. D. (2009). Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*. *Oecologia*, 160(4), 807–816. <https://doi.org/10.1007/s00442-009-1345-6>
- Bergsten, A., Bodin, Ö., & Ecke, F. (2013). Protected areas in a landscape dominated by logging – A connectivity analysis that integrates varying protection levels with competition–colonization tradeoffs. *Biological Conservation*, 160, 279–288. <https://doi.org/10.1016/j.biocon.2013.01.016>
- Beyer, L., Blume, H.-P., Elsner, D.-C., & Willnow, A. (1995). Soil organic matter composition and microbial activity in urban soils. *Science of The Total Environment*, 168(3), 267–278. [https://doi.org/10.1016/0048-9697\(95\)04704-5](https://doi.org/10.1016/0048-9697(95)04704-5)

Bosatta, E., & Ågren, G. I. (1994). Theoretical analysis of microbial biomass dynamics in soils. *Soil Biology and Biochemistry*, 26(1), 143–148. [https://doi.org/10.1016/0038-0717\(94\)90206-2](https://doi.org/10.1016/0038-0717(94)90206-2)

Brundrett, M. C., Piché, Y., & Peterson, R. L. (2011). A new method for observing the morphology of vesicular–arbuscular mycorrhizae. *Canadian Journal of Botany*. <https://doi.org/10.1139/b84-290>

Burke, D. J. (2008). Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. *American Journal of Botany*, 95(11), 1416–1425. <https://doi.org/10.3732/ajb.0800184>

Cairney, J. W. G., & Chambers, S. M. (2013). *Ectomycorrhizal Fungi: Key Genera in Profile*. Springer Science & Business Media.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., ... Knight, R. (2010a). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335–336. <https://doi.org/10.1038/nmeth.f.303>

Cavagnaro, T. R., Gao, L.-L., Smith, F. A., & Smith, S. E. (2001). Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytologist*, 151(2), 469–475. <https://doi.org/10.1046/j.0028-646x.2001.00191.x>

Chen, M., Arato, M., Borghi, L., Nouri, E., & Reinhardt, D. (2018). Beneficial Services of Arbuscular Mycorrhizal Fungi – From Ecology to Application. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.01270>

Corradi, N., & Bonfante, P. (2012). The Arbuscular Mycorrhizal Symbiosis: Origin and Evolution of a Beneficial Plant Infection. *PLOS Pathogens*, 8(4), e1002600. <https://doi.org/10.1371/journal.ppat.1002600>

Craul, P. J. (1985). A Description of Urban Soils and Their Desired Characteristics. *Journal of Arboriculture*, 11(11), 330–339.

David J. Nowak & Daniel E. Crane. (1998). The Urban Forest Effects (UFORE) Model: Quantifying Urban Forest Structure and Functions. *USDA Forest Service*, 714–720.

- Davison, J., Öpik, M., Daniell, T. J., Moora, M., & Zobel, M. (2011). Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiology Ecology*, 78(1), 103–115. <https://doi.org/10.1111/j.1574-6941.2011.01103.x>
- Dedysh, S. N. (2019). Bryobacter. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–5). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781118960608.gbm01667>
- Deepika, S., & Kothamasi, D. (2015). Soil moisture—A regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. *Mycorrhiza*, 25(1), 67–75. <https://doi.org/10.1007/s00572-014-0596-1>
- Epp Schmidt, D. J., Pouyat, R., Szlavecz, K., Setälä, H., Kotze, D. J., Yesilonis, I., Cilliers, S., Hornung, E., Dombos, M., & Yarwood, S. A. (2017). Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nature Ecology & Evolution*, 1(5), 1–9. <https://doi.org/10.1038/s41559-017-0123>
- Fellbaum, C. R., Mensah, J. A., Cloos, A. J., Strahan, G. E., Pfeffer, P. E., Kiers, E. T., & Bücking, H. (2014). Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, 203(2), 646–656. <https://doi.org/10.1111/nph.12827>
- Fierer, N., Jackson, J. A., Vilgalys, R., & Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, 71(7), 4117–4120. <https://doi.org/10.1128/AEM.71.7.4117-4120.2005>
- Florian Walder, Helge Niemann, Mathimaran Natarajan, Moritz F. Lehmann, Thomas Boller, & Andres Wiemken. (2012). Mycorrhizal Networks: Common Goods of Plants Shared under Unequal Terms of Trade | Plant Physiology | Oxford Academic. *Plant Physiology*, 159(2), 789–797. <https://doi-org.proxy-um.researchport.umd.edu/10.1104/pp.112.195727>
- Frank, J., Barry, S., & Madden, J. (n.d.). *Oaks Belowground: Mycorrhizas, Truffles, and Small Mammals*. 8.
- Gift, D., Groffman, P., Kaushal, S., & Mayer, P. (2008). Denitrification Potential, Root Biomass, and Organic Matter in Degraded and Restored Urban Riparian Zones. *Restoration Ecology*, 18, 113–120. <https://doi.org/10.1111/j.1526-100X.2008.00438.x>
- Groffman, P. M., Bain, D. J., Band, L. E., Belt, K. T., Brush, G. S., Grove, M. J., Yesilonis, I. C., & Zipperer, W. C. (2003). Down by the riverside: Urban riparian ecology—Groffman—2003—Frontiers in Ecology and the Environment—Wiley Online Library. *Frontiers in Ecology and the Environment*, 1(6), 315–321.

- Groffman, P. M., Pouyat, R. V., McDonnell, M. J., Pickett, S. T. A., & Zipperer, W. C. (1995). Carbon Pools and Trace Gas Fluxes in Urban Forest Soils. In *Soil Management and Greenhouse Effect*. CRC Press.
- Gupta, M. M. (2020). Arbuscular Mycorrhizal Fungi: The Potential Soil Health Indicators. In B. Giri & A. Varma (Eds.), *Soil Health* (pp. 183–195). Springer International Publishing. https://doi.org/10.1007/978-3-030-44364-1_11
- Hawkes, C. V., Kivlin, S. N., Rocca, J. D., Huguet, V., Thomsen, M. A., & Suttle, K. B. (2011). Fungal community responses to precipitation. *Global Change Biology*, 17(4), 1637–1645. <https://doi.org/10.1111/j.1365-2486.2010.02327.x>
- Henry, S., Baudoin, E., López-Gutiérrez, J. C., Martin-Laurent, F., Brauman, A., & Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *Journal of Microbiological Methods*, 59(3), 327–335. <https://doi.org/10.1016/j.mimet.2004.07.002>
- Hobbie, E. A. (2006). Carbon Allocation to Ectomycorrhizal Fungi Correlates with Belowground Allocation in Culture Studies. *Ecology*, 87(3), 563–569. <https://doi.org/10.1890/05-0755>
- Högberg, M. N., & Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, 154(3), 791–795. <https://doi.org/10.1046/j.1469-8137.2002.00417.x>
- Horwath, W. r., & Paul, E. a. (1994). Microbial Biomass. In *Methods of Soil Analysis* (pp. 753–773). John Wiley & Sons, Ltd. <https://doi.org/10.2136/sssabookser5.2.c36>
- Hristova, K., & Six, J. (2006). Linking N Cycling to Microbial Community Function Within Soil Microenvironments in Cover Crop Systems. *Kearney Foundation of Soil Science*, 10.
- Jansa, J., Forczek, S. T., Rozmoš, M., Püschel, D., Bukovská, P., & Hřelová, H. (2019). Arbuscular mycorrhiza and soil organic nitrogen: Network of players and interactions. *Chemical and Biological Technologies in Agriculture*, 6(1), 10. <https://doi.org/10.1186/s40538-019-0147-2>
- Karpati, A. S., Handel, S. N., Dighton, J., & Horton, T. R. (2010). Quercus rubra-associated ectomycorrhizal fungal communities of disturbed urban sites and mature forests. *Mycorrhiza*, 21(6), 537–547.
- Kivlin, S. N., Hawkes, C. V., & Treseder, K. K. (2011). Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 43(11), 2294–2303. <https://doi.org/10.1016/j.soilbio.2011.07.012>

- Kranabetter, J. M., Durall, D. M., & MacKenzie, W. H. (2009). Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. *Mycorrhiza*, 19(2), 99–111. <https://doi.org/10.1007/s00572-008-0208-z>
- Latterell, J. J., Scott Bechtold, J., O’keefe, T. C., Van Pelt, R., & Naiman, R. J. (2006). Dynamic patch mosaics and channel movement in an unconfined river valley of the Olympic Mountains. *Freshwater Biology*, 51(3), 523–544. <https://doi.org/10.1111/j.1365-2427.2006.01513.x>
- Learn about i-Tree | i-Tree*. (n.d.). Retrieved October 4, 2021, from <https://www.itreetools.org/>
- Lenoir, I., Fontaine, J., & Lounès-Hadj Sahraoui, A. (2016). Arbuscular mycorrhizal fungal responses to abiotic stresses: A review. *Phytochemistry*, 123, 4–15. <https://doi.org/10.1016/j.phytochem.2016.01.002>
- Leyval, C., Joner, E. J., del Val, C., & Haselwandter, K. (2002). Potential of arbuscular mycorrhizal fungi for bioremediation. In S. Gianinazzi, H. Schüepp, J. M. Barea, & K. Haselwandter (Eds.), *Mycorrhizal Technology in Agriculture: From Genes to Bioproducts* (pp. 175–186). Birkhäuser. https://doi.org/10.1007/978-3-0348-8117-3_14
- Liu, R., Wang, M., Chen, W., & Peng, C. (2016). Spatial pattern of heavy metals accumulation risk in urban soils of Beijing and its influencing factors. *Environmental Pollution*, 210, 174–181. <https://doi.org/10.1016/j.envpol.2015.11.044>
- Livesley, S. J., McPherson, E. G., & Calfapietra, C. (2016). The Urban Forest and Ecosystem Services: Impacts on Urban Water, Heat, and Pollution Cycles at the Tree, Street, and City Scale. *Journal of Environmental Quality*, 45(1), 119–124. <https://doi.org/10.2134/jeq2015.11.0567>
- Luebs, R. E., Stanford, G., & Scott, A. D. (1956). Relation of Available Potassium to Soil Moisture. *Soil Science Society of America Journal*, 20(1), 45–50. <https://doi.org/10.2136/sssaj1956.03615995002000010011x>
- Männistö, M. K., Rawat, S., Starovoytov, V., & Häggblom, M. M. Y. 2012. (n.d.). *Granulicella arctica* sp. Nov., *Granulicella mallensis* sp. Nov., *Granulicella tundricola* sp. Nov. And *Granulicella sapmiensis* sp. Nov., novel acidobacteria from tundra soil. *International Journal of Systematic and Evolutionary Microbiology*, 62(Pt_9), 2097–2106. <https://doi.org/10.1099/ijs.0.031864-0>
- Marcel G. A. van der Heijden, Richard D. Bardgett, & Nico M. van Straalen. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11, 296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>

