How Mechanical Disturbances Affect Soil Respiration Rates in Urban Forests Five to Eleven Years After Development

by

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ABSTRACT

How Mechanical Disturbances Affect Soil Respiration Rates in Urban Forests
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Urban forests are a valuable resource which provide ecological services and functions. The integrity of an urban forest patch can be affected long-term by soil disturbances associated with urban land development, such as: topsoil clearing and soil compaction. The purpose of this exploratory study is to analyze soil microbial activity of forested urban areas following land development with known disturbance histories. Three sites in Guelph, ON, with soil disturbance histories between 5 and 11 years ago were used to compare soil microbial activity. Soils from forests after mechanical disturbance and controls were measured for respiration rates in forest patches using a 24-hour soil CO₂ test. Disturbed soils from 5, 7, and 11 years after disturbance had lower respiration values compared to controls. Impacted soil biology can be easily tested for and should be considered by landscape architects to plan for more resilient urban forests.

Key words: Urban Land Development, Solvita 24hr CO₂ test, Landscape Architecture, Soil Microbiome
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Landscape architects can be involved in urban forest planning and design. Examples of relevant projects include: afforestation, reforestation, park and trail design, and other efforts to increase tree canopy cover within urban areas. However, typical urban land development practices which often occur prior to urban forest re-establishment can result in functional impairments to physical, chemical, and biological soil conditions (Chen et al., 2013). Impaired soil conditions can lead to altered ecosystem functioning (Geissen et al., 2012; Viall, Gentry, Hopkins, Ganguli, & Stahl, 2014; Wurst & Ohgushi, 2015). This has implications for urban forests because, from a functional standpoint, they are equivalent to any other forest ecosystem.

Forest ecosystems are characterized by the occurrence of ecosystem functions such as water, carbon, and nutrient cycling (Waring & Running, 2010). These forest ecosystem functions are largely a result of the living organisms that exist in the lowest soil horizons to the uppermost vegetative canopies. Landscape architects are faced with a practical problem when working on urban forestry projects, and the purpose of this chapter is to define the problem and to explain the expected results of this exploratory study. This exploratory study focuses on urban forests in Guelph, Ontario, and is concerned with how mechanical disturbances affect soil biological functioning. This introductory chapter will begin with a brief explanation on the value of urban forests, followed by a description of disturbance and of typical urban land development, and will conclude with information about soil microbiology and soil microbiological testing methods.

1.1. Urban Forests

Urbanization trends could result in an increasing amount of urban forests to be converted to different land uses with different land cover types. However, urban forests are valuable. Urban forests are generally perceived to have inherent value, by providing ecological, economic, environmental, and social benefits (City of Guelph, 2012; Firehock, 2015; Tsunetsuga et al., 2013). According to Brommer (2015), urban forests in Guelph, Ontario, contribute to an estimated 28.6% canopy cover average in the city. US forestry services suggest that a 40%
canopy cover in cities will maximize the benefits and ensure the sustainability of urban forests (City of Toronto, 2013). Guelph's urban forest is valuable for the city and its residents.

Urban forests function like any forest ecosystem, but also face increased disturbance frequency and severity (City of Toronto, 2013). For example, in Canada an estimated 1.2 million street trees are *Fraxinus spp.* (McKenney et al., 2012). Emerald ash borer infestations, which affect *Fraxinus spp.*, have resulted in urban forest canopy reductions and have an estimated impact of $524 - $890 million by the year 2012 (McKenney et al., 2012). The emerald ash borer infestation impacted economic, environmental, and human health measures (Donovan et al., 2013). Dutch elm disease had a similar effect on urban forests in many regions of Canada, which highlights how biotic disturbances can be detrimental to urban forests. The popular use of *Fraxinus* and *Ulmus spp.* in urban forest planting resulted in urban forests being severely impacted across many parts of North America.

### 1.2. Disturbance and Typical Urban Land Development

The negative impacts of biotic disturbances such as the emerald ash borer or Dutch elm disease are visually evident. However, abiotic or human caused disturbances also affect urban forests, and may be more difficult to perceive. Abiotic disturbance such as topsoil clearing, subsoil compaction, and other mechanisms can bring physical, chemical, and biological changes to site conditions (Hagan et al., 2011; Moora et al., 2014). Abiotic disturbances, such as mechanical disturbances caused by the use of heavy machinery, are common during typical land development practices for site preparation and construction (Chen et al., 2013). Urban land development often involves land use and land cover change, of which both can be detrimental to soil properties.

Soil both figuratively and literally forms the base of forest ecosystems. Soil disturbance mechanisms associated with land use and land cover change are analogous to those that occur during urban land development, and have been extensively studied in agriculture (Kaschuk, Alberton, & Hungria, 2011; Postma-Blaauw, De Goede, Bloem, Faber, & Brussaard, 2010; Thomson et al., 2015), forestry (Hartmann et al., 2012), and ecological restoration (Bach, Baer, Meyer, & Six, 2010). Soil compaction and topsoil removal have particularly been identified as
having negative impacts for agricultural, forest, and other ecosystems. This concern has not been addressed to the same degree for urban land development. Landscape architects oversee and utilize typical land development practices, which are often associated with soil disturbances. Unlike biotic disturbances, steps taken during urban forest planning can directly prevent potential mechanical soil disturbances.

1.3. Soil Microbiology and Legacy Effects

Soil microbes are responsible for many ecosystem functions which benefit plant communities. High numbers of soil microbes can be found in upper soil horizons such as topsoil (Aber & Melillo, 2001). Soil microbial populations can be extremely biodiverse (Berendsen, Pieterse, & Bakker, 2012), but are primarily composed of bacteria and fungi in forest ecosystems (Mendes, Garbeva, & Raaijmakers, 2013). Soil microbial populations, although composed of different microbial lifeforms, function together and are collectively known as the soil microbiome. Through symbiotic plant-microbe interactions, the plant provides fuel for microbes in the form of simple sugars, and in return the soil microorganisms enhance biochemical processes which can increase plant growth (Lugtenberg & Kamilova, 2009; Nadeem, Ahmad, Zahir, Javaid, & Ashraf; Schlaeppi & Bulgarelli, 2015) and improve disease resistance (Berendsen et al., 2012; Pieterse et al., 2014).

Soil disturbances have been shown to disrupt microbial populations. Intensive land uses affect the composition and the functioning of the soil microbiome (Moora et al., 2014; Oehl et al., 2010; Postma-Blaauw et al., 2010). These changes can alter soil microbiological functioning and subsequent nutrient, carbon, and energy cycles (Busse et al., 2006; Enloe, Lockaby, Zipperer, & Somers, 2015). This could lead to decreased plant productivity (Larney, Janzen, Olsen & Olsen, 2009), and less resilient and resistant ecosystems when stressed (Vries et al., 2012). These effects are seen immediately following disturbance, with soil microbial functional and compositional changes that can persist for multiple years.

Persistent functional and compositional changes which occur in ecosystems after disturbance are called legacy effects. Legacies of disturbance have been observed in many ecosystem types, including forests (Song et al., 2015). Functional and compositional changes to
the soil microbiome have been documented to last anywhere from 1-100 years after a disturbance regimen (Fichtner, von Oheimb, Hardtle, Wilken, & Gutknecht, 2014). Long-lasting effects are often found after abiotic disturbances analogous to those which occur during urban land development. In order to study disturbance and legacy, microbiological testing methods are typically used as indicators of soil biological composition and function.

1.4. Microbiological Soil Testing Methods

Microbiological soil testing methods can be used for a variety of desired analyses. However, functional metrics will be focused on for the purpose of this study. Tests of soil microbial function can be used to analyze ecosystem functions that are provided by the soil microbiome. A common methodology is soil respiration. Soil respiration is a measurement of the CO$_2$ efflux from soil samples. Effluxes of soil carbon can be used to measure levels of soil microbiome functioning because soil microorganisms respire CO$_2$ as a product of metabolic activity (Pell, Stenstrom, & Granhall, 2005). Soil respiration is correlated with other soil and microbial parameters (Mariani, Chang, & Kabzems, 2006). In general, healthy and intact microbial populations respire more CO$_2$ from soil compared to disturbed populations.

