The Influence of Urbanization on Microbial Diversity and Composition at Urban Vegetation Restoration Sites

SHABNAM TEJANI, MARC CADOTTE
UNIVERSITY OF TORONTO, SCARBOROUGH

ABSTRACT

Urbanization is a major driver of global change, leading to rapid biodiversity loss, habitat fragmentation and species extinctions. Vegetative restoration of degraded ecosystems is a important strategy for restoring ecosystem function, but the effects of restoration efforts on soil microbial communities is not well understood. To understand the impacts of restoration efforts on microbial diversity and composition, we explored the ecological processes and abiotic influences that shape microbial communities across restorative gradients. While microbial species diversity was similar across sites, species composition was varied and influenced by intersecting environmental factors. Species richness increased with distance from Toronto City Centre, likely as a result of soil alkalinity and imperviousness in urbanized areas.

INTRODUCTION

- Restoration initiatives are employed to facilitate the rehabilitation of sites where anthropogenic disturbances inhibit natural unassisted vegetative regeneration.
- Facilitated native vegetative restoration is the preliminary step in improving soil quality; regaining nutrient acquisition, ecosystem functions, and supporting high levels of biodiversity.
- The growth of new vegetation can increase the input of organic matter into the soil providing a new source of nutrient cycling and improving soil physical and chemical properties, which promotes ecosystem functioning and processes.
- Soil microbes play a crucial role in ecosystem functioning, and changes in soil microbial communities can have significant impacts on soil quality and plant productivity, and ecosystem functioning.

HYPOTHESIS: ***Soil microbial diversity and composition in actively restored forests decreases along a gradient of urbanization and is moderated by factors such as :***

- ***Restoration site age***
  TIME SINCE CESSATION OF PREVIOUS LAND USES (IE. AGRICULTURE OR URBAN DEVELOPMENT) + NUMBER OF RESTORATION PLANTINGS = MORE ESTABLISHED ECOSYSTEM
- ***Less Urbanized Landscapes***
  URBANIZED LANDSCAPES ARE ASSOCIATED WITH METAL CONTAMINATION AND MORE ALKALINE SOIL
- ***Planted restoration community diversity and composition***
  HOW DOES % COVER, TREE DIVERSITY AND TARGET SPECIES SHAPE BIOREMEDIATION TRAJECTORIES

STUDY LOCATION

As of 2021, **81.56%** of Canada's population lives in major cities, while rural communities continue to decline. Major cities inherently facilitate rapid urbanization and habitat fragmentation due to land clearing, immense infrastructure construction, and dense human habitation.

Figure 1: Historical land use change of The Greater Toronto Area between 1939 and 2017, illustrates the extent to which urbanization has led to ecosystem fragmentation.

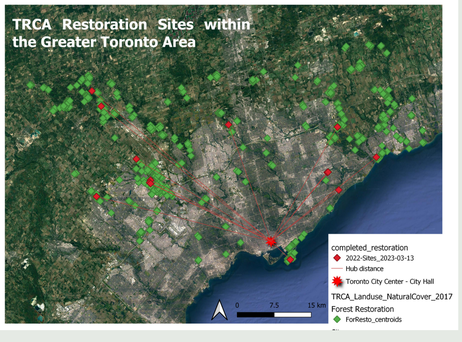

Figure 2: Toronto and Region Conservation Authority (TRCA) restoration sites in the GTA (green) assessed during this study (red), and their relative distances from the Toronto city centre (The hub).

METHODS & RESULTS

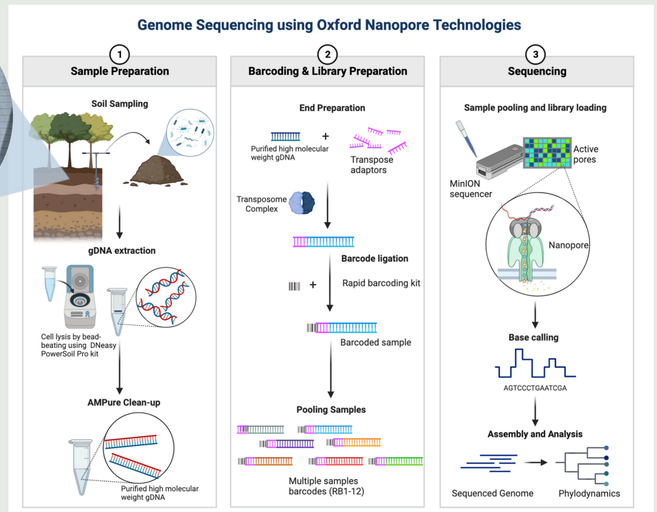

Figure 3: We utilized **Oxford's MinION Nanopore sequencer to provide species-level taxonomic resolution of soil microbiomes** of various restoration sites. Soil samples were first assessed using a NanoDrop™ 2000 Spectrophotometer to assess DNA quality before extraction. Samples were prepared for DNA sequencing via tagging with barcoded primers to allow differentiation of DNA samples.