Mark E. Harmon, Knute J. Nadelhoffer, & John M. Blair. (2017, March 1). *Measuring decomposition, nutrient turnover, and stores in plant litter*. H.J. Andrews Experimental Forest - Oregon State University. <https://andrewsforest.oregonstate.edu/publications/2713>

Mavimbela, L. Z., Sieben, E. J. J., & Procheş, Ş. (2018). Invasive alien plant species, fragmentation and scale effects on urban forest community composition in Durban, South Africa. *New Zealand Journal of Forestry Science*, 48(1), 19. <https://doi.org/10.1186/s40490-018-0124-8>

McDonnell, M. J., Pickett, S. T. A., Groffman, P., Bohlen, P., Pouyat, R. V., Zipperer, W. C., Parmelee, R. W., Carreiro, M. M., & Medley, K. (1997). Ecosystem processes along an urban-to-rural gradient. *Urban Ecosystems*, 1(1), 21–36. <https://doi.org/10.1023/A:1014359024275>

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular—Arbuscular mycorrhizal fungi. *New Phytologist*, 115(3), 495–501. <https://doi.org/10.1111/j.1469-8137.1990.tb00476.x>

McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, 52(10), 883–890. [https://doi.org/10.1641/0006-3568\(2002\)052\[0883:UBAC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2)

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, 8(4), e61217. <https://doi.org/10.1371/journal.pone.0061217>

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, 15(12), 1409–1416. <https://doi.org/10.1080/00103628409367568>

Mitchell, R. J., Hester, A. J., Campbell, C. D., Chapman, S. J., Cameron, C. M., Hewison, R. L., & Potts, J. M. (2010). Is vegetation composition or soil chemistry the best predictor of the soil microbial community? *Plant and Soil*, 333(1), 417–430. <https://doi.org/10.1007/s11104-010-0357-7>

Moss, J. L., Doick, K. J., Smith, S., & Shahrestani, M. (2019). Influence of evaporative cooling by urban forests on cooling demand in cities. *Urban Forestry & Urban Greening*, 37, 65–73. <https://doi.org/10.1016/j.ufug.2018.07.023>

Nawaz, M. F., Bourrié, G., & Trolard, F. (2013). Soil compaction impact and modelling. A review. *Agronomy for Sustainable Development*, 33(2), 291–309. <https://doi.org/10.1007/s13593-011-0071-8>

- Neher, D. A. (2001). Role of Nematodes in Soil Health and Their Use as Indicators. *Journal of Nematology*, 33(4), 161–168.
- Nehls, U. (2008). Mastering ectomycorrhizal symbiosis: The impact of carbohydrates. *Journal of Experimental Botany*, 59(5), 1097–1108. <https://doi.org/10.1093/jxb/erm334>
- Newbound, M., McCarthy, M. A., & Lebel, T. (2010). Fungi and the urban environment: A review. *Landscape and Urban Planning*, 96(3), 138–145. <https://doi.org/10.1016/j.landurbplan.2010.04.005>
- Newsham, K. K., Fitter, A. H., & Watkinson, A. R. (1995). Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology & Evolution*, 10(10), 407–411. [https://doi.org/10.1016/S0169-5347\(00\)89157-0](https://doi.org/10.1016/S0169-5347(00)89157-0)
- Nocker, A., & Camper, A. K. (2006). Selective Removal of DNA from Dead Cells of Mixed Bacterial Communities by Use of Ethidium Monoazide. *Applied and Environmental Microbiology*, 72(3), 1997–2004. <https://doi.org/10.1128/AEM.72.3.1997-2004.2006>
- Nowak, D. J. (n.d.). *i-Tree: Global tools to assess tree benefits and risks to improve forest management*. 4.
- Nowak, D. J., & Crane, D. E. (2000). *The Urban Forest Effects (UFORE) model: Quantifying urban forest structure and functions*. <https://www.nrs.fs.fed.us/pubs/8420>
- Nowak, D. J., Hirabayashi, S., Doyle, M., McGovern, M., & Pasher, J. (2018). Air pollution removal by urban forests in Canada and its effect on air quality and human health. *Urban Forestry & Urban Greening*, 29, 40–48. <https://doi.org/10.1016/j.ufug.2017.10.019>
- Nowak, D., & Walton, J. (2005). Projected Urban Growth (2000–2050) and Its Estimated Impact on the US Forest Resource. *Journal of Forestry*, 103, 383–389.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O’Hara, R., Simpson, G., Solymos, P., Stevens, M., Szöcs, E., & Wagner, H. (2020). *Vegan community ecology package version 2.5-7 November 2020*.
- Plyaskina, O. V., & Ladonin, D. V. (2009). Heavy metal pollution of urban soils. *Eurasian Soil Science*, 42(7), 816–823. <https://doi.org/10.1134/S1064229309070138>
- Pöhlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., Nguyen, N., Kjoller, R., Bates, S. T., Baldrian, P., Frøslev, T. G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., ... Tedersoo, L. (2020). FungalTraits: A user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, 105(1), 1–16. <https://doi.org/10.1007/s13225-020-00466-2>

Pouyat, R. V., & Trammell, T. L. E. (2019). Chapter 10—Climate change and urban forest soils. *Developments in Soil Science*, 36, 189–211. <https://doi.org/10.1016/B978-0-444-63998-1.00010-0>

Rahman, M. A., Moser, A., Gold, A., Rötzer, T., & Pauleit, S. (2018). Vertical air temperature gradients under the shade of two contrasting urban tree species during different types of summer days. *Science of The Total Environment*, 633, 100–111. <https://doi.org/10.1016/j.scitotenv.2018.03.168>

Read, D. J., Leake, J. R., & Perez-Moreno, J. (2011). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*. <https://doi.org/10.1139/b04-123>

Robertson, G. P., & Groffman, P. (2015). *Nitrogen transformations* (pp. 421–446). <https://doi.org/10.1016/B978-0-12-415955-6.00014-1>

Rosenblatt, A. E., Gold, A. J., Stolt, M. H., Groffman, P. M., & Kellogg, D. Q. (2001). Identifying Riparian Sinks for Watershed Nitrate using Soil Surveys. *Journal of Environmental Quality*, 30(5), 1596–1604. <https://doi.org/10.2134/jeq2001.3051596x>

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17, e00547. <https://doi.org/10.1016/j.gecco.2019.e00547>

Selosse, M.-A., Richard, F., He, X., & Simard, S. W. (2006). Mycorrhizal networks: Des liaisons dangereuses? *Trends in Ecology & Evolution*, 21(11), 621–628. <https://doi.org/10.1016/j.tree.2006.07.003>

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal Symbiosis*. Academic Press.

Sparks, D. L., Soil Science Society of America, & American Society of Agronomy. (1996). *Methods of soil analysis. Part 3, Part 3*,. Soil Science Society of America : American Society of Agronomy.

Sun, Y., & Zhao, S. (2016). Leaf litter decomposition in urban forests: Test of the home-field advantage hypothesis. *Annals of Forest Science*, 73(4), 1063–1072. <https://doi.org/10.1007/s13595-016-0577-y>

Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). Decomposition in terrestrial ecosystems. *Studies in Ecology (USA)*. https://scholar.google.com/scholar_lookup?title=Decomposition+in+terrestrial+ecosystems&author=Swift%2C+M.+J.&publication_year=1979

- Tibbett, M., & Sanders, F. E. (2002). Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. *Annals of Botany*, 89(6), 783–789. <https://doi.org/10.1093/aob/mcf129>
- Ullah, S., & Faulkner, S. P. (2006). Denitrification Potential of Different Land-use Types in an Agricultural Watershed, Lower Mississippi Valley. *Ecological Engineering*, 28, 131–140. <https://doi.org/10.1016/j.ecoleng.2006.05.007>
- Urban, D. L., O'Neill, R. V., & Shugart, H. H. (1987). Landscape Ecology. *BioScience*, 37(2), 119–127. <https://doi.org/10.2307/1310366>
- Van Der Heijden, M. G. A., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3), 296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>
- Vandekerckhove, T. T., Willems, A., Gillis, M., & Coomans, A. 2000. (n.d.). Occurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in *Xiphinema americanum*-group species (Nematoda, Longidoridae). *International Journal of Systematic and Evolutionary Microbiology*, 50(6), 2197–2205. <https://doi.org/10.1099/00207713-50-6-2197>
- Vitousek, P. M. (1994). Beyond Global Warming: Ecology and Global Change. *Ecology*, 75(7).
- Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human Domination of Earth's Ecosystems. *Science*, 277(5325), 494–499. <https://doi.org/10.1126/science.277.5325.494>
- Vitousek, P. M., Turner, D. R., Parton, W. J., & Sanford, R. L. (1994). Litter Decomposition on the Mauna Loa Environmental Matrix, Hawai'i: Patterns, Mechanisms, and Models. *Ecology*, 75(2), 418–429. <https://doi.org/10.2307/1939545>
- Wallace, K. J., Laughlin, D. C., Clarkson, B. D., & Schipper, L. A. (2018). Forest canopy restoration has indirect effects on litter decomposition and no effect on denitrification. *Ecosphere*, 9(12), e02534. <https://doi.org/10.1002/ecs2.2534>
- Wang, C., Liu, D., & Bai, E. (2018). Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biology and Biochemistry*, 120, 126–133. <https://doi.org/10.1016/j.soilbio.2018.02.003>
- Wang, Y., Wang, M., Li, Y., Wu, A., & Huang, J. (2018). Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. *PLOS ONE*, 13(4), e0196408. <https://doi.org/10.1371/journal.pone.0196408>
- Wanxiao Wang, Jincai Shi, Qiuji Xie, Yina Jiang, Nan Yu, & Ertao Wang. (n.d.). *Nutrient Exchange and Regulation in Arbuscular Mycorrhizal Symbiosis: Molecular*

Plant. Retrieved October 4, 2021, from [https://www.cell.com/molecular-plant/fulltext/S1674-2052\(17\)30228-9?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1674205217302289%3Fshowall%3Dtrue](https://www.cell.com/molecular-plant/fulltext/S1674-2052(17)30228-9?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1674205217302289%3Fshowall%3Dtrue)