There are different methodologies for measuring soil respiration, including lab based and field testing techniques. An available option is a 24-hour soil respiration test, which has been used in previous studies to assess soil functionality (Munoz-Rojas, Erickson, Dixon, & Merritt, 2016). For the purpose of this study, a simple to use and inexpensive methodology is required. Commercially available tests such as the 24-hour methodology have previously been used to assess soil functionality after disturbance, with accurate results in comparison to related lab-based techniques (Haney, Brinton, & Evans, 2008).

1.5. Expected Outcomes and Hypotheses

This particular study will use a 24-hour soil respiration test to measure soil microbiome functioning in urban forest soils with histories of disturbance. Soil respiration values of disturbed soil will be compared to undisturbed forest soils in close proximity.
The primary hypothesis is that disturbed urban forest soils are expected to have lower levels of soil respiration, which is a measure of soil microbial functioning, compared to undisturbed soils 5, 7, and 11 years after mechanical disturbance. A secondary hypothesis is that disturbed forest soils with longer durations since disturbance are expected to show signs of recovery, which would be reflected in more similar soil respiration values in comparison to undisturbed levels.
Chapter 2: Literature Review

This chapter reviews literature relevant to this study. Section 2.1 defines and details the importance of urban forests. Section 2.2 introduces studies of the soil microbiome. Sections 2.3 and 2.4 highlight functions of the microbiome. The chapter concludes with sections 2.5 and 2.6, which describe factors that affect the soil microbiome.

2.1. Urban Forests: Definition, Benefits, and Health

For some, an urban forest is the only space where they can regularly access a natural environment. In a rapidly urbanizing world, urban forests are now becoming increasingly valued. This is because forests can provide numerous social, economic, and environmental benefits, especially in urban areas. Landscape architects can have a role in creating and ensuring the integrity of urban forest environments through design. This section will define the urban forest, describe the provided benefits for humans, and summarize the determinants of urban forest health and integrity.

Urban forests come in all shapes and sizes. However, some cities have officially defined the ‘urban forest’. For example, according to Guelph’s Urban Forestry Management Plan (2012):

The City’s urban forest is comprised of the individual trees and all the treed areas that occur within its boundaries. These include treed natural areas, as well as individual or small groups of trees in parks, along roadways, and on residential, industrial, commercial and institutional properties. (p.12).

This definition, while specific to Guelph, Ontario, Canada, is generalizable to most towns, suburbs, and cities. Space for urban forest can be limited in dense urban areas, thus urban forests are generally smaller in size in comparison to natural and managed forests outside of city boundaries.

According to some global projections, urban land is expected to triple in size between the years 2000-2030 (Seto, Guneralp, & Hutrya, 2012). Planning for urban forests requires knowledge of forest ecosystems at scales typically found within city boundaries. Most of our knowledge of forest ecosystems comes from studies of natural and managed forests outside of the city. However, these studies typically use a spatial scale for analysis known as a forest stand (Waring & Running, 2010). Waring & Running (2010) state that a study forest stand is generally recognized as being under one hectare in size, which is spatially equivalent to many urban
forests. Most research on forest stands is therefore applicable to urban forests, irrespective of the greater landscape context.

Forest ecosystems provide many ecological benefits. In an urban context, a forest can also provide economic, environmental, and social benefits which can help to improve human health. For example, Firehock (2015) lists economic benefits provided by urban forests such as: higher property values and reduced energy use for homes and other buildings protected by tree canopies. Ecosystem services provided by urban forests are now commonly assigned monetary value and are recognized as having economic importance. This includes ecosystem services which aid to filter air, water, and sunlight, as well as sequester carbon and reduce the urban heat island effect (City of Guelph, 2012). Finally, in a social sense, urban forests are of value because they are spaces where people can meet, interact, and play. In fact, just being in a natural setting, such as an urban forest, can be advantageous for an individual’s physiological and psychological well-being (Tsunetsuga et al., 2013). Ultimately, urban forests benefit humans.

With an understanding of the value of urban forests comes a responsibility to properly plan for and to manage these spaces. Proper management should ensure health and integrity of urban forests. Many variables are correlated with a healthy forest, such as: absence of disease and invasive species, canopy structure and vertical stratification, tree species composition and stand age, etc. (Waring & Running, 2010). However, urban forests are ecosystems. This means they are characterized by many biotic and abiotic interactions, across biological trophic levels, and are reliant on proper ecosystem functioning. The most important variables for a healthy forest are, in some way, related to ecosystem functions such as water, carbon, and nutrient cycles. Proper ecosystem functioning, and ultimately urban forest health and integrity, starts in the soil; where microscopic organisms have an undeniable influence on both belowground and aboveground processes.

2.2. The Soil Microbiome

Plants have, and continue, to coevolve with soil microorganisms. Soil is a substrate for a highly diverse community of microbes, which can live symbiotically with plants and can have
effects on their hosts that range from beneficial to parasitic. The soil microbial genes and cell densities associated with a plant far outnumber the plant's own genes and cell densities (Mendes et al., 2013). Soil microbiology is complex, as Berendsen et al. (2012) explain, “Soil microbial communities represent the greatest reservoir of biological diversity known in the world so far” (p.478). Although phylogenetically diverse, soil microorganisms can be studied as a functioning community, that is, from an ecological perspective. Thus, it is important to distinguish between and to define the terms soil microorganism, soil microbiota, and soil microbiome.

Soil microorganisms, or soil microbes, include bacteria, archaea, protozoa, algae, fungi, and viruses. These microorganisms are microscopic in scale, invisible to the naked eye. Soil microbiology generally involves analyzing individual microorganism species and populations, often providing important biological information. Next, soil microbiota refers to the ecological community of microorganisms, thus how microorganisms within this community interact with each other and with their physical surroundings. Schlaeppi & Bulgarelli (2015) define microbiota as the, “Totality of microbes (bacteria, archaea, and fungi) in a particular environment. Refers to taxonomy and abundance of community members” (p 213). Thus, profiling a soil microbiota can provide insights on community structure and on phylogenetic profiles of plant associated soil microbes.

Finally, as Mendes et al. (2013) explain, “The collective communities of plant-associated microorganisms are referred to as the plant microbiome or as the plants’ other genome” (p. 634). Differentiating between the terms soil microbiota and soil microbiome is important because of the desired outcomes of their respective analyses. Both soil microbiota and soil microbiome analyses utilize similar techniques, but soil microbiome analysis is focused on providing functional information about plant-microbe-environment interactions. The study of soil microbiota is generally concerned with identifying what life forms compose a microbial population, whereas the study of the soil microbiome is concerned with what the microbial population is doing. Examples of ecological processes studied through soil microbiome analysis are nutrient cycling, carbon sequestration, and nitrogen fixation.
Plant-microbe interactions are not confined to soils and roots. But, the area surrounding the plant root is characterized by high microbial abundance and activity in contrast with other aboveground plant microenvironments (Berg et al., 2014), and in comparison with bulk soil (Berendsen et al. 2012). For example, concentrations of bacteria in the rhizosphere can be from 10 to 1000 times higher than in bulk soil (Lugtenberg & Kamilova, 2009). The area of soil influenced by plant roots is called the rhizosphere (rhizο=root). The rhizosphere was first defined in 1904 by soil bacteriologist Lorenz Hiltner (Curl & Truelove, 1986). Thus, the idea of plant-microbe symbioses is not new. Technological advances and theoretical paradigms have advanced, resulting in a greater understanding of the rhizosphere, and how plants are able to shape their microbiome.

Plants, through their roots, are able to recruit beneficial microbes within the rhizosphere by exuding simple organic compounds that stimulate microbial activity (Aber & Melillo, 2001). A substantial amount of fixed carbon, measured experimentally between 5-21%, is secreted as root exudates (Marschner, 1995). In other words, plants can shape a microbiome from the existing microbial population (microbiota) in the soil. The simple organic compounds, which are products of photosynthesis, are an easy source of energy for soil microorganisms and create a rich substrate for microbial growth in the rhizosphere. The host-plant can then benefit from the symbiotic relationship through microbial mechanisms which support drought tolerance, increased nutrient availability and uptake, disease resistance, among other benefits. However, the symbiotic relationship does come at a carbon cost for the host-plant. Using photosynthetically produced energy to support soil microbial growth instead of other plant functions can be viewed as a carbon cost to plant benefit ratio. Ultimately, optimizing this ratio is important for ecosystem health and function.