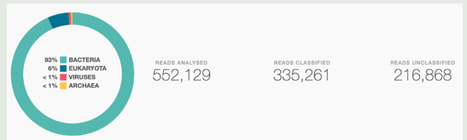

Figure 4: Genome sequences of bacterial, archaea, fungal and viral species were assembled using the rapid gDNA library preparation and real-time WIMP analysis workflow by EPI2ME. Barcoded gDNA strands were converted to basecalls using nanopore technology resulting in 335,261 eligible base-call reads, classified using the NCBI database producing rapid species identification.

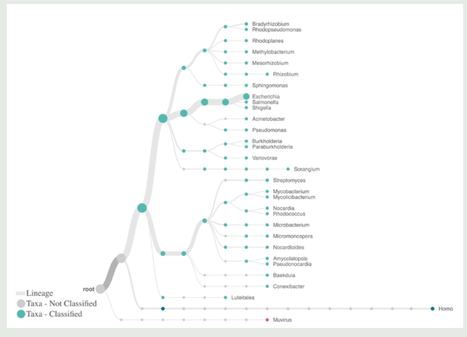

Figure 5: Taxonomic tree of top 30 genera with a 0.1% minimum abundance cutoff. Thickness of the branches displayed correlates to the relative read counts for each genus. Represented from samples are species from 12 orders, 8 classes, 5 phyla, and 3 superkingdoms - bacteria, Eukarya and Virus.

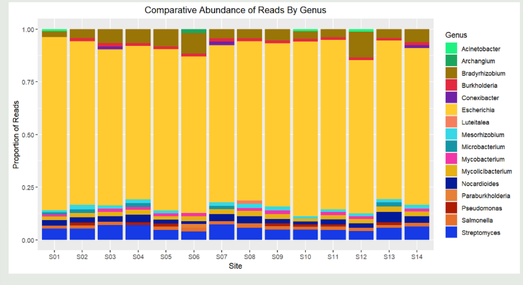

Figure 6: Relative abundance bar plot displaying comparative abundance of reads by genus per site. Illustrating the composition of bacterial species per site. Escherichia being the highest in abundance across all sites, followed by Bradyrhizobium and Streptomyces.

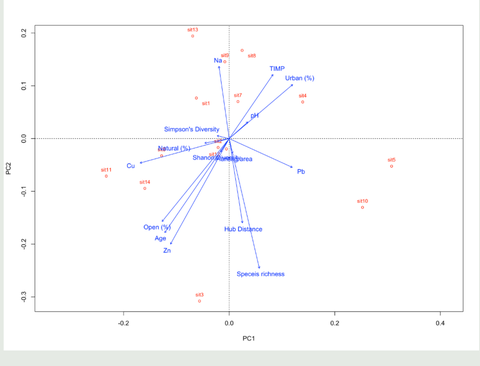

Figure 7: Non-metric Dimensional Scaling (NMDS) plot demonstrating the relative effect of environmental factors on species composition across sites. Table 1 provides further insight of each factor influencing species composition.

Variables of Beta diversity Measure affecting Composition of Microbial Species	
Sit1-14	*Species composition of Restoration sites 1-14*
TIMP	*A cumulative value of impervious (i.e., paved, bricked, and/or concrete) surfaces at 100 meters. A measure of urbanization intensity employed to establish an urbanization gradient*
Urban %	*Land use categories classified as urban (residential, industrial, commercial, etc.). were summed for the total percentage of the urban area.*
Natural %	*Natural land uses total percentages (i.e., forest, meadow, riverine, etc.)*
Open %	*Open land uses total percentages (i.e., Agriculture, cemetery, etc.)*
Age	*Age of restoration (Time between cessation of previous land use and time of sampling.*
Planting area	*Size or quantity of area planted in the project. This may be an indication of the restoration effort as a greater number of trees and/or funds are applied to larger projects.*
Na, Cu, Pb, Zn	*Presence of sodium, copper, lead, and zinc*
Hub Distance	*Distance (km) from the hub of the city i.e., Toronto City Hall*