Waterlot, C., Bidar, G., Pelfrène, A., Roussel, H., Fourrier, H., & Douay, F. (2013). Contamination, Fractionation and Availability of Metals in Urban Soils in the Vicinity of Former Lead and Zinc Smelters, France. *Pedosphere*, 23(2), 143–159. [https://doi.org/10.1016/S1002-0160\(13\)60002-8](https://doi.org/10.1016/S1002-0160(13)60002-8)

White, C. S., & McDonnell, M. J. (1988). Nitrogen cycling processes and soil characteristics in an urban versus rural forest. *Biogeochemistry*, 5(2), 243–262. <https://doi.org/10.1007/BF02180230>

Whitney, K. (2010). Living Lawns, Dying Waters: The Suburban Boom, Nitrogenous Fertilizers, and the Nonpoint Source Pollution Dilemma. *Technology and Culture*, 51(3), 652–674.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., ... Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>

Willms, I. M., Rudolph, A. Y., Göschel, I., Bolz, S. H., Schneider, D., Penone, C., Poehlein, A., Schöning, I., & Nacke, H. (n.d.). Globally Abundant “Candidatus Udaeobacter” Benefits from Release of Antibiotics in Soil and Potentially Performs Trace Gas Scavenging. *MSphere*, 5(4), e00186-20. <https://doi.org/10.1128/mSphere.00186-20>

Wortley, L., Hero, J.-M., & Howes, M. (2013). Evaluating Ecological Restoration Success: A Review of the Literature. *Restoration Ecology*, 21(5), 537–543. <https://doi.org/10.1111/rec.12028>

Zhang, Y., Ji, G., Wu, T., & Qiu, J. (2020). Urbanization significantly impacts the connectivity of soil microbes involved in nitrogen dynamics at a watershed scale. *Environmental Pollution*, 258, 113708. <https://doi.org/10.1016/j.envpol.2019.113708>

Chapter 4: Conclusions

The overall goals of this research were to investigate how urban stream restoration affects riparian microbial communities and to explore the possible link between forest health and microbial communities, including AMF root colonization. Methods typically used to characterize stream and forest health, such as benthic macroinvertebrates and plant communities, may be slow to change after a disturbance. My research evaluated if microbial communities within the riparian and forest soils were sensitive to restoration and urbanization impacts, and therefore could be used as biological indicators to gauge stream restoration success and healthy forest ecosystems.

The first study evaluated the soil and microbial differences between restored, degraded, and unimpacted (i.e. reference) riparian sites by quantifying soil bacterial 16S rRNA and fungal ITS gene quantities and characterizing community composition based on sequencing. I also quantified two functional genes (*nirK* and *nirS*) involved in bacterial denitrification. The second study examined the bacterial and fungal communities of different quality levels of forested i-Tree Eco sites. Once again bacterial 16S rRNA and fungal ITS genes were quantified and sequenced. Additionally, I measured the microbial biomass based on chloroform fumigation and quantified AMF root colonization between the forest quality types. Both studies examined the relationship between the bacterial and fungal communities and a selection of soil chemical properties that included pH, SOM percentage, and nutrient content.

In the riparian soils, restoration changed many key soil properties, and those properties were correlated to microbial community structure. The SOM was significantly higher in the post restoration sites than the pre or reference sites. Higher organic matter

content in soils can lower the oxygen levels in the floodplain and create anaerobic conditions suitable for denitrification (Tiedje, 1982; Gift et al, 2010). Restored riparian floodplains typically have lower levels of organic matter, and therefore lower levels of denitrification when compared to reference floodplain soils. Overtime, however, restored riparian zones have been shown to have higher levels of soil organic matter compared to younger restoration sites (McMillan and Noe, 2017). This study's restorations having significantly higher levels of SOM compared to the pre and reference soils is an important finding that could indicate riparian floodplain conditions are suitable for denitrification to occur. Soil texture was a significant factor for differentiating bacterial and fungal communities between restoration sites, specifically clay content. The post-restoration soils also had the most clay content compared to the pre and reference sites and were the highest pH. Soil pH also significantly correlated to the differences in the bacterial and fungal community composition.

The quantities of *nirK* and *nirS* genes also differed between restoration treatments. I found that after restoration, the riparian soils had significantly higher quantities of *nirS* gene copies than the reference soils. The opposite was true for *nirK*, where reference sites had significantly higher gene copies. These two genes code for homologs of nitrite reductase but are usually found in different denitrifying bacteria. Previous studies have observed that *nirK* organisms are less likely to carry the *nosZI* gene (nitrous oxide reductase), and therefore may produce more nitrous oxide (Graf et al. 2014). If restorations, such as those included here, favor denitrifiers that carry *nirS* this is positive from a greenhouse gas perspective because more complete denitrification may be occurring (Bowen et al. 2020).

The riparian restorations were designed to improve hydrological connection in the riparian zone. They changed soil texture, SOM content, and increased soil pH. Although I did not measure denitrification activity, differences in the microbial community and soil properties are indicative of greater complete denitrification and therefore enhanced N removal. Future monitoring of stream restoration sites should include determining some soil chemical properties (SOM, pH) and quantification of *nirK* and *nirS* genes. The gene quantification should be compared to denitrification potential assays to strengthen the supposed relationship between microbial community structure and function.

In contrast to the differences in soil properties observed in the riparian sites, few differences were observed between forest quality types. Counter to my expectations SOM did not differ between the forest quality types, though the high-quality sites tended to have more SOM compared to mid and low quality. Soil texture also did not differ between the forest quality sites or correlate to differences in microbial community composition. Overall, few differences were observed between high, mid, and low-quality forest stands in terms of soil properties or differences in the fungal and bacterial community composition.

The noteworthy exception was in the case of mycorrhizal fungi. The percent of AMF root colonization and the relative abundance of ECM fungal sequences positively correlated to forest quality scores. Both AMF and ECM fungi are important drivers of forest ecosystem services. They can improve plant health directly through nutrient and water uptake and indirectly by lowering disease incidence and improving soil structure, increasing aggregation for instance.

Urbanization effects that lowered forest quality scores included increased impervious surface, more invasive species cover, and lower tree health and establishment. All these factors are interrelated to mycorrhizal colonization. Since mycorrhiza can be critical for tree establishment, future management and research goals should include evaluating the use inoculums at tree planting. This is outside the scope of the current study, but maybe a valuable tool to improve urban soil health.

The conclusions of this research are constrained by sample number and access to sites, particularly in the case i-Tree Eco sites where we had a low response rate from private homeowners. Future research should increase statistical power by including more samples within each group to better capture heterogeneity. Of course, there was only one 10-year riparian restoration, as this practice was not as common in the past. If future research is done, many more restoration will be available. Here too only a single time point was sampled, but a longitudinal study would also yield important information. In the case of forest sites, the root colonization yielded significant results between the low and high quality forest types, however, statistical power could be further increased by sampling roots from more trees with each plot, as opposed to one per plot. Future research could also investigate AMF and ECM root colonization in the riparian floodplains before and after stream restoration. Finally, future research should investigate the use of microbial inoculums to encourage colonization of beneficial microorganisms like AMF that could help to improve plant and soil health.

In summary, this work supports including microbial methods in monitoring and evaluating urban ecosystems. In both the riparian and forest sites, fungal communities were sensitive to disturbance and human impacts. Rather than focus on characterization

of the entire fungal community however, I would suggest focusing on mycorrhizal abundance either through microscopic quantification of root tips or targeted molecular approaches. There are at least some primer sets specific to AMF for example. In evaluating restoration success, we always must be clear what the original goal was. Fairfax County, VA has invested in stream restoration in large part to improve water quality. The data collected here point to some success in establishing the functional community needed to remove N in urban runoff and these metrics should continue to be evaluated and compared across different restoration types.

References

- Bowen, Holly, Jude E. Maul, Michel A. Cavigelli, and Stephanie Yarwood. “Denitrifier Abundance and Community Composition Linked to Denitrification Activity in an Agricultural and Wetland Soil.” *Applied Soil Ecology* 151 (July 2020): 103521. <https://doi.org/10.1016/j.apsoil.2020.103521>.
- Gift, D. M., Groffman, P. M., Kaushal, S. S., & Mayer, P. M. (2010). Denitrification Potential, Root Biomass, and Organic Matter in Degraded and Restored Urban Riparian Zones. *Restoration Ecology*, 18(1), 113–120. <https://doi.org/10.1111/j.1526-100X.2008.00438.x>
- Graf, Daniel R. H., Christopher M. Jones, and Sara Hallin. “Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N₂O Emissions.” Edited by Valerie de Cr  cy-Lagard. *PLoS ONE* 9, no. 12 (December 1, 2014): e114118. <https://doi.org/10.1371/journal.pone.0114118>.
- McMillan, S. K., & Noe, G. B. (2017). Increasing floodplain connectivity through urban stream restoration increases nutrient and sediment retention. *Ecological Engineering*, 108, 284–295. <https://doi.org/10.1016/j.ecoleng.2017.08.006>
- Tiedje, J.M. 1982. Denitification. Pages 1011-1025 in *Methods of soil analysis, Part 2: microbiological and biochemical properties*. 1 edition. ASA and SSA, Madison, Wisconsin.

Appendices

Appendix I. Chapter 2 supplemental information

Table S1: Sample information including the stream branch that was sampled, the central, downstream, or upstream location, and the restoration type.