2.3. Microbiome Effects on Plant Growth

The soil microbiome helps plants to grow through a variety of mechanisms. Research in agriculture, silviculture, horticulture, and plant science has resulted in a better understanding of the soil microbiome and its potential for applications in related professions. For example, understanding and manipulating soil microbiology has been deemed the next frontier in sustainable agriculture (Schlaeppi & Bulgarelli, 2015; Zahir et al., 2003). Also, sustainable
forestry methods are relying more on microbiological science to ensure the success of replanting after harvests such as clear cutting (Stanturf & Madsen, 2016). Despite a growing focus on functional contributions of the soil microbiome in current research, many research articles of the past rely on microbiological techniques that are intended to analyze particular constituents of the soil microbiome, such as bacteria or fungus. As a result of this, research findings in this section will first detail the effects and mechanisms of bacteria, and then the effects and mechanisms of fungus for plant growth. Keeping in mind that these constituents of the microbiome work together, this section will conclude with a description of microbial interactions in the soil and the influence on plant growth.

Bacterial plant growth promotion is a phenomenon which has received increased attention, and is a topic of many recent studies on soil microbiology. Research on plant-growth promoting bacteria in the rhizosphere (PGPR) is generally focused on identifying taxonomy and the mechanisms of how these bacterial populations can help plants to grow. Bacteria are the most abundant microorganisms present in the rhizosphere (Kaymak, 2010) and a large variety of bacterial genera have not only been identified, but have also been understood to have pronounced effects on plant growth (Nadeem et al., 2014). PGPR, through both direct and indirect mechanisms, have been found to enhance plant growth and development under non-stress and stress conditions (Glick et al., 2007; Nadeem et al., 2010). Direct mechanisms supporting plant growth include: enhanced plant nutrition via facilitative nutrient uptake from soil (Richardson, Barea, Mcneil, & Prigent-Combaret, 2009), the production of plant growth regulators, and the production of protective enzymes (Nadeem et al., 2013). Indirect mechanisms of plant growth by PGPR involve protection from harmful pathogens, and will be discussed in later sections. It is well understood that plant associated bacteria are able to benefit plant growth.

In addition to bacteria, fungi represent a significant portion of the soil microbiome and have also been found to enhance plant growth. Fungi are able to support plant growth through a symbiotic association with plant roots, called a mycorrhiza, which literally means ‘fungus root’ (Dighton, 2003). According to Giovanetti et al. (2006), about 80% of all terrestrial plants are able to establish this type of symbiotic association. By associating with fungi, plants are able to
benefit from a number of fungal mechanisms which can help to improve nitrogen fixation, to enhance photosynthesis rates, to produce secondary metabolites, and to protect the plant during osmotic stress (Ruiz-Lozano, 2003). Perhaps the most pronounced effects on plant growth are a result of the fungal structures being much smaller in diameter than the finest plant roots: with fungal filaments measuring around 5-6 µm compared with fine plant roots at roughly 1-2 mm (Harley, 1969). The fungal filaments have large surface to volume ratios and add a significant amount of root surface area, therefore enabling the plant to absorb water and nutrients more efficiently from soil (Nadeem et al. 2013). So, fungi provide both structural and functional benefits for plants, which is advantageous for plant growth.

As alluded to before, most of the information about how the soil microbiome can influence plant growth comes from studies which have teased apart the functions of constituent microorganisms, such as the individual effects of bacteria or fungi on particular growth mechanisms. However, the availability of new methodologies in microbiology has allowed researchers to be able to study the soil microbiome in a more holistic manner. For example, in a recent article by Pohjanen et al. (2014), the researchers detailed how mycorrhizal fungi and endophytic bacteria can together produce interactive effects for promoting plant growth and nutrient uptake in Pinus sylvestris seedlings. When both fungi and bacteria benefit plant growth through similar biological mechanisms, it is understandable how interactive effects could be achieved. Interactions between soil microorganisms have also been shown to indirectly improve plant growth by collectively inhibiting the growth of soil pathogens and enhancing growth mechanisms (Fitter & Garbaye 1994, Cardoso et al. 2013). To conclude, assemblies of beneficial microbes can have synergistic effects on plant growth, which hints at the influence the soil microbiome can have on plant productivity and health.

2.4. Plant Health: Disease Resistance and Stress Tolerance

The soil microbiome is important for plant health. Evolved plant-microbe symbioses can be beneficial for resisting a number of detrimental biotic and abiotic stressors which plants are commonly subjected to. An increasing body of evidence points to how the entire complex of rhizosphere associated microbes, and their interactions, can help plants to tolerate stress and disease (Daffonchio, Hirt, & Berg, 2014; Pieterse et al. 2014; Glick, 2015). Once again, the
majority of research comes from the domains of agriculture, plant science, and microbiology. But, compared to plant-growth mechanisms, the relationships between the soil microbiome and plant health are generally attributed to the total interactive functions of microbial mechanisms. This section will briefly describe both biotic and abiotic stressors which affect plant health and the corresponding indirect and direct soil microbial mechanisms which have evolved to protect the plant.

A multitude of pests and pathogens attack plants in nature. The above and belowground biotic attackers which can seriously threaten plant health include: bacterial and fungal pathogens, plant viruses, insects and nematodes (Glick, 2015). However, in addition to a plant’s immune system, the soil microbiome is critical for defending plants against biotic stressors through direct and indirect protective mechanisms. In 1991, three research groups independently provided evidence of an indirect plant protective mechanism in which bacterial strains in the soil promoted plant health through stimulation of the plant immune system (Pieterse et al. 2014). This mechanism has been termed induced systemic resistance (ISR). Walters, Ratsep, & Havis (2013) explain that ISR offers an enhanced general level of protection against a broad spectrum of attackers. Basically, ISR is characterized by a faster and stronger protective response from the plant following a biotic stress.

Besides the ability to confer general systemic resistance, the soil microbiome can help to protect plants against pathogens and pests through more direct mechanisms. For example, a number of reports indicate that beneficial plant associated soil microbes can have antagonistic effects towards pathogens, which increases plant resistance (Berg et al., 2005). Essentially, the beneficial microbes out-compete the pathogenic organisms for nutrient resources. The microbiome can also act to inhibit the functioning of plant pathogenic organisms by producing antibiotics or degrading enzymes (Glick, 2015). This production of antimicrobial and metabolites works to directly suppress parasitic microorganism populations which could harm the plant. Ultimately, the soil microbiome is involved in protecting the plant-host from biological pathogens and is also capable of providing protection from abiotic stress.

The soil microbiome provides plants with increased regulatory potential to rapidly adapt to their abiotic environment. Environmental stressors that are non-biological are considered
abiotic stressors. For example: drought, salinity, flood, and heavy metal toxicity are common abiotic stressors which can harm a plant. The soil microbiome has been found to mitigate the effects of many abiotic stressors, but this section will focus on drought and salinity because according to (Daffonchio et al., 2014) they are the two major abiotic factors that limit plant development and growth.

First, microbes can alleviate drought stress by eliciting a variety of physiological and nutritional plant responses (Benabdellah, Abbas, Abourouh, Aroca, & Azcon, 2011). The microbes of the microbiome are themselves drought tolerant, because they are adapted to dry conditions, and therefore aid the plant by initiating compensatory mechanisms. In turn, these compensatory mechanisms not only protect plant health but can also allow the plant to continue to grow in environmentally stressed conditions.