DISCUSSION

- Remediation and rehabilitation of plant communities facilitates microbial species recovery, promoting resilience to future disturbances. Operative effects include microclimate variations, improving soil biogeochemical properties, and increasing biotic interactions.
- Simpson and Shannon indices showed relatively similar bacterial diversity throughout sites, however, species composition differed between sites.
- Restoration age, proportion of canopy cover and zinc concentrations were highly correlated across sites. Zinc is a key component in driving metabolic reactions by catalyzing activation of enzymes for plant protein synthesis, enabling growth regulation, and is often used as crop fertilizer. However, high concentrations of zinc can be detrimental to soil microbial survival, as it disrupts photo-oxidative and -catalytic processes. Greater microbial diversity at these sites could thus indicate pollution induced community tolerace
- Sites with high pH were closely associated with greater impervious surface and urban proportionality in the surrounding area, likely as soil disturbances (i.e compaction and contamination), vehicle exhaust pollution and urban liming can increase soil alkalinity (Yang & Zhang, 2015)
- Copper concentrations were correlated with less urban sites indicating successful naturalization to pre-existing soil states. Although anthropogenic inputs can cause flux in heavy metal levels within the soil (i.e. Zn, Pb, Cu), average metal content is indicative of natural rather than urbanized landscapes (Mirzaei et al. 2015).
- Muvirus, more specifically phage Mu of Escherichia known to insert its DNA into host E. coli chromosomes, was the among the top 30 genera present at the restoration sites, most likely due to the natural interplay between bacterial and viral agents.

Constant exposure to extreme human induced disturbances is detrimental to what once used to be natural habitat. **Natural habitat fragments within urban ecosystems consist of novel altered species interactions, biogeochemical conditions, and unprecedented community compositions shaped by anthropogenic forcings.**
For community managers seeking to guide restoration projects, **land use history, distance from urban hub and metal contaminations have strong implications for soil microbial communities** and therefore overall site restoration goals. **Assessing microbial needs of a site can optimize restoration efforts** to achieve outcomes critical for sustainable management and conservation frameworks, and streamline resource application

FURTHER RESEARCH

- Incorporating above and below ground vegetation and microbe functional groups to better understand contributions to ecosystem functioning processes.
- Exploring the impact of phylogenetic diversity and taxonomic distance on species functional groups and overall community composition.
- Exploration of detailed land use classes and their correlation to soil abiotic conditions and impacts on microbial communities.

REFERENCES

Bhattacharyya, S., Soniat, M.M., Walker, D., Jang, S., Finkelstein, I.J., and Harshey, R.M. Phage Mu Gam protein promotes NHEJ in concert with Escherichia coli ligase. Proc. Natl. Acad. Sci. Dec 11;115(50): E11614-E11622. doi: 10.1073/pnas.1816606115

Bowd, E., Blanchard, W., McBurney, L., & Lindenmayer, D. (2021). Direct and indirect disturbance impacts on forest biodiversity. Ecosphere, 12(12), e03823. https://doi.org/10.1002/ecs2.3823

Liu, L., Zhu, K., Krause, S. M. B., Li, S., Wang, X., Zhang, Z., Shen, M., Yang, Q., Lian, J., Wang, X., Ye, W., & Zhang, J. (2021). Changes in assembly processes of soil microbial communities during secondary succession in two subtropical forests. Soil Biology and Biochemistry, 154, 108144. https://doi.org/10.1016/j.soilbio.2021.108144

Matsuo, Y., Komiya, S., Yasumizu, Y., Yasuoka, Y., Mizushima, K., Takagi, T., Kryukov, K., Fukuda, A., Morimoto, Y., Naito, Y., Okada, H., Bono, H., Nakagawa, S., & Hirota, K. (2021). Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinIONTM nanopore sequencing confers species-level resolution. BMC Microbiology, 21(1), 35. https://doi.org/10.1186/s12866-021-02094-5

Singh, J. S., & Gupta, V. K. (2018). Soil microbial biomass: A key soil driver in management of ecosystem functioning. Science of The Total Environment, 634, 497–500. https://doi.org/10.1016/j.scitotenv.2018.03.373

Yang, J.-L., & Zhang, G.-L. (2015). Formation, characteristics and eco-environmental implications of urban soils – A review. Soil Science and Plant Nutrition, 61(sup1), 30–46. https://doi.org/10.1080/00380768.2015.1035622

BioRender. (n.d.). Retrieved March 27, 2023, from https://app.biorender.com/illustrations/641d1147cbfcdb08d12cdcc1

Gautam, R., (2022) Evaluating Soil Trace Metal Content in Urban Restoration Sites Across the Greater Toronto Area. Department of Physical and Environmental Sciences, UTSC. CHMD90 Thesis in Completion of Environmental Chemistry Specialist (ongoing)

CUBES LAB; CADOTTE URBAN BIODIVERSITY & ECOSYSTEM SERVICES LAB
@TejaniShabnam Shabnam.tejani@mail.utoromto.ca @Shabnam Tejani