Sample	Branch	Sample_code	Restoration
1	Brittenford	BT_C_R_34.5	Pre
2	Brittenford	BT_U_0	Pre
3	Brittenford	BT_C_L_34.5	Pre
4	Brittenford	BT_C_0	Pre
5	Brittenford	BT_D_L_34.5	Pre
6	Walney	Wal_U_0	Reference
7	Walney	Wal_U_R_34.5	Reference
8	Walney	Wal_C_L_34.5	Reference
9	Walney	Wal_D_C_0	Reference
10	Flatlick3	FL_C_L_72	Pre
11	Flatlick3	FL_D_L_34.2	Pre
12	Flatlick3	FL_C_L_34.2	Pre
13	Flatlick3	FL_U_L_34.2	Pre
14	Flatlick3	FL_C_0	Pre
15	Wolf Trap	WT_D_R_34.2	Post
16	Wolf Trap	WT_U_0	Post
17	Wolf Trap	WT_U_R_34.2	Post
18	Wolf Trap	WT_C_0	Post
19	Wolf Trap	WT_D_0	Post
20	South Lakes	SL_U_R_34.2	Post
21	South Lakes	SL_C_R_34.2	Post
22	South Lakes	SL_D_L_34.2	Post
23	South Lakes	SL_D_R_34.2	Post
24	Banks	BNK_U_R_34.2	Post
25	Banks	BNK_U_R_0	Post
26	Banks	BNK_C_L_34.2	Post
27	Banks	BNK_D_0	Post
28	Banks	BNK_C_0	Post
29	Crook Branch	CB_D_L_34.2	Pre
30	Crook Branch	CB_D_R_49	Pre
31	Crook Branch	CB_D_0	Pre
32	Crook Branch	CB_C_R_34.2	Pre
33	Crook Branch	CB_U_R_34.2	Pre
34	Rabbit Branch	RB_U_0	Pre
35	Rabbit Branch	RB_U_R_34.3	Pre
36	Rabbit Branch	RB_D_L_34.5	Pre
37	Rabbit Branch	RB_D_0	Pre

38	Rabbit Branch	RB_C_0	Pre
39	Bull Neck	BN_C_L_34.2	Pre
40	Bull Neck	BN_D_L-34.2	Pre
41	Bull Neck	BN_D_0	Pre
42	Bull Neck	BN_U_0	Pre
43	Bull Neck	BN_U_R_34.2	Pre
44	Dead Run SV	DRSV_U_0	Pre
45	Dead Run SV	DRSV_U_R_34.2	Pre
46	Dead Run SV	DRSV_C_0_R	Pre
47	Dead Run SV	DRSV_C_R_34.2	Pre
48	Dead Run SV	DRSV_C_0_L	Pre
49	Dead Run SV	DRSV_D_R_34.2	Pre
50	Flag Run	FR_D_R_34.2	Pre
51	Flag Run	FR_C_R_34.2	Pre
52	Flag Run	FR_D_0	Pre
53	Flag Run	FR_U_0	Pre
54	Poplar Springs	POP_U_R_34.5	Post_10
55	Poplar Springs	POP_U_L_34.5	Post_10
56	Poplar Springs	POP_U_0	Post_10
57	Poplar Springs	POP_D_R_34.5	Post_10
58	Poplar Springs	POP_C_0	Post_10
59	Poplar Springs	POP_C_R_34.5	Post_10
60	Big Rocky Run	BRR_C_R_34.5	Post
61	Big Rocky Run	BRR_D_R_34.5	Post
62	Big Rocky Run	BRR_U_0	Post
63	Big Rocky Run	BRR_C_0	Post
64	Big Rocky Run	BRR_D_0	Post
65	Paul Spring GMP	GMP_D_R_34.5	Post
66	Paul Spring GMP	GMP_C_0	Post
67	Paul Spring GMP	GMP_U_0	Post
68	Pohick at Guinea	PG_D_0	Post
69	Pohick at Guinea	PG_D_R_34.2	Post
70	Pohick at Guinea	PG_U_R_34.2	Post
71	Pohick at Guinea	PG_U_0	Post
72	Pohick at Guinea	PG_C_0	Post
73	Greentree	GT_C_0	Reference
74	Greentree	GT_C_L_34.2	Reference
75	Greentree	GT_U_R_34.2	Reference
76	Greentree	GT_D_L_34.2	Reference
77	Greentree	GT_D_R_34.2	Reference
78	Banshee Reeks	BAR_C_R_34.5	Reference
79	Banshee Reeks	BAR_C_0	Reference
80	Banshee Reeks	BAR_D_0	Reference

81	Banshee Reeks	BAR_D_R_34.5	Reference
82	Banshee Reeks	BAR_U_L_34.5	Reference
83	Banshee Reeks	BAR_U_0	Reference

Table S2: qPCR Reaction Conditions

Target Gene	Target	Pure Culture	Primers & References (Forward / Reverse)	Thermocycler Conditions (Acquisition Step Bolded)	Number of Cycles	Plasmid standard and soil correction efficiency (%) (r^2 values)
EUB	Bacteria 16S rRNA gene	<i>Escherichia coli</i>	EUB338 EUB518 (Fierer et al, 2005)	95°C for 5 min 95°C for 5 s / 55°C for 15 s / 72°C for 10 s	1 40	93- 98% soil = 90% All $r^2 \geq 97\%$
ITS	Fungal ITS	<i>Haematonecretia haematococca</i>	ITS1F ITS 5.8S (Fierer et al, 2005)	95°C for 5 min 95°C for 5 s / 55°C for 15 s / 72°C for 10 s	1 40	95-99% soil = 93% All $r^2 \geq 99\%$ except soil $r^2 = 87\%$
<i>nirK</i>	Nitric oxide reductase	<i>Sinorhizobium meliloti</i>	nirk876 nirK1040 (Henry et al, 2004)	95°C for 5 min 95°C for 15 s / 63-59°C for 60 s 95°C for 15 s / 58°C for 60 s	1 5 (TD) 40	91-96% soil = 95% All $r^2 \geq 97\%$ except soil $r^2 = 91\%$
<i>nirS</i>	Nitric oxide reductase	<i>Pseudomonas stutzeri</i>	nirSCD3aF nirSR3cd (Hristova and Six, 2006)	95°C for 5 min 95°C for 15 s / 63-59°C for 75 s 95°C for 15 s / 58°C for 75 s	1 5 (TD) 41	91-109%, soil = 97% All $r^2 \geq 97\%$

Appendix II. Chapter 3 supplemental information

Table S1: Fairfax plot health variable data sheet created by the Urban Forest Management Division of Fairfax County, VA. Scores from each section are totaled with a max score of 82.5 for each i-Tree Eco plot. A high score in each section is deemed as a higher quality forested stand, less impacted by urbanization.

Plot ID: _____ Date: _____ Survey Team: _____

***If selected, skip rest of survey**

Overall Plot

Impervious*	Dominant impervious	Impervious/vegetation or lawn	Trees with lawn	Partially forested	Forested
0	1	2	3	4	5
6	7	8	9	10	

Human Impact

Site cleared; impervious	Extensive or imminent development	>50% of plot impacted	Moderate; active restoration activities	Light; no construction	Minimal or none
0	1	2	3	4	5
6	7	8	9	10	

Tree Age Class & Size Distribution

No trees	Even age or single tree	Limited age structure	Transition forest	Mixed age	Ideal composition
0	1	2	3	4	5
6	7	8	9	10	

Tree Establishment & Maintenance/Stand Management

No trees	Dead trees; lack of tree maintenance	Negligent/Stressed	Acceptable	Unmanaged or restored	Natural forest/Ideal
0	1	2	3	4	5
6	7	8	9	10	

Tree Structure/Health

Dead tree(s) or no trees	>75% of trees in plot stressed	>50% stressed	Less than half stressed	Mostly healthy	Normal/Healthy
0	1	2	3	4	5
6	7	8	9	10	

Invasive Plants

Completely overgrown	>80% of plot colonized	>50% colonized	Equal (~50%) native/invasive	Patchy invasives	Majority native
0	1	2	3	4	5
6	7	8	9	10	

Forest pests/diseases

Majority Dead Tree(s) or no trees	Significant	>50% of trees affected	Moderate	Light or temporary (e.g. defoliation)	Superficial or none
0	1	2	3	4	5
6	7	8	9	10	

Deer browse

~100% of plot affected	Lack of shrubs & understory	>50% affected; non-palatable plants	Dispersed or transient damage	<25% impacted; palatable plants present	Minimal (<10%) or none; regeneration
0	1	2	3	4	5
6	7	8	9	10	

Total Score:
(out of 82.5)

Supplemental Questions:

1 Impact of forest pests and type:

- ☐ none (0.5 points)
- ☐ scale insects/aphids
- ☐ defoliators
- ☐ disease/cankers
- ☐ borers
- ☐ other

List species, if known: _____

2 Evidence of poor tree care:

- ☐ yes
- ☐ no (0.5 points)

3 Is the plot mostly comprised of ash trees?

- ☐ yes
- ☐ no (0.5 points)

4 Evidence of ecosystem management?

- ☐ yes (0.5 points)
- ☐ no

5 Is there a distinct deer browse line?

- ☐ yes
- ☐ no (0.5 points)

Table S2: qPCR Reaction Conditions

Target Gene	Target	Pure Culture	Primers & References (Forward / Reverse)	Thermocycler Conditions (Acquisition Step Bolded)	Number of Cycles	Plasmid standard and soil correction efficiency (%) (r^2 values)
EUB	Bacteria 16S rRNA gene	<i>Escherichia coli</i>	EUB338 EUB518 (Fierer et al, 2005)	95°C for 5 min 95°C for 5 s / 55°C for 15 s / 72°C for 10 s	1 40	99%, soil = 83 % All $r^2 \geq 93\%$
ITS	Fungal ITS	<i>Haematonecretia haematococca</i>	ITS1F ITS 5.8S (Fierer et al, 2005)	95°C for 5 min 95°C for 5 s / 55°C for 15 s / 72°C for 10 s	1 40	93%, soil = 93% All $r^2 \geq 99$ except soil $r^2 = 87\%$

Bibliography

- Alexander, H. D., & Arthur, M. A. (2014). Increasing Red Maple Leaf Litter Alters Decomposition Rates and Nitrogen Cycling in Historically Oak-Dominated Forests of the Eastern U.S. *Ecosystems*, 17(8), 1371–1383. <https://doi.org/10.1007/s10021-014-9802-4>
- Alvey, A. A. (2006). Promoting and preserving biodiversity in the urban forest. *Urban Forestry & Urban Greening*, 5(4), 195–201. <https://doi.org/10.1016/j.ufug.2006.09.003>
- Andersen, K., Bird, K. L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjær, K. H., Orlando, L., Gilbert, M. T. P., & Willerslev, E. (2012). Meta-barcoding of ‘dirt’ DNA from soil reflects vertebrate biodiversity. *Molecular Ecology*, 21(8), 1966–1979. <https://doi.org/10.1111/j.1365-294X.2011.05261.x>
- Anderson, I. C., & Cairney, J. W. G. (2007). Ectomycorrhizal fungi: Exploring the mycelial frontier. *FEMS Microbiology Reviews*, 31(4), 388–406. <https://doi.org/10.1111/j.1574-6976.2007.00073.x>
- Andersen, R., Chapman, S. J., & Artz, R. R. E. (2013). Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*, 57, 979–994. <https://doi.org/10.1016/j.soilbio.2012.10.003>
- Andrew F. Smith & Sally E. Smith. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? | SpringerLink. *Plant and Soil*, 348. <https://link.springer.com/article/10.1007%2Fs11104-011-0865-0>
- Ao, L., Zhao, M., Li, X., & Sun, G. (2021). Different Urban Forest Tree Species Affect the Assembly of the Soil Bacterial and Fungal Community. *Microbial Ecology*. <https://doi.org/10.1007/s00248-021-01754-3>
- Arroyo, P., Sáenz de Miera, L. E., & Ansola, G. (2015). Influence of environmental variables on the structure and composition of soil bacterial communities in natural and constructed wetlands. *Science of The Total Environment*, 506–507, 380–390. <https://doi.org/10.1016/j.scitotenv.2014.11.039>
- Babikova, Z., Johnson, D., Bruce, T., Pickett, J., & Gilbert, L. (2014). Underground allies: How and why do mycelial networks help plants defend themselves? *BioEssays*, 36(1), 21–26. <https://doi.org/10.1002/bies.201300092>
- Balser, T. C., McMahon, K. D., Bart, D., Bronson, D., Coyle, D. R., Craig, N., Flores-Mangual, M. L., Forshay, K., Jones, S. E., Kent, A. E., & Shade, A. L. (2006). Bridging the gap between micro—And macro-scale perspectives on the role of microbial communities in global change ecology. *Plant and Soil*, 289(1), 59–70. <https://doi.org/10.1007/s11104-006-9104-5>