Salinity, or salt stress, is another common environmental factor that can affect plant health. Salt stress in plants can be caused by a variety of sources including: recycled and saline irrigation water, aerosols in coastal areas, and de-icing salts used on sidewalks and roadways in winter months. Salinity can cause significant damage to plants by making water uptake by roots very difficult, produce an excess level of sodium in leaves, and affect protein synthesis, lipid metabolism, and photoynthesis (Hinghole & Pathak, 2016). The soil microbiome can alleviate symptoms of salinity by producing hormones and other chemicals which help the plant to regulate water uptake and osmotic pressure (Podolich, Ardanov, Zaets, Pirtila, & Kozyrovska, 2014). In fact, inoculating the soil with salt tolerant microbes has been successful for inducing salt tolerance in plants (Hinghole & Pathak, 2016). To conclude, the microbiome can initiate and provide protective mechanisms against salt stress in plants.

A functional soil microbiome can allow a plant to adapt to a number of environmental stresses. This includes biotic and abiotic stresses that commonly affect plant health. In the absence of associated microbes, some plants are unable to withstand these stressful conditions (Daffonchio et al., 2014). Therefore, the soil microbiome is a key determinant of plant health and productivity. Details about how the microbiome can influence plant health continue to surface, with applications for biocontrol of diseases and stress already being tested and marketed, albeit with mixed results. It is important to note that the ability to optimize plant
health using an introduced microbiome is not completely understood, but nevertheless a functional microbiome is generally understood to benefit plant health.

2.5. Environmental and Anthropogenic Factors which Affect the Soil Microbiome

The soil microbiome is a dynamic superorganism continuously adapting to changing environmental conditions. The belowground microbial community is shaped through time and space by several external factors. For example, according to Meyer et al. (2013), major drivers for microbial performance in soil are not only limited to site-specific parameters such as climatic conditions or soil texture, but also to land use and management practices. Terrestrial ecosystems are affected by natural and anthropogenic disturbances, and implications for the microbiome are beginning to be explored, especially for intensive land-use practices. This section describes how both environmental and anthropogenic factors can result in soil microbiome spatial and functional variability.

Microbial populations are not evenly distributed in soil. Properties of soil vary spatially, and these differences can dictate the structure of the soil microbial community across a range of scales. While the microbiome can be studied as a functional whole, it is useful to separate how different soil properties contribute to the spatial distribution of both bacteria and fungi. This is because of inherent differences required for each type of life-form to thrive. First, soil pH is a major factor for influencing bacterial community structure (Uroz et al., 2016). In fact, Fierer & Jackson (2006) reported a 60% higher bacterial richness in forest soil with a near-neutral pH, compared to acidic soil with a pH of 5.1. In contrast, fungal population distribution is correlated more with C:N ratios and available nutrient pools in the soil environment (Lauber, Strickland, Bradford, & Fierer, 2008). Thus, spatial variance of soil conditions can affect the microbial composition of a microbiome, based on how well specific life forms are adapted to different soil conditions.

Besides soil condition factors, the soil microbiome is actively shaped by plants. As alluded to earlier, plants recruit soil microbes through a variety of biochemical mechanisms. However, recruitment is selective. Individual plant species are generally associated with populations composed of similar microbial species. In fact, Lejon et al. (2005) highlighted how tree species play a significant role in the differentiation of bacterial communities in soil.
Numerous studies have also been able to demonstrate how tree host specificity is an important driver of symbiotic and decomposer fungi populations (Zhou & Hyde, 2001; Lang, Seven, & Polle, 2010; Uroz et al., 2016). Ultimately, tree and other plant forms actively shape the microbiome, which corresponds with spatial and functional differences.

Some of the most well-studied spatial gradients in biological diversity are those existing across terrestrial biomes. Similar cross-biome inquiries have recently been applied to further understand microbiome ecology on a broad scale. According to Moora et al. (2014), microbial community composition and diversity differ among broad habitat types such as arable fields, grasslands, and forests. In a study by Fierer et al. (2012), a cross-biome genetic analysis produced similar results: soil microbiome phylogenetic and functional diversity are highly variable across 16 different biome types. To conclude, microbiome composition and function vary across major terrestrial biomes due to a number of below and above-ground factors. A distinction has surfaced in soil microbiome ecology which categorizes environmental factors affecting the soil microbial populations according to either forested or open habitat types. The term habitat type refers to the structural (mostly vertical) aspects of a biome. Forested habitats have canopy cover and include tropical, deciduous, and boreal forests. To contrast, open habitats would include biomes such as grasslands and arable fields. The distinction between forested and open habitats is important because multiple factors can be categorized, and changes to the soil microbiome can be observed according to a natural gradient. Factors which affect the microbiome can be organized, and interactive effects can be understood when classifying habitats as either forested or open.

The impact of previously mentioned factors such as soil conditions and plant-microbe specificity are better understood when they are associated with the environmental particularities of contrasting biomes. In other words, some variables affect the soil microbiome more in different habitats. A major benefit of distinguishing between forested and open habitats is the ability to understand how the inherent microclimatic differences a canopy versus no canopy can provide, and how this can affect the soil microbiome. For example, Moora et al. (2014), found significant variation in fungal community composition between forested and open habitats. The differences were attributed to how a tree canopy can result in soil and
climatic variations when compared to open habitats (2014). To contrast, a study by Nuccio et al. (2016), highlighted that climatic factors played a larger role than soil characteristics in shaping microbial communities in open habitats. So, understanding how environmental factors shape/affect microbiome composition may be better understood when the structure of biomes are considered.

The soil microbiome has to not only adapt to changing environmental conditions, but also to anthropogenic disturbances. The factors which were previously described as having effects on the soil microbiome were generally extrapolated from observing low management land uses, such as forests, grasslands, or other biomes with low anthropogenic impacts. However, the remainder of this section will describe how high intensity management practices can alter the composition and function of the soil microbiome. Most of the research comes from agriculture and forestry, but understanding the mechanisms behind the management practices in question can provide insight for professionals in landscape architecture, who may engage in similar soil altering practices. Since the majority of the soil microbiome exists in upper soil horizons, understanding the effects of mechanical disturbance may prove to be beneficial for reevaluating the use of high intensity land altering practices and management.

Some agricultural and forestry practices can alter soil conditions and the microbiome. For example, an article by Postma-Blaauw et al. (2010) details how the soil biota community is affected by agricultural intensification because of soil disturbance, increased fertilization levels, and crop diversity reduction. This can ultimately lead to changes in agroecosystem functioning. Although forest soils are generally less managed in comparison to agricultural soils, microbial communities are still impacted by forestry practices (Hartmann, 2012). This is because soil biota can be disturbed by forest harvesting procedures which can often result in compaction of soil, organic matter removal, and soil physical-chemical alterations (Uroz et al., 2016). So, soil microbiome composition and function are significantly altered by select high intensity agriculture and forestry practices involving mechanical disturbance and other associated impacts.

Land use change is also associated with major disturbances to the soil microbiome. An article by Fichtner et al. (2014) details how changing land use can significantly alter soil
characteristics and aboveground species dynamics with soil microbes. Different soil microbial communities can be more strongly impacted after land use change, depending on the types of disturbances. For example, Uroz et al. (2016) suggest land conversion is a major factor which alters fungal communities. These land use driven changes affect ecosystem functionality, including nutrient cycling and plant species productivity and diversity (Fichtner et al., 2014). In fact, multiple ecosystem functions may be impaired by biodiversity loss and simplification of community composition in soil (Wagg, Bender, Widmer, & van der Heijden, 2014). Not only is the soil microbiome composition and function altered by land use change, but these changes can have lasting effects on ecosystem functions which require long periods of time to be restored.

2.6. Disturbance Resistance, Resilience, and Legacy

The previous section described differences in soil microbiome composition and function, according to soil condition and land-use intensity gradients. Changes to soil conditions and land use intensity may alter to the soil microbiome. Any significant change over time to the aforementioned factors which determine soil microbiome composition and function can be considered a disturbance. Disturbances can be natural or anthropogenically caused, and according to Suleiman, Manoeli, Boldo, Pereira, & Roesch (2012), microbial populations may be resistant, resilient, or remain altered following a disturbance. The soil microbiome is typically resistant or resilient to natural disturbances, but this depends on severity and type. On the other hand, anthropogenic disturbances, such as high intensity agricultural or forestry practices, can have long-lasting effects on soil microbial composition and function.