Barber, N. A., Lamagdeleine-Dent, K. A., Willand, J. E., Jones, H. P., & McCravy, K. W. (2017). Species and functional trait re-assembly of ground beetle communities in restored grasslands. *Biodiversity and Conservation*, 26(14), 3481–3498. <https://doi.org/10.1007/s10531-017-1417-6>

Bauhus, J., & Khanna, P. (1999). *The significance of microbial biomass in forest soils* (pp. 77–110).

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Frontiers in Plant Science*, 10. <https://www.frontiersin.org/article/10.3389/fpls.2019.01068>

Bennett, A. E., & Bever, J. D. (2009). Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*. *Oecologia*, 160(4), 807–816. <https://doi.org/10.1007/s00442-009-1345-6>

Bergsten, A., Bodin, Ö., & Ecke, F. (2013). Protected areas in a landscape dominated by logging – A connectivity analysis that integrates varying protection levels with competition–colonization tradeoffs. *Biological Conservation*, 160, 279–288. <https://doi.org/10.1016/j.biocon.2013.01.016>

Bernhardt, E. S., & Palmer, M. A. (2007). Restoring streams in an urbanizing world. *Freshwater Biology*, 52(4), 738–751. <https://doi.org/10.1111/j.1365-2427.2006.01718.x>

Bernhardt, E. S., Palmer, M. A., Allen, J. D., Alexander, G., Barnas, K., Brooks, S., Carr, J., Clayton, S., Dahm, C., Follstad-Shah, J., Galat, D., Gloss, S., Goodwin, P., Hart, D., Hassett, B., Jenkinson R., Katz, S., Knodolf, G. M., Lake, P. S., ... Sudduth, E. (2005). Synthesizing U.S. River Restoration Efforts. *Science*, 308(5722), 636–637. <https://doi.org/10.1126/science.1109769>

Beyer, L., Blume, H.-P., Elsner, D.-C., & Willnow, A. (1995). Soil organic matter composition and microbial activity in urban soils. *Science of The Total Environment*, 168(3), 267–278. [https://doi.org/10.1016/0048-9697\(95\)04704-5](https://doi.org/10.1016/0048-9697(95)04704-5)

Bodelier, P. (2011). Toward Understanding, Managing, and Protecting Microbial Ecosystems. *Frontiers in Microbiology*, 2, 80. <https://doi.org/10.3389/fmicb.2011.00080>

Booth, D. B. (1990). Stream-channel Incision Following Drainage-Basin Urbanization. *Journal of the American Water Resources Association*. <https://onlinelibrary-wiley-com.proxy-um.researchport.umd.edu/doi/abs/10.1111/j.1752-1688.1990.tb01380.x>

Bosatta, E., & Ågren, G. I. (1994). Theoretical analysis of microbial biomass dynamics in soils. *Soil Biology and Biochemistry*, 26(1), 143–148. [https://doi.org/10.1016/0038-0717\(94\)90206-2](https://doi.org/10.1016/0038-0717(94)90206-2)

Bossio, D. A., Fleck, J. A., Scow, K. M., & Fujii, R. (2006). Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biology and Biochemistry*, 38(6), 1223–1233. <https://doi.org/10.1016/j.soilbio.2005.09.027>

Bowen, H. (2016). *The Distribution and Function of Denitrification Genes: Exploring Agricultural Management and Soil Chemical Implications*. 138.

Bowen, Holly, Jude E. Maul, Michel A. Cavigelli, and Stephanie Yarwood. “Denitrifier Abundance and Community Composition Linked to Denitrification Activity in an Agricultural and Wetland Soil.” *Applied Soil Ecology* 151 (July 2020): 103521. <https://doi.org/10.1016/j.apsoil.2020.103521>.

Brown, S., Miltner, E., & Cogger, C. (2012). Carbon Sequestration Potential in Urban Soils. In R. Lal & B. Augustin (Eds.), *Carbon Sequestration in Urban Ecosystems* (pp. 173–196). Springer Netherlands. https://doi.org/10.1007/978-94-007-2366-5_9

Brundrett, M. C., Piché, Y., & Peterson, R. L. (2011). A new method for observing the morphology of vesicular–arbuscular mycorrhizae. *Canadian Journal of Botany*. <https://doi.org/10.1139/b84-290>

Burchell II, M. R., Skaggs, R. W., Lee, C. R., Broome, S., Chescheir, G. M., & Osborne, J. (2007). Substrate Organic Matter to Improve Nitrate Removal in Surface-Flow Constructed Wetlands. *Journal of Environmental Quality*, 36(1), 194–207. <https://doi.org/10.2134/jeq2006.0022>

Burke, D. J. (2008). Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. *American Journal of Botany*, 95(11), 1416–1425. <https://doi.org/10.3732/ajb.0800184>

Cairney, J. W. G., & Chambers, S. M. (2013). *Ectomycorrhizal Fungi: Key Genera in Profile*. Springer Science & Business Media.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., ... Knight, R. (2010a). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335–336. <https://doi.org/10.1038/nmeth.f.303>

Cavagnaro, T. R., Gao, L.-L., Smith, F. A., & Smith, S. E. (2001). Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytologist*, 151(2), 469–475. <https://doi.org/10.1046/j.0028-646x.2001.00191.x>

Chen, M., Arato, M., Borghi, L., Nouri, E., & Reinhardt, D. (2018). Beneficial Services of Arbuscular Mycorrhizal Fungi – From Ecology to Application. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.01270>

Corradi, N., & Bonfante, P. (2012). The Arbuscular Mycorrhizal Symbiosis: Origin and Evolution of a Beneficial Plant Infection. *PLOS Pathogens*, 8(4), e1002600. <https://doi.org/10.1371/journal.ppat.1002600>

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, 19(4), 988–995. <https://doi.org/10.1111/gcb.12113>

Coyne, M. S., Arunakumari, A., Averill, B. A., & Tiedje, J. M. (1989). Immunological identification and distribution of dissimilatory heme cd1 and nonheme copper nitrite reductases in denitrifying bacteria. *Applied and Environmental Microbiology*, 55(11), 2924–2931. <https://doi.org/10.1128/aem.55.11.2924-2931.1989>

Craul, P. J. (1985). A Description of Urban Soils and Their Desired Characteristics. *Journal of Arboriculture*, 11(11), 330–339.

David J. Nowak & Daniel E. Crane. (1998). The Urban Forest Effects (UFORE) Model: Quantifying Urban Forest Structure and Functions. *USDA Forest Service*, 714–720.

Davison, J., Öpik, M., Daniell, T. J., Moora, M., & Zobel, M. (2011). Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiology Ecology*, 78(1), 103–115. <https://doi.org/10.1111/j.1574-6941.2011.01103.x>

Dedysh, S. N. (2019). Bryobacter. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–5). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781118960608.gbm01667>

Deepika, S., & Kothamasi, D. (2015). Soil moisture—A regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. *Mycorrhiza*, 25(1), 67–75. <https://doi.org/10.1007/s00572-014-0596-1>

Dosskey, M. G., Vidon, P., Gurwick, N. P., Allan, C. J., Duval, T. P., & Lowrance, R. (2010). The Role of Riparian Vegetation in Protecting and Improving Chemical Water Quality in Streams ¹: T H E R O L E O F R I P A R I A N V E G E T A T I O N I N P R O T E C T I N G A N D I M P R O V I N G C H E M I C A L W A T E R Q U A L I T Y I N S T R E A M S. *JAWRA Journal of the American*

Water Resources Association, 46(2), 261–277. <https://doi.org/10.1111/j.1752-1688.2010.00419.x>

Elmore, A. J., & Kaushal, S. S. (2008). Disappearing headwaters: Patterns of stream burial due to urbanization. *Frontiers in Ecology and the Environment*, 6(6), 308–312. <https://doi.org/10.1890/070101>

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., & Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature Communications*, 10(1), 2369. <https://doi.org/10.1038/s41467-019-10373-z>

Epp Schmidt, D. J., Pouyat, R., Szlavecz, K., Setälä, H., Kotze, D. J., Yesilonis, I., Cilliers, S., Hornung, E., Dombos, M., & Yarwood, S. A. (2017). Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nature Ecology & Evolution*, 1(5), 1–9. <https://doi.org/10.1038/s41559-017-0123>

Fellbaum, C. R., Mensah, J. A., Cloos, A. J., Strahan, G. E., Pfeffer, P. E., Kiers, E. T., & Bücking, H. (2014). Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, 203(2), 646–656. <https://doi.org/10.1111/nph.12827>

Fellows, C. S., Hunter, H. M., Eccleston, C. E. A., De Hayr, R. W., Rassam, D. W., Beard, N. J., & Bloesch, P. M. (2011). Denitrification potential of intermittently saturated floodplain soils from a subtropical perennial stream and an ephemeral tributary. *Soil Biology and Biochemistry*, 43(2), 324–332. <https://doi.org/10.1016/j.soilbio.2010.10.019>

Fierer, N., Jackson, J. A., Vilgalys, R., & Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, 71(7), 4117–4120. <https://doi.org/10.1128/AEM.71.7.4117-4120.2005>

Florian Walder, Helge Niemann, Mathimaran Natarajan, Moritz F. Lehmann, Thomas Boller, & Andres Wiemken. (2012). Mycorrhizal Networks: Common Goods of Plants Shared under Unequal Terms of Trade | Plant Physiology | Oxford Academic. *Plant Physiology*, 159(2), 789–797. <https://doi-org.proxy-um.researchport.umd.edu/10.1104/pp.112.195727>

Frank, J., Barry, S., & Madden, J. (n.d.). *Oaks Belowground: Mycorrhizas, Truffles, and Small Mammals*. 8.