Although disturbance can be short in duration, the soil microbiome can take an disproportionate amount of time to recover. Long-term disturbance legacies are now a common topic for research, since soil-plant feedbacks determine the success of ecological restoration efforts in terms of plant health and ecosystem functions. Also, many of the anthropogenic disturbances which have been studied from an agriculture and forestry perspective are analogous to disturbances that are associated with urban development and other land use changes. This can have implications for planning and design professionals, especially landscape architects. This section describes the temporal effects of soil disturbance.
First, resistance, resilience, and legacy will be defined as they pertain to disturbances to the soil microbiome. This will be followed by a review of recovery timelines and mechanisms following both natural and anthropogenic disturbances. This section will conclude with a description of disturbance analogues, with reasons why they are significant for sustainable development.

Resistance, resilience, and legacy are terms commonly used in ecology to describe how an ecosystem, or its constituent biological populations, respond to disturbance. Microbial populations are part of healthy ecosystems, and their responses to disturbance are categorized by being either resistant, resilient, or remaining changed (legacy). According to Aber & Melillo (2001), a resistant population would remain relatively unchanged following a disturbance. A severe disturbance would be required to alter a resistant population. In contrast, a resilient population can be changed relatively easily, but can rapidly return to its initial state (Aber & Melillo, 2001). Finally, legacy is when a soil microbial population remains altered for an extended period of time following a disturbance. This usually corresponds with persistent functional changes, such as impacted nutrient cycling, for example (Wurst & Ohgushi, 2015). So, understanding how the soil microbiome can respond to disturbance can be useful for ensuring ecosystem integrity and health.

Soil microbiome resistance and resilience have been primarily studied with relation to natural disturbances such as drought, flood, fire, and disease. Recent research efforts have sought to understand why microbial populations can be either resistant or resilient to disturbance. The overall ability of the soil microbiome to respond to natural disturbances is determined by factors such as microbial traits, biomass, composition, and diversity (Orwin, Dickie, Wood, Bonner, & Holdaway, 2015). Factorial variations are correlated with the efficacy of a soil microbiome to be either resistant or resilient to disturbance. In other words, some variables are correlated with a microbiome which is more resistant to disturbance, whereas other variables are correlated with a microbiome being more resilient to disturbance.

For example, compositional factors such as fungal to bacterial ratios could determine how a microbiome responds to a disturbance. A microbiome with a higher fungal ratio may be better suited to resist disturbance. This is because of the evolved ability of a fungal population to buffer abiotic and biotic stress, which is a result of the life-strategy of fungus: slow, but
robust growth. In contrast, a microbiome with higher bacterial ratios may be less resistant, but more resilient to disturbance. This is because bacteria are more sensitive to environmental stresses, but can re-grow much faster than fungus. Resistance and resilience mechanisms are able to quickly respond to disturbance, resulting in short-term alterations to the soil microbiome.

If a disturbance is too drastic, the soil microbiome can be altered long-term. Anthropogenically caused disturbances, often associated with land use change, may represent events beyond the adaptive capacity of an ecological community to recover within a reasonable timeframe (Larney et al., 2016). While most studies have dealt with short-term effects of soil and plant disturbance, research within the last ten years has begun to address long-term soil microbial legacy effects. While there is no clear consensus on how long legacy effects persist, some articles document changes to microbial populations and functions that last in excess of one hundred years (Elgersma, Ehrenfeld, Yu, & Vor, 2011; Fichtner et al., 2013). However, this depends on disturbance type and severity, along with existing soil conditions.

Legacy effects are usually studied after land has been converted from high intensity land uses into a less anthropogenically impacted state, for example: from agriculture to forest. As time passes, researchers analyze the soil microbiome to determine if composition and function remain altered after disturbance regimens have ceased. Fichtner et al. (2013), specified how legacy effects have been observed for more than fifty years after conversion from agricultural cultivation to forests in other studies. They also found direct evidence of legacy effects in soil microbial communities one hundred years after afforestation (2013). When soils from afforested areas were compared to nearby reference forest sites, microbial biomass and activity remained significantly altered. The re-establishment of traditional land use alone is generally insufficient for the microbiome restoration.

Long-lasting soil microbiome disturbance legacies can, in turn, lead to altered primary productivity and other ecosystem functions. While planning a for a healthy and functioning landscape, the implications of soil legacies should ideally be considered. Soil disturbance studies typically come from agricultural and forestry perspectives, but natural and anthropogenic disturbances are not limited to these land uses. In fact, many similar disturbance
regimes occur during urban development, with some anthropogenic disturbances having direct analogues to those common in agriculture and forestry. For example, urban land development practices can include land clearing, topsoil removal, surface grading, compaction, and building construction, which can all lead to degraded soil conditions (Chen et al., 2013). Many of these disturbances have been studied in other land use contexts, but the effects on soil biota and consequent ecosystem functions are similar.

Topsoil removal, which commonly occurs during urban land development, has been studied in agriculture for the subsequent effects on plant growth and health, which are a result of soil disturbance legacies. For example, a study by Larney et al. (2009), revealed that grain yield reductions persisted for sixteen years, which was the entire length of the study. In fact, “Average grain yield reductions during the sixteen year period were 10% for 5 cm, 20% for 10 cm, 29% for 15 cm, and 39% for 20 cm of topsoil removal” (p.178). Topsoil removal of similar depth ranges occur during urban land use development. Results from similar soil de-surfacing experiments can be useful for anticipating any biogeochemical limitations of soil, which can be barriers to landscape design.

Forestry practices such as harvesting and compaction have been found to induce soil disturbance legacies. Harvesting, like clear-cutting, is associated with organic material removal and loss of stored carbon (Uroz et al., 2016). Soil compaction can also significantly alter soil microbial communities and function. Both of these disturbances can result in long-term alterations to soil biota. Essentially, forestry harvesting practices are analogous to land clearing in urban land development. Soil carbon levels are affected in both scenarios. On the other hand, soil compaction via the use of heavy equipment in forestry is analogous to soil compaction associated with urban land development. There are other disturbance analogues which can have legacy effects on the soil microbiome. Some circumstances require intensive restoration efforts in order for the soil microbiome to fully recover. Otherwise, legacy effects may continue to alter soil microbial community structure, function, and related ecosystem functions for longer than a human lifespan.

No studies have yet compared forest soil microbiomes associated with disturbance from landscape development projects. The extent and duration of change to soil microbiomes, especially when establishing long-lived urban forests, could be valuable information for more sustainable landscape designs.
Methods

3.0. Introduction & Approach

An inexpensive, commercially available, soil respiration testing methodology was used to compare soil CO2 values between disturbed and undisturbed areas in recent urban development projects. The study sites are all located within the City of Guelph, in southern Ontario. Guelph was chosen as the study area because it is a local, relatively small geographic area at 87 km², with many recent urban development projects (Statistics Canada, 2016). High resolution aerial photography is widely available since the year 2006, which allowed for potential study sites to be identified and documented. Three sites were chosen based on visual evidence of soil disturbances which occurred between 5-11 years ago. This chapter details the methodology in two separate sections: site selection and soil sampling.

3.1. Site Selection Methodology

3.1.1 Data Sources. Maps, aerial photographs, and computer programs were used to discover and to document soil disturbance. High resolution aerial photographs were available through the geographic information system (GIS) database Scholars Geoportal. The photographs are from the South Western Orthophotography Project, which is abbreviated as SWOOP (Ontario Ministry of Natural Resources, 2015). SWOOP data were collected in the years 2006, 2010, and 2015. The SWOOP datasets consist of remotely-sensed digital aerial photography with a spatial resolution of 20 cm. Adobe Photoshop was used to create digital overlays of SWOOP aerial photography to compare sequential change. Lastly, Google Earth Street view was used to further investigate potential sample sites.

3.1.2 Selection Procedure. Study sites were selected by searching for evidence of visible soil disturbance within the boundaries of the City of Guelph. Compiled SWOOP aerial photography was used in order to search for visual evidence of topsoil clearing and soil compaction. Visual evidence consisted of bare or graded soils and/or the presence of earth moving equipment such as bulldozers or scrapers. The documented visual evidence was used to infer soil disturbance. Analysis of SWOOP photography was undertaken according to the City of Guelph’s ward system, in order to simplify the process. The locations of the study sites, and how the city's ward system is laid out, can be seen within the context of the City of Guelph in figure 3.1. Peripheral soil disturbance was expected to be more common than disturbance in the city core.
3.1.3 Clair Road East site. SWOOP aerial photography was first analyzed to determine an appropriate study site with a disturbance history from five years ago. The chosen site is located off Clair Road East, in ward 6, and can be seen in figure 3.2.
The Clair Road site is adjacent to Pergola Commons, which is a newly developed commercial area within the city's south end. Currently, residential development is still undergoing to the north of the study location.

The study property is 12,750 m$^2$ (3.2 acres) which consists of an existing woodlot and newly constructed stormwater management pond. The image from 2010, in figure 3.2, shows an undisturbed site. The stormwater pond was completed in 2012, but there is only low quality, clouded aerial photography available on Google Earth. From the collection of images, there is evidence of surface regrading with subsequent soil disturbance. Trees, shrubs, and grasses were planted around the stormwater pond after construction, with the majority being planted in the south-west corner of the study site.

3.1.4. Watson Parkway North site. A site located off of Watson Parkway North, in ward 2, is a site which was disturbed seven years ago. The study site is adjacent to a newly developed commercial and residential area, which is in the north end of Guelph, and can be seen in figure 3.3. The site is across Watson Parkway North from the Eastview community pollinators park.

Figure 3.3. Aerial photograph comparison documenting the 7 year disturbance history of the Watson Parkway North site. The black dotted outline represents the study area within the context of the development. The white dashed line delineates the undisturbed area of the site. Image Source: SWOOP.
The study area is 10,150 m² (2.5 acres) in size. The study site contains a stormwater management pond. The banks of the stormwater pond were planted with a mix of deciduous trees, coniferous trees, and grasses following grading and construction. Prior to 2010, the site was undisturbed by construction practices, according to available aerial photography. The site was regraded to accommodate the stormwater management pond, which resulted in soil disturbance.

3.1.5. Summit Ridge Drive site. A site located in the Summit Ridge residential development was chosen for the eleven year study condition, and can be seen in figure 3.4. The study site is a stormwater management pond within a naturalized area, located to the south of the Summit Ridge Drive, in the ward 1. The study area is in proximity to Summit Ridge Park and Hadati Creek.

The study area is 30,500 m² (7.5 acres) in size. Photographic evidence of soil disturbance during construction is clear in the aerial photograph from 2006. The light toned areas on the photograph is exposed subsoil. Topsoil had been removed and the subsoil was regraded to create the stormwater management pond. Vegetation was reestablished after construction, with trees, shrubs, and grasses being planted. A patch of forest was clearly preserved at the north end of the stormwater pond.

Figure 3.4. Aerial photograph comparison documenting the 11 year disturbance history of the Summit Ridge Drive site. The black dotted outline represents the study area within the context of the development. The white dashed line delineates the undisturbed area of the site. Image Source: SWOOP.
3.2. Soil Sampling Methodology

3.2.1 Materials. A Solvita® twenty-four hour soil CO2 test was used to determine soil respiration levels. The Solvita® twenty-four hour soil CO2 field test is a USDA-approved method for determining soil respiration. The test kits are manufactured by Woods End Laboratory (Mt. Vernon, Maine) and consist of: (i.) plastic CO2 probes with indicator gel surfaces (ii.) 8 ounce polystyrene plastic jars fitted with gas tight rubber seals, and (iii.) procedural instructions and test interpretation documents. Twelve polystyrene jars and twelve CO2 probes were purchased. The CO2 probes were refrigerated at 4°C until use. A metal soil trowel with an inscribed ruler for determining soil depth was used for soil sampling.

Google Maps was used in order to document the GPS coordinates of sample locations. Test instructions were provided and adhered to. 6mm (¼”) wire mesh was used for sieving the soil samples, in order to homogenize soil and to remove plant debris, while minimally disturbing the sample. A digital scale, with 1 gram accuracy, was used to measure and to standardize soil sample weight. Soil samples were placed in a dark room which was kept at 20°C. Finally, fluorescent lighting was used when comparing CO2 probe colours to the interpretative key.

3.2.2 Sampling Design. Soil respiration values were compared between two sample conditions: disturbed soil and undisturbed soil. The disturbed soil condition had three disturbance periods: five, seven, and eleven years after disturbance. Four soil samples were taken at each site; two for each condition, for a total of twelve samples. Undisturbed soil conditions were controlled for by selecting areas within the site boundary which have photographic evidence of remaining undisturbed prior to and post land development. Disturbed soil conditions were controlled for by selecting areas within the site boundary which have photographic evidence of disturbance.

3.2.3 Procedure. Soil sampling occurred between February 6th, 2017- February 27th, 2017. The protocol provided for the Solvita® twenty-four hour soil CO2 field testing was used, which has been elaborated on in this section and is available from the Solvita website (https://solvita.com/shop/category/soil-field-test/). Soil was sampled on days with air temperatures above 0°C, to avoid frozen soil. In order to ensure a proper soil moisture range, soil was sampled after a minimum of two days since precipitation. Both rain and snow were considered precipitation. GPS coordinates were documented using a mobile phone. The locations can be seen in Figures 3.5-3.7.
Figure 3.5. Soil sampling locations at Clair Road East site (43°30’10.5" N, 80°10’59.9" W, Guelph, ON). Samples were collected 5 years after disturbance. 'C' indicates control, and 'D' indicates disturbed soil. The numbers correspond with soil sample numbers. Aerial photograph source: SWOOP 2015.

Figure 3.6. Soil sampling locations at Watson Parkway North site, 43°34’44.1" N, 80°13’25.6" W, Guelph, ON. Samples were collected 7 years after disturbance. Samples were collected 5 years after disturbance. 'C' indicates control, and 'D' indicates disturbed soil. The numbers correspond with soil sample numbers. Aerial photograph source: SWOOP 2015.
Soil sampling locations at Summit Ridge site, 43°34’11.9” N, 80°13’38.1” W, Guelph, ON. Samples were collected 10 years after disturbance. The numbers on the map correspond with soil sample numbers. Aerial photograph source: SWOOP 2015.

Soil samples for the control conditions were taken in areas that were undisturbed, as deemed by aerial photography analysis. For the disturbed conditions, soil samples were taken in re-forested areas which resembled the undisturbed areas as close as possible in terms of vegetation density, vertical stratification, and ecological successional level. The surface vegetation and humus layer were first removed with the soil trowel. Soil samples were then collected up to a depth of 15 cm. The samples were sieved in the field in order to homogenize the sample and to remove any organic matter such as roots or leaf litter.

Soil samples were placed into large sealable plastic bags and labelled. The samples were transported back to the testing location in a cooler. Using a digital scale, the soil samples were weighed to a standard of 90 g. The 90 g samples were labelled and placed into individual polystyrene jars, along with a CO2 gel paddle, and then sealed. The samples were then placed in a dark room which was kept at 20°C. After the allotted time of 24-hours had passed, the CO2 gel paddles were removed and compared to the provided colour interpretation key, under fluorescent light. Gel paddle colour results were recorded. Results from the twelve samples representing three site disturbance conditions and periods are reported and discussed in the following chapters.
Chapter 4: Results

Soil respiration test results will be presented in this chapter. The primary findings, which are derived from a comparison between respiration values of disturbed soil and undisturbed soil, will be described first in section 4.1. Sections 4.2-4.4 are a site by site analysis which further describes the soil test results. Soil testing results are reported in ranges of respiration, specifically grams of CO$_2$/m$^2$/day. Also reported are the corresponding descriptions of microbial activity in soil, which range from very low to very high.