Gift, D., Groffman, P., Kaushal, S., & Mayer, P. (2008). Denitrification Potential, Root Biomass, and Organic Matter in Degraded and Restored Urban Riparian Zones. *Restoration Ecology*, 18, 113–120. <https://doi.org/10.1111/j.1526-100X.2008.00438.x>

Graf, Daniel R. H., Christopher M. Jones, and Sara Hallin. “Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N₂O Emissions.” Edited by Valerie de Crécy-Lagard. PLoS ONE 9, no. 12 (December 1, 2014): e114118.

<https://doi.org/10.1371/journal.pone.0114118>.

Griffith, M. B., & McManus, M. G. (2020). Consideration of spatial and temporal scales in stream restorations and biotic monitoring to assess restoration outcomes: A literature review, part 1. *River Research and Applications*, 36(8), 1385–1397.

<https://doi.org/10.1002/rra.3692>

Groffman, P. M., Bain, D. J., Band, L. E., Belt, K. T., Brush, G. S., Grove, M. J., Yesilonis, I. C., & Zipperer, W. C. (2003). Down by the riverside: Urban riparian ecology—Groffman—2003—Frontiers in Ecology and the Environment—Wiley Online Library. *Frontiers in Ecology and the Environment*, 1(6), 315–321.

Groffman, P. M., Boulware, N. J., Zipperer, W. C., Pouyat, R. V., Band, L. E., & Colosimo, M. F. (2002). Soil Nitrogen Cycle Processes in Urban Riparian Zones | Environmental Science & Technology. *Environmental Science & Technology*, 36(21), 4547–4552.

Groffman, P. M., & Crawford, M. K. (2003). Denitrification Potential in Urban Riparian Zones. *Journal of Environmental Quality*, 32(3), 1144–1149.

<https://doi.org/10.2134/jeq2003.1144>

Groffman, P. M., Pouyat, R. V., McDonnell, M. J., Pickett, S. T. A., & Zipperer, W. C. (1995). Carbon Pools and Trace Gas Fluxes in Urban Forest Soils. In *Soil Management and Greenhouse Effect*. CRC Press.

Gupta, M. M. (2020). Arbuscular Mycorrhizal Fungi: The Potential Soil Health Indicators. In B. Giri & A. Varma (Eds.), *Soil Health* (pp. 183–195). Springer International Publishing. https://doi.org/10.1007/978-3-030-44364-1_11

Harris, J. A. (2003). Measurements of the soil microbial community for estimating the success of restoration. *European Journal of Soil Science*, 54(4), 801–808.

<https://doi.org/10.1046/j.1351-0754.2003.0559.x>

Hawkes, C. V., Kivlin, S. N., Rocca, J. D., Huguet, V., Thomsen, M. A., & Suttle, K. B. (2011). Fungal community responses to precipitation. *Global Change Biology*, 17(4), 1637–1645. <https://doi.org/10.1111/j.1365-2486.2010.02327.x>

Henry, S., Baudoin, E., López-Gutiérrez, J. C., Martin-Laurent, F., Brauman, A., & Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *Journal of Microbiological Methods*, 59(3), 327–335.

<https://doi.org/10.1016/j.mimet.2004.07.002>

Heylen, K., Gevers, D., Vanparys, B., Wittebolle, L., Geets, J., Boon, N., & De Vos, P. (2006). The incidence of nirS and nirK and their genetic heterogeneity in cultivated denitrifiers. *Environmental Microbiology*, 8(11), 2012–2021. <https://doi.org/10.1111/j.1462-2920.2006.01081.x>

Hobbie, E. A. (2006). Carbon Allocation to Ectomycorrhizal Fungi Correlates with Belowground Allocation in Culture Studies. *Ecology*, 87(3), 563–569. <https://doi.org/10.1890/05-0755>

Högberg, M. N., & Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, 154(3), 791–795. <https://doi.org/10.1046/j.1469-8137.2002.00417.x>

Horwath, W. r., & Paul, E. a. (1994). Microbial Biomass. In *Methods of Soil Analysis* (pp. 753–773). John Wiley & Sons, Ltd. <https://doi.org/10.2136/sssabookser5.2.c36>

Hristova, K., & Six, J. (2006). Linking N Cycling to Microbial Community Function Within Soil Microenvironments in Cover Crop Systems. *Kearney Foundation of Soil Science*, 10.

Jansa, J., Forczek, S. T., Rozmoš, M., Püschel, D., Bukovská, P., & Hřelová, H. (2019). Arbuscular mycorrhiza and soil organic nitrogen: Network of players and interactions. *Chemical and Biological Technologies in Agriculture*, 6(1), 10. <https://doi.org/10.1186/s40538-019-0147-2>

Kang, E., Li, Y., Zhang, X., Yan, Z., Wu, H., Li, M., Yan, L., Zhang, K., Wang, J., & Kang, X. (2021). Soil pH and nutrients shape the vertical distribution of microbial communities in an alpine wetland. *Science of The Total Environment*, 774, 145780. <https://doi.org/10.1016/j.scitotenv.2021.145780>

Karpati, A. S., Handel, S. N., Dighton, J., & Horton, T. R. (2010). *Quercus rubra*-associated ectomycorrhizal fungal communities of disturbed urban sites and mature forests. *Mycorrhiza*, 21(6), 537–547.

Kaushal, S. S., Groffman, P. M., Mayer, P. M., Striz, E., & Gold, A. J. (2008). Effects of Stream Restoration on Denitrification in an Urbanizing Watershed. *Ecological Applications*, 18(3), 789–804. <https://doi.org/10.1890/07-1159.1>

Kaushal, S. S., & Belt, K. T. (2012). The urban watershed continuum: Evolving spatial and temporal dimensions. *Urban Ecosystems*, 15(2), 409–435. <https://doi.org/10.1007/s11252-012-0226-7>

Kivlin, S. N., Hawkes, C. V., & Treseder, K. K. (2011). Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 43(11), 2294–2303. <https://doi.org/10.1016/j.soilbio.2011.07.012>

- Kranabetter, J. M., Durall, D. M., & MacKenzie, W. H. (2009). Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. *Mycorrhiza*, 19(2), 99–111. <https://doi.org/10.1007/s00572-008-0208-z>
- Latterell, J. J., Scott Bechtold, J., O’keefe, T. C., Van Pelt, R., & Naiman, R. J. (2006). Dynamic patch mosaics and channel movement in an unconfined river valley of the Olympic Mountains. *Freshwater Biology*, 51(3), 523–544. <https://doi.org/10.1111/j.1365-2427.2006.01513.x>
- Learn about i-Tree | i-Tree. (n.d.). Retrieved October 4, 2021, from <https://www.itreetools.org/>
- Lenoir, I., Fontaine, J., & Lounès-Hadj Sahraoui, A. (2016). Arbuscular mycorrhizal fungal responses to abiotic stresses: A review. *Phytochemistry*, 123, 4–15. <https://doi.org/10.1016/j.phytochem.2016.01.002>
- Leopold, L. B., Huppman, R., & Miller, A. (2005). Geomorphic Effects of Urbanization in Forty-One Years of Observation. *Proceedings of the American Philosophical Society*, 149(3), 349–371.
- Leyval, C., Joner, E. J., del Val, C., & Haselwandter, K. (2002). Potential of arbuscular mycorrhizal fungi for bioremediation. In S. Gianinazzi, H. Schüepp, J. M. Barea, & K. Haselwandter (Eds.), *Mycorrhizal Technology in Agriculture: From Genes to Bioproducts* (pp. 175–186). Birkhäuser. https://doi.org/10.1007/978-3-0348-8117-3_14
- Linde-Medina, M., & Marcucio, R. (2018). Living tissues are more than cell clusters: The extracellular matrix as a driving force in morphogenesis. *Progress in Biophysics and Molecular Biology*, 137, 46–51. <https://doi.org/10.1016/j.pbiomolbio.2018.01.009>
- Liu, R., Wang, M., Chen, W., & Peng, C. (2016). Spatial pattern of heavy metals accumulation risk in urban soils of Beijing and its influencing factors. *Environmental Pollution*, 210, 174–181. <https://doi.org/10.1016/j.envpol.2015.11.044>
- Livesley, S. J., McPherson, E. G., & Calfapietra, C. (2016). The Urban Forest and Ecosystem Services: Impacts on Urban Water, Heat, and Pollution Cycles at the Tree, Street, and City Scale. *Journal of Environmental Quality*, 45(1), 119–124. <https://doi.org/10.2134/jeq2015.11.0567>
- Luebs, R. E., Stanford, G., & Scott, A. D. (1956). Relation of Available Potassium to Soil Moisture. *Soil Science Society of America Journal*, 20(1), 45–50. <https://doi.org/10.2136/sssaj1956.03615995002000010011x>
- Männistö, M. K., Rawat, S., Starovoytov, V., & Häggblom, M. M. Y. 2012. (n.d.). *Granulicella arctica* sp. Nov., *Granulicella mallensis* sp. Nov., *Granulicella tundricola* sp. Nov. And *Granulicella sapmiensis* sp. Nov., novel acidobacteria from tundra soil.