4.1. Primary Findings

Areas of soil which were mechanically disturbed 5, 7, and 11 years ago all had lower soil respiration values in comparison to areas of undisturbed soil, in sampled urban forest sites. All undisturbed soil samples had higher soil respiration rates than any disturbed soils sampled, which is depicted in figure 4.1. The difference in respiration values indicates reductions of soil functioning persisting up to 11 years after the initial mechanical disturbance. All soil respiration values were within the Solvita® categories of medium-low to very high soil microbial activity levels, so no samples were severely impaired.

![Figure 4.1](image-url)  

*Figure 4.1. Soil microbial activity comparisons by urban forest sample sites. Soil microbial activity categories correspond with Solvita® soil respiration field test interpretation documents. Each bar represents the average range of two soil tests per condition.*
4.2. Site 1: Clair Road East

The soil at the Clair Road east site was disturbed roughly five years ago, which is the shortest duration since disturbance tested in this study. Soil samples D₁ and D₂ from the disturbed areas of the site were within the range of 10-25 g CO₂/m²/day, which is categorized according to the Solvita® field test interpretation document as being medium-high soil microbial activity. In comparison, soil samples C₁ and C₂ from the undisturbed areas of the site had greater respiration values, within the range of 25-65 g CO₂/m²/day, which is categorized as very high soil microbial activity. These values can be seen in figure 4.2.

The soil respiration values follow a trend of being lower in the disturbed areas of the site (as seen in figure 4.1). However, the disturbed soil samples from the Clair Road East site had the highest respiration values in comparison to the other sites with longer periods since disturbance. The soil respiration values from the Clair Road East site were also the most similar to the undisturbed areas. In other words, the microbial activity in the disturbed areas of the site were the closest to the undisturbed areas five years after disturbance.

4.3. Site 2: Watson Parkway North

Soil disturbance occurred seven years ago at the Watson Parkway North site. Soil samples D₁ and D₂, from the disturbed areas of the site, were both in the respiration range of 2-6 g CO₂/m²/day, and can be seen in figure 4.2. This range is considered low-medium soil microbial activity. In comparison, soil samples C₁ and C₂ from the undisturbed areas of the site were higher, in the range of 25-65 g CO₂/m²/day. These values are correlated with very high soil microbial activity levels.

In disturbed urban forest soils, respiration values were lowest seven years after disturbance. This indicates that the lowest amounts of soil microbial activity, of any sampled soils, occurred seven years after soil disturbance. The soil samples from disturbed areas of the Watson Parkway North site have the largest difference in respiration values, when compared to the undisturbed control. This indicates a relatively small amount of recovery to soil microbial activity following a seven year time period.
<table>
<thead>
<tr>
<th>Site</th>
<th>Years Since Disturbance</th>
<th>Undisturbed Soil Condition</th>
<th>Disturbed Soil Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clair Road East (CRE)</td>
<td>5</td>
<td><img src="#" alt="C1" /></td>
<td><img src="#" alt="D1" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="#" alt="C2" /></td>
<td><img src="#" alt="D2" /></td>
</tr>
<tr>
<td>Watson Parkway North (WPN)</td>
<td>7</td>
<td><img src="#" alt="C1" /></td>
<td><img src="#" alt="D1" /></td>
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<tr>
<td></td>
<td></td>
<td><img src="#" alt="C2" /></td>
<td><img src="#" alt="D2" /></td>
</tr>
<tr>
<td>Summit Ridge Drive (SRD)</td>
<td>11</td>
<td><img src="#" alt="C1" /></td>
<td><img src="#" alt="D1" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td><img src="#" alt="C2" /></td>
<td><img src="#" alt="D2" /></td>
</tr>
</tbody>
</table>

**Legend**

- ![2-6 g CO₂/m²/day](#)
- ![6-10 g CO₂/m²/day](#)
- ![10-25 g CO₂/m²/day](#)
- ![25-65 g CO₂/m²/day](#)

Figure 4.2. Soil respiration values from soil tests in urban forests in Guelph, Ontario. The soil tests compare microbial activity between undisturbed (control) and mechanically disturbed conditions from 5, 7, and 11 years ago. Respiration values are given in g CO₂/m²/day.
4.4. Site 3: Summit Ridge Drive

Soil at the Summit Ridge Drive site was disturbed eleven years ago. Soil samples D1 and D2, from the disturbed area of the site (see figure 4.2), both respired in the range of 6-10 g CO$_2$/m$^2$/day. This respiration range is considered ideal activity for plant growth. In comparison, soil samples C$_1$ and C$_2$ from the undisturbed areas of the site had higher respiration values at 10-25 g CO$_2$/m$^2$/day and 25-65 g CO$_2$/m$^2$/day, respectively. Sample 1 is considered medium-high soil microbial activity, whereas sample 3 is considered very high soil microbial activity.

The soil at the Summit Ridge Drive site had the longest time since disturbance. The soil samples had respiration values which were approaching the estimated pre-disturbance levels, which were established by the control samples. This could be indicative of recovery of soil microbial function in the disturbed areas of the site. Also, the control values from the undisturbed area of the site varied. One value was medium-high soil microbial activity, and the other was very high soil microbial activity. While this was the only site where control values differed, this was not entirely unexpected, as the samples were taken from different locations within the undisturbed area of the site.

4.5. Summary of Results

Disturbed soil had less microbial activity compared to undisturbed soil, in the sampled urban forest locations within Guelph, Ontario. The disturbed soil from the Clair Road East site (site 1) had the highest respiration values compared to the other sites with longer durations since disturbance, which was unexpected. All control samples had very high levels of microbial activity, and there was still a discrepancy in soil respiration 5, 7, and 11 years after mechanical soil disturbance which occurred during development. The results presented in this chapter will be further interpreted in the following discussion section.
Chapter 5: Discussion

The following chapter interprets the results of this study and compares them to relevant soil disturbance literature. The chapter begins by building on the main findings in sections 5.1 and 5.2. Next, the results are broken down site-by-site in section 5.3. The chapter concludes with an explanation of study limitations in section 5.4.

5.1. Disturbance and Soil Microbial Response

Results from soil respiration testing support the original hypothesis, which was: following urban land development, soil respiration values from urban forest soils are expected to be lower in disturbed areas compared to undisturbed control areas. The primary findings of this exploratory study were that all disturbed soil samples respired less in comparison to undisturbed controls. This suggests continued impairment to soil microbiome functioning in mechanically disturbed soils 5, 7, and 11 years after disturbance. Although soil respiration tests are primarily used to determine levels of soil microbial functioning, values are also highly correlated with soil microbial biomass (Holden & Treseder, 2013). So, a decrease in soil respiration would be highly correlated with a decrease in soil microbial biomass. The lower soil CO₂ efflux values of disturbed soils could therefore be a result of lower microbial biomass, among other soil microbial responses to disturbance.

The primary findings of this exploratory study are consistent with the results of a meta-analysis performed by Holden & Tresender in 2013. Their article compiled results from 139 published papers on soil microbial responses to forest disturbances, of which 38 studies used soil respiration as a metric for determining microbial functioning following disturbance. According to Holden and Tresender (2013), forest disturbances significantly reduced soil respiration values. In addition to reduced soil respiration values following disturbance, an average biomass reduction of 29.4% was reported following all types of forest disturbances. This reduction was even greater for abiotic disturbances such as storms, fire, and tree harvesting, which are more closely related to the disturbance types analyzed in this study.

The primary findings are also consistent with a study by Chen et al. (2013). Their study analyzed the effects of typical land development practices on various soil carbon pools. One of
the studied soil carbon pools was soil microbial biomass, which was measured using a related soil testing protocol called chloroform fumigation extraction. Chen et al. (2013) reported lower soil microbial respiration values in disturbed soils in comparison to an undisturbed control, four years following disturbance. This finding is consistent with results from the five year disturbance condition in this study. The disturbances studied by Chen et al. are analogous to the disturbances analyzed in this study.

Chen et al. (2013) defined typical practice for urban land development as a removal of the soil A-horizon (topsoil), and subsequent compaction of subsoil by heavy machinery. This definition of typical practice was similar to the operational definition used in this study. Also, the soil was tested at a depth of 5-10 cm, after being stockpiled and redistributed over the regraded subsoil. This article is one of few which used a similar disturbance history type, timeframe, and soil microbial testing method. Although the tested areas from their study were not specifically in urban forests, it was noted that the study site was replanted with five different species of trees.