International Journal of Systematic and Evolutionary Microbiology, 62(Pt_9), 2097–2106. <https://doi.org/10.1099/ijs.0.031864-0>

Marcel G. A. van der Heijden, Richard D. Bardgett, & Nico M. van Straalen. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11, 296–310. <https://doi.org/0.1111/j.1461-0248.2007.01139.x>

Mark E. Harmon, Knute J. Nadelhoffer, & John M. Blair. (2017, March 1). *Measuring decomposition, nutrient turnover, and stores in plant litter*. H.J. Andrews Experimental Forest - Oregon State University. <https://andrewsforest.oregonstate.edu/publications/2713>

Mavimbela, L. Z., Sieben, E. J. J., & Procheş, Ş. (2018). Invasive alien plant species, fragmentation and scale effects on urban forest community composition in Durban, South Africa. *New Zealand Journal of Forestry Science*, 48(1), 19. <https://doi.org/10.1186/s40490-018-0124-8>

McDonnell, M. J., Pickett, S. T. A., Groffman, P., Bohlen, P., Pouyat, R. V., Zipperer, W. C., Parmelee, R. W., Carreiro, M. M., & Medley, K. (1997). Ecosystem processes along an urban-to-rural gradient. *Urban Ecosystems*, 1(1), 21–36. <https://doi.org/10.1023/A:1014359024275>

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular—Arbuscular mycorrhizal fungi. *New Phytologist*, 115(3), 495–501. <https://doi.org/10.1111/j.1469-8137.1990.tb00476.x>

McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, 52(10), 883–890. [https://doi.org/10.1641/0006-3568\(2002\)052\[0883:UBAC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2)

McMillan, S. K., & Noe, G. B. (2017). Increasing floodplain connectivity through urban stream restoration increases nutrient and sediment retention. *Ecological Engineering*, 108, 284–295. <https://doi.org/10.1016/j.ecoleng.2017.08.006>

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, 8(4), e61217. <https://doi.org/10.1371/journal.pone.0061217>

McNeish, R. E., Benbow, M. E., & McEwan, R. W. (2017). Removal of the Invasive Shrub, *Lonicera maackii* (Amur Honeysuckle), from a Headwater Stream Riparian Zone Shifts Taxonomic and Functional Composition of the Aquatic Biota. *Invasive Plant Science and Management*, 10(3), 232–246. <https://doi.org/10.1017/inp.2017.22>

- Meeds, J. A., Marty Kranabetter, J., Zigg, I., Dunn, D., Miros, F., Shipley, P., & Jones, M. D. (2021). Phosphorus deficiencies invoke optimal allocation of exoenzymes by ectomycorrhizas. *The ISME Journal*, 15(5), 1478–1489. <https://doi.org/10.1038/s41396-020-00864-z>
- Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, 15(12), 1409–1416. <https://doi.org/10.1080/00103628409367568>
- MiSeq System Denature and Dilute Libraries Guide (15039740)*. (n.d.).
- Mitchell, R. J., Hester, A. J., Campbell, C. D., Chapman, S. J., Cameron, C. M., Hewison, R. L., & Potts, J. M. (2010). Is vegetation composition or soil chemistry the best predictor of the soil microbial community? *Plant and Soil*, 333(1), 417–430. <https://doi.org/10.1007/s11104-010-0357-7>
- Mohammadi, K., Heidari, G., Khalesro, S., & Sohrabi, Y. (2011). Soil management, microorganisms and organic matter interactions: A review. *AFRICAN JOURNAL OF BIOTECHNOLOGY*, 10, 19840–19849. <https://doi.org/10.5897/AJBX11.006>
- Moreno-Mateos, D., Barbier, E. B., Jones, P. C., Jones, H. P., Aronson, J., López-López, J. A., McCrackin, M. L., Meli, P., Montoya, D., & Rey Benayas, J. M. (2017). Anthropogenic ecosystem disturbance and the recovery debt. *Nature Communications*, 8(1), 1–6. <https://doi.org/10.1038/ncomms14163>
- Moss, J. L., Doick, K. J., Smith, S., & Shahrestani, M. (2019). Influence of evaporative cooling by urban forests on cooling demand in cities. *Urban Forestry & Urban Greening*, 37, 65–73. <https://doi.org/10.1016/j.ufug.2018.07.023>
- Nawaz, M. F., Bourrié, G., & Trolard, F. (2013). Soil compaction impact and modelling. A review. *Agronomy for Sustainable Development*, 33(2), 291–309. <https://doi.org/10.1007/s13593-011-0071-8>
- Neher, D. A. (2001). Role of Nematodes in Soil Health and Their Use as Indicators. *Journal of Nematology*, 33(4), 161–168.
- Nehls, U. (2008). Mastering ectomycorrhizal symbiosis: The impact of carbohydrates. *Journal of Experimental Botany*, 59(5), 1097–1108. <https://doi.org/10.1093/jxb/erm334>
- Newbound, M., McCarthy, M. A., & Lebel, T. (2010). Fungi and the urban environment: A review. *Landscape and Urban Planning*, 96(3), 138–145. <https://doi.org/10.1016/j.landurbplan.2010.04.005>
- Newsham, K. K., Fitter, A. H., & Watkinson, A. R. (1995). Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology & Evolution*, 10(10), 407–411. [https://doi.org/10.1016/S0169-5347\(00\)89157-0](https://doi.org/10.1016/S0169-5347(00)89157-0)

Nocker, A., & Camper, A. K. (2006). Selective Removal of DNA from Dead Cells of Mixed Bacterial Communities by Use of Ethidium Monoazide. *Applied and Environmental Microbiology*, 72(3), 1997–2004. <https://doi.org/10.1128/AEM.72.3.1997-2004.2006>

Nowak, D. J. (n.d.). *i-Tree: Global tools to assess tree benefits and risks to improve forest management*. 4.

Nowak, D. J., & Crane, D. E. (2000). *The Urban Forest Effects (UFORE) model: Quantifying urban forest structure and functions*. <https://www.nrs.fs.fed.us/pubs/8420>

Nowak, D. J., & Crane, D. E. (2002). Carbon storage and sequestration by urban trees in the USA. *Environmental Pollution*, 116(3), 381–389. [https://doi.org/10.1016/S0269-7491\(01\)00214-7](https://doi.org/10.1016/S0269-7491(01)00214-7)

Nowak, D. J., Hirabayashi, S., Doyle, M., McGovern, M., & Pasher, J. (2018). Air pollution removal by urban forests in Canada and its effect on air quality and human health. *Urban Forestry & Urban Greening*, 29, 40–48. <https://doi.org/10.1016/j.ufug.2017.10.019>

Nowak, D., & Walton, J. (2005). Projected Urban Growth (2000–2050) and Its Estimated Impact on the US Forest Resource. *Journal of Forestry*, 103, 383–389.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M., Szöcs, E., & Wagner, H. (2020). *Vegan community ecology package version 2.5-7 November 2020*.

Peay, K. G., Kennedy, P. G., & Bruns, T. D. (2011). Rethinking ectomycorrhizal succession: Are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecology*, 4(3), 233–240. <https://doi.org/10.1016/j.funeco.2010.09.010>

Philippot, L., Hallin, S., & Schlöter, M. (2007). Ecology of Denitrifying Prokaryotes in Agricultural Soil. *Advances in Agronomy*, 96, 249–305. [https://doi.org/10.1016/S0065-2113\(07\)96003-4](https://doi.org/10.1016/S0065-2113(07)96003-4)

Phillips, R. P., Brzostek, E., & Midgley, M. G. (2013). The mycorrhizal-associated nutrient economy: A new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist*, 199(1), 41–51. <https://doi.org/10.1111/nph.12221>

Plyaskina, O. V., & Ladonin, D. V. (2009). Heavy metal pollution of urban soils. *Eurasian Soil Science*, 42(7), 816–823. <https://doi.org/10.1134/S1064229309070138>

Pöhlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., Nguyen, N., Kjoller, R., Bates, S. T., Baldrian, P., Frøslev, T. G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., ...

Tedersoo, L. (2020). FungalTraits: A user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, 105(1), 1–16. <https://doi.org/10.1007/s13225-020-00466-2>

Pouyat, R. V., & Trammell, T. L. E. (2019). Chapter 10—Climate change and urban forest soils. *Developments in Soil Science*, 36, 189–211. <https://doi.org/10.1016/B978-0-444-63998-1.00010-0>

Rahman, M. A., Moser, A., Gold, A., Rötzer, T., & Pauleit, S. (2018). Vertical air temperature gradients under the shade of two contrasting urban tree species during different types of summer days. *Science of The Total Environment*, 633, 100–111. <https://doi.org/10.1016/j.scitotenv.2018.03.168>

Read, D. J., Leake, J. R., & Perez-Moreno, J. (2011). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*. <https://doi.org/10.1139/b04-123>

Rice, C. W., Moorman, T. B., & Beare, M. (1997). Role of Microbial Biomass Carbon and Nitrogen in Soil Quality. In *Methods for Assessing Soil Quality* (pp. 203–215). John Wiley & Sons, Ltd. <https://doi.org/10.2136/sssaspecpub49.c12>

Rillig, M. C., Wright, S. F., Nichols, K. A., & Schmidt, W. F. (n.d.). *Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils*. 11.

Robertson, G. P., & Groffman, P. (2015). *Nitrogen transformations* (pp. 421–446). <https://doi.org/10.1016/B978-0-12-415955-6.00014-1>

Roley, S. S., Tank, J. L., Stephen, M. L., Johnson, L. T., Beaulieu, J. J., & Witter, J. D. (2012). Floodplain restoration enhances denitrification and reach-scale nitrogen removal in an agricultural stream. *Ecological Applications*, 22(1), 281–297. <https://doi.org/10.1890/11-0381.1>

Rosenblatt, A. E., Gold, A. J., Stolt, M. H., Groffman, P. M., & Kellogg, D. Q. (2001). Identifying Riparian Sinks for Watershed Nitrate using Soil Surveys. *Journal of Environmental Quality*, 30(5), 1596–1604. <https://doi.org/10.2134/jeq2001.3051596x>

Roy, S., Byrne, J., & Pickering, C. (2012). A systematic quantitative review of urban tree benefits, costs, and assessment methods across cities in different climatic zones. *Urban Forestry & Urban Greening*, 11(4), 351–363. <https://doi.org/10.1016/j.ufug.2012.06.006>

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17, e00547. <https://doi.org/10.1016/j.gecco.2019.e00547>

Selosse, M.-A., Richard, F., He, X., & Simard, S. W. (2006). Mycorrhizal networks: Des liaisons dangereuses? *Trends in Ecology & Evolution*, 21(11), 621–628.
<https://doi.org/10.1016/j.tree.2006.07.003>

Serrana, J. M., Li, B., Sumi, T., Takemon, Y., & Watanabe, K. (2021). Profiling the microbial community structure and functional diversity of a dam-regulated river undergoing gravel bar restoration. *Freshwater Biology*, 66(11), 2170–2184.
<https://doi.org/10.1111/fwb.13824>

Shapleigh, J. P. (2013). Denitrifying Prokaryotes. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The Prokaryotes: Prokaryotic Physiology and Biochemistry* (pp. 405–425). Springer. https://doi.org/10.1007/978-3-642-30141-4_71

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal Symbiosis*. Academic Press.

Song, P., Kim, G., Mayer, A., He, R., & Tian, G. (2020). Assessing the Ecosystem Services of Various Types of Urban Green Spaces Based on i-Tree Eco. *Sustainability*, 12(4), 1630. <https://doi.org/10.3390/su12041630>

Sparks, D. L., Soil Science Society of America, & American Society of Agronomy. (1996). *Methods of soil analysis. Part 3, Part 3*. Soil Science Society of America : American Society of Agronomy.

Staley, C., Unno, T., Gould, T. j., Jarvis, B., Phillips, J., Cotner, J. b., & Sadowsky, M. j. (2013). Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. *Journal of Applied Microbiology*, 115(5), 1147–1158. <https://doi.org/10.1111/jam.12323>

Steenberg, J. W. N., Duinker, P. N., & Nitoslawski, S. A. (2019). Ecosystem-based management revisited: Updating the concepts for urban forests. *Landscape and Urban Planning*, 186, 24–35. <https://doi.org/10.1016/j.landurbplan.2019.02.006>

Sun, Y., & Zhao, S. (2016). Leaf litter decomposition in urban forests: Test of the home-field advantage hypothesis. *Annals of Forest Science*, 73(4), 1063–1072.
<https://doi.org/10.1007/s13595-016-0577-y>

Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). Decomposition in terrestrial ecosystems. *Studies in Ecology (USA)*.
https://scholar.google.com/scholar_lookup?title=Decomposition+in+terrestrial+ecosystems&author=Swift%2C+M.+J.&publication_year=1979

Sweeney, B. W., Bott, T. L., Jackson, J. K., Kaplan, L. A., Newbold, J. D., Standley, L. J., Hession, W. C., & Horwitz, R. J. (2004). Riparian deforestation, stream narrowing, and loss of stream ecosystem services. *Proceedings of the National Academy of Sciences*, 101(39), 14132–14137. <https://doi.org/10.1073/pnas.0405895101>

- Tajik, S., Ayoubi, S., & Lorenz, N. (2020). Soil microbial communities affected by vegetation, topography and soil properties in a forest ecosystem. *Applied Soil Ecology*, 149, 103514. <https://doi.org/10.1016/j.apsoil.2020.103514>
- Tedersoo, L., & Smith, M. E. (2013). Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, 27(3), 83–99. <https://doi.org/10.1016/j.fbr.2013.09.001>
- Teste, F. P., Lambers, H., Enowashu, E. E., Laliberté, E., Marhan, S., & Kandeler, E. (2021). Soil microbial communities are driven by the declining availability of cations and phosphorus during ecosystem retrogression. *Soil Biology and Biochemistry*, 163, 108430. <https://doi.org/10.1016/j.soilbio.2021.108430>
- Thompson, J., Pelc, C. E., Brogan, W. R., & Jordan, T. E. (2018). The multiscale effects of stream restoration on water quality. *Ecological Engineering*, 124, 7–18. <https://doi.org/10.1016/j.ecoleng.2018.09.016>
- Tibbett, M., & Sanders, F. E. (2002). Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. *Annals of Botany*, 89(6), 783–789. <https://doi.org/10.1093/aob/mcf129>
- Tiedje, J.M. 1982. Denitification. Pages 1011-1025 in Methods of soil analysis, Part 2: microbiological and biochemical properties. 1 edition. ASA and SSA, Madison, Wisconsin.
- Tuttle, A. K., McMillan, S. K., Gardner, A., & Jennings, G. D. (2014). Channel complexity and nitrate concentrations drive denitrification rates in urban restored and unrestored streams. *Ecological Engineering*, 73, 770–777. <https://doi.org/10.1016/j.ecoleng.2014.09.066>
- Ullah, S., & Faulkner, S. P. (2006). Denitrification Potential of Different Land-use Types in an Agricultural Watershed, Lower Mississippi Valley. *Ecological Engineering*, 28, 131–140. <https://doi.org/10.1016/j.ecoleng.2006.05.007>
- Urban, D. L., O'Neill, R. V., & Shugart, H. H. (1987). Landscape Ecology. *BioScience*, 37(2), 119–127. <https://doi.org/10.2307/1310366>
- United Nations World Water Assessment Programme (WWAP) (2010) Water for sustainable urban human settlements. Briefing note. United Nations Human Settlements Programme (UN-HABITAT)
- Van Der Heijden, M. G. A., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3), 296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>

Vandekerckhove, T. T., Willems, A., Gillis, M., & Coomans, A. 2000. (n.d.). Occurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in *Xiphinema americanum*-group species (Nematoda, Longidoridae). *International Journal of Systematic and Evolutionary Microbiology*, 50(6), 2197–2205.

<https://doi.org/10.1099/00207713-50-6-2197>

van der Linde, S., Suz, L. M., Orme, C. D. L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H. S., Hansen, K., Jacob, F., Kristöfel, F., ... Bidartondo, M. I. (2018). Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature*, 558(7709), 243–248. <https://doi.org/10.1038/s41586-018-0189-9>

Vannote, R. L., Minshall, W. G., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*. <https://doi.org/10.1139/f80-017>

Violin, C. R., Cada, P., Sudduth, E. B., Hassett, B. A., Penrose, D. L., & Bernhardt, E. S. (2011). Effects of urbanization and urban stream restoration on the physical and biological structure of stream ecosystems. *Ecological Applications*, 21(6), 1932–1949. <https://doi.org/10.1890/10-1551.1>

Vitousek, P. M. (1994). Beyond Global Warming: Ecology and Global Change. *Ecology*, 75(7).

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., & Tilman, D. G. (1997). Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications*, 7(3), 737–750. [https://doi.org/10.1890/1051-0761\(1997\)007\[0737:HAOTGN\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2)

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human Domination of Earth's Ecosystems. *Science*, 277(5325), 494–499. <https://doi.org/10.1126/science.277.5325.494>

Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20(1), 5–15. <https://doi.org/10.1890/08-0127.1>

Vitousek, P. M., Turner, D. R., Parton, W. J., & Sanford, R. L. (1994). Litter Decomposition on the Mauna Loa Environmental Matrix, Hawai'i: Patterns, Mechanisms, and Models. *Ecology*, 75(2), 418–429. <https://doi.org/10.2307/1939545>

Wallace, K. J., Laughlin, D. C., Clarkson, B. D., & Schipper, L. A. (2018). Forest canopy restoration has indirect effects on litter decomposition and no effect on denitrification. *Ecosphere*, 9(12), e02534. <https://doi.org/10.1002/ecs2.2534>

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & II, R. P. M. (2015). The urban stream syndrome: Current knowledge and the search for a cure. *Journal of the North American Benthological Society*. <https://doi.org/10.1899/04-028.1>

Wang, C., Liu, D., & Bai, E. (2018). Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biology and Biochemistry*, 120, 126–133. <https://doi.org/10.1016/j.soilbio.2018.02.003>

Wang, W. J., Dalal, R. C., Moody, P. W., & Smith, C. J. (2003). Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biology and Biochemistry*, 35(2), 273–284. [https://doi.org/10.1016/S0038-0717\(02\)00274-2](https://doi.org/10.1016/S0038-0717(02)00274-2)

Wang, Y., Wang, M., Li, Y., Wu, A., & Huang, J. (2018). Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. *PLOS ONE*, 13(4), e0196408. <https://doi.org/10.1371/journal.pone.0196408>

Wanxiao Wang, Jincai Shi, Qiujin Xie, Yina Jiang, Nan Yu, & Ertao Wang. (n.d.). *Nutrient Exchange and Regulation in Arbuscular Mycorrhizal Symbiosis: Molecular Plant*. Retrieved October 4, 2021, from [https://www.cell.com/molecular-plant/fulltext/S1674-2052\(17\)30228-9?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1674205217302289%3Fshowall%3Dtrue](https://www.cell.com/molecular-plant/fulltext/S1674-2052(17)30228-9?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1674205217302289%3Fshowall%3Dtrue)

Waterlot, C., Bidar, G., Pelfrène, A., Roussel, H., Fourrier, H., & Douay, F. (2013). Contamination, Fractionation and Availability of Metals in Urban Soils in the Vicinity of Former Lead and Zinc Smelters, France. *Pedosphere*, 23(2), 143–159. [https://doi.org/10.1016/S1002-0160\(13\)60002-8](https://doi.org/10.1016/S1002-0160(13)60002-8)

Webb, M. (1953). Effects of Magnesium on Cellular Division in Bacteria. *Science*, 118(3073), 607–611. <https://doi.org/10.1126/science.118.3073.607>

Wemheuer, F., Taylor, J. A., Daniel, R., Johnston, E., Meinicke, P., Thomas, T., & Wemheuer, B. (2020). Tax4Fun2: Prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. *Environmental Microbiome*, 15(1), 11. <https://doi.org/10.1186/s40793-020-00358-7>

White, C. S., & McDonnell, M. J. (1988). Nitrogen cycling processes and soil characteristics in an urban versus rural forest. *Biogeochemistry*, 5(2), 243–262. <https://doi.org/10.1007/BF02180230>

Whitney, K. (2010). Living Lawns, Dying Waters: The Suburban Boom, Nitrogenous Fertilizers, and the Nonpoint Source Pollution Dilemma. *Technology and Culture*, 51(3), 652–674.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemond, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E.,

- Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., ... Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>
- Willms, I. M., Rudolph, A. Y., Göschel, I., Bolz, S. H., Schneider, D., Penone, C., Poehlein, A., Schöning, I., & Nacke, H. (n.d.). Globally Abundant “Candidatus Udaeobacter” Benefits from Release of Antibiotics in Soil and Potentially Performs Trace Gas Scavenging. *MSphere*, 5(4), e00186-20. <https://doi.org/10.1128/mSphere.00186-20>
- Wiseman, P. E., & Wells, C. (2005). Soil Inoculum Potential and Arbuscular Mycorrhizal Colonization of *Acer Rubrum* in Forested and Developed Landscapes 7.
- Wortley, L., Hero, J.-M., & Howes, M. (2013). Evaluating Ecological Restoration Success: A Review of the Literature. *Restoration Ecology*, 21(5), 537–543. <https://doi.org/10.1111/rec.12028>
- Yan, L., Xie, C., Xu, X., & Che, S. (2019). The influence of revetment types on soil denitrification in the adjacent tidal urban riparian zones. *Journal of Hydrology*, 574, 398–407. <https://doi.org/10.1016/j.jhydrol.2019.04.071>
- Yochum, S. (2018). Guidance for Stream Restoration. *United States Department of Agriculture*, 112.
- Zhang, Y., Ji, G., Wu, T., & Qiu, J. (2020). Urbanization significantly impacts the connectivity of soil microbes involved in nitrogen dynamics at a watershed scale. *Environmental Pollution*, 258, 113708. <https://doi.org/10.1016/j.envpol.2019.113708>