When compared to the results from this study, both the meta-analysis by Holden & Tresender (2013) and the study by Chen et al. (2013) reported similar disturbance effects on soil microbial populations. Soil disturbances resulted in decreases to soil microbial activity and to soil microbial population numbers, and when viewing the accumulation of results from similar studies, the trend is a decrease in these metrics. Not all studies of this type have consistent results, with reports of: increases, no effect, and decreases of soil microbiome function and population numbers following abiotic disturbance (Holden & Tresender, 2013). Soil microbial activity is thought to be impaired following disturbance because of the accumulation of the effects of both soil compaction and topsoil removal, which have been extensively studied in the domains of agriculture and forestry.

5.2. Soil Compaction and Topsoil Removal

Soil compaction studies use a more specific definition of soil disturbance compared to urban land development studies. Although not equivalent, the results from soil compaction studies offer a useful comparison to the results from this study, by helping to explain the
microbial response to a more specific disturbance type. The definition for disturbance used in this study includes subsoil compaction, and there is a large amount of literature which describes what happens to microbiome functioning and structure following compaction. Soil compaction studies have produced inconsistent results, some of which support what was found and some of which are contrary to the findings of this study.

Beylich et al. (2009) evaluated literature on the effects of soil compaction on soil organisms and soil biological processes. Their meta-analysis found contrasting results with respect to the effects of compaction on soil microbial biomass. In tests performed in the field, soil microbial biomass slightly increased after compaction (Beylich et al., 2009). In contrast, tests that were performed in a laboratory setting resulted in considerable reductions of soil microbial biomass (Beylich, 2009). If operating on the premise that soil microbial biomass is highly correlated with soil microbial respiration, the results of the meta-analysis can be compared to the results of this study. However, because respiration testing was performed under controlled conditions, the findings of this study are inconsistent with the results of the meta-analysis.

The test results that were compiled for the Beylich et al. (2009) meta-analysis varied considerably, which will be important for interpreting site-by-site results later in this chapter. The variability in the effects of soil compaction on microbial biomass can be explained for a number of reasons. Firstly, the difference between field and lab results can be attributed to the higher number of variables, both biotic and abiotic, in field studies. Soil properties, climate, water, and oxygen levels can be controlled for in a laboratory study. In comparison, field conditions can be influenced by previous site conditions, microbial population resistance and resiliency, and a conducive environment for microbial growth.

Another soil compaction study by Busse et al. (2005), reported a relatively mild effect on soil microbial biomass following a significant reduction in soil pore space. The inconsistency of soil compaction studies on microbial biomass, where microbial biomass has been shown either to increase, to remain the same, or to decrease, may be a result of the small size of soil microorganisms when compared to soil pore space. While soil compaction could impact the
relatively larger fungi mycelia, bacteria in the soil may be unaffected. Busse et al. (2005), in the same study, also found that removal of the uppermost layer (15 cm) of the A soil horizon resulted in significantly lower microbial biomass. While soil microorganisms have varied responses to soil compaction, topsoil removal seems to have a clear negative effect on microbial activity.

Maio, Zhou, Sui, Zhang, & Liu (2015) studied the impact of topsoil removal on soil CO₂ efflux and found that soil CO₂ emissions were 17-28% lower, eight years after topsoil removal with no subsequent amendments or profile rebuilding. Maio et al. (2015) attributed the reduction in overall CO₂ emissions to a decrease in soil respiration. Many soil disturbance studies, which include topsoil removal as a type of disturbance, also analyze the ability of organic soil amendments and soil profile rebuilding to restore soil carbon and nutrient cycles after disturbance (Carlson et al., 2015; Chen et al., 2013; Larney et al., 2016; Larney et al., 2009; & Maio et al., 2015). Such studies inherently operate on the premise that topsoil removal will decrease microbial carbon levels.

Topsoil removal reduces soil microbial activity because the majority of soil microbial populations are found within the upper soil horizons (Uroz et al., 2016). Humus and topsoil layers provide an ideal environment for soil microorganisms to thrive. When the uppermost soil horizons are removed, so are the majority of soil microorganisms and the environment which supports biological life in soil (Larney et al., 2016). Soil physical-chemical properties are also altered following topsoil removal as a result of exposing mineral soil horizons. The deeper soil profiles are not as conducive for microbial activity and growth.

The primary findings of this study can be partly attributed to the effects of soil compaction and of topsoil removal. While results of soil compaction on soil microbial functioning may not be as straightforward as topsoil removal, both can be used to partially explain the observed effect in this study. Both soil compaction and topsoil removal were observed to have occurred at each study site, to some degree, based on aerial photo evidence. The individual effects of soil compaction and topsoil removal can be useful for understanding the impact of urban land development practices on soil microbial functioning. However, a more
broad definition of disturbance which includes both compaction and topsoil removal, may be more appropriate for understanding the combined effects of typical land development practices on urban forest soils.

5.3. Site-by-Site Analyses

Although soil respiration values remained impaired 5, 7, and 11 years after disturbance, it was expected *a priori* that over time these values would begin to normalize towards pre-disturbance levels. The logic was based on evidence that soil microbial populations can be resilient, which generally leads to some degree of recovery after disturbance, and progresses with time (Aber & Melillo, 2001). The soil respiration results from 7 and 11 years following disturbance are in accordance with this *a priori* prediction. 11 years after disturbance, soil respiration values are more similar to control when compared to 7 years after disturbance.

The results from the 7 and 11 year after disturbance conditions are trending towards being consistent with a study by Larney et al. (2016) which documented soil resilience following topsoil removal. Larney et al. (2016) found measures of soil carbon, including soil microbial biomass carbon, had normalized 14 years after disturbance. The team concluded that the normalization to pre-disturbance levels rendered the removal of topsoil insignificant in terms of a long-lasting effect on soil quality parameters. However, the study by Larney et al. included a one time manure amendment, which could have contributed to a faster recovery period in comparison to soils which are not amended following disturbance.

The results from the 5 year since disturbance condition at the Clair Road East site were unexpected, based on the prediction that soil respiration values would normalize to pre-disturbance levels over time. Respiration rates from disturbed soils are ranked highest to lowest: 5 >11 >7. The 5 year condition was expected to have the greatest difference in soil respiration values between disturbed and undisturbed areas. Theoretically, the shortest duration since disturbance would correspond with the least amount of time for the soil microbiome to recover. This did not hold true, with the highest soil respiration values from disturbed areas occurring 5 years after disturbance.
High soil respiration results measured 5 years after disturbance might be a consequence of altered physical-chemical soil properties. Not all changes to soil physical-chemical properties are correlated with decreases in soil microbial activity. Depending on the type of physical-chemical alteration, measures of soil microbial activity may actually increase immediately after disturbance (Holden & Treseder 2013). The increased microbial activity has been attributed to higher oxygen levels after some soil disturbance regimes (Solvita, 2016), and also to higher soil temperatures caused by direct sunlight warming topsoil after removal of stratified vegetation layers (Holden & Treseder). This higher soil respiration output effect, which usually occurs directly after disturbance, may not be able to fully explain the measured effect in this study.

Changes to soil physical-chemical properties can result in short-term increases of soil respiration, which may partially explain the high respiration values found 5 years after disturbance. However, Mariani et al. (2006), found that soil respiration values remained higher than control three years after tree harvesting and significant soil compaction, despite a reduction in microbial biomass. Higher soil respiration values were attributed to changes in microbial functional efficiency, because reduced microbial biomass numbers cannot explain the legacy of increased soil respiration in the Mariani et al. (2006) study. The relatively higher soil respiration values found in this study may therefore be a result of increased microbial efficiency, and not a reflection of increased biomass numbers. This illustrates a limitation of soil respiration testing, which will be discussed later in this chapter.

It is possible that soil respiration was high 5 years after disturbance because of the young age of the re-forested area. The re-forested area may actually be more similar to a grassland ecosystem than a forest ecosystem. The Clair Road East site is extensively covered with grass. According to Golubiewski (2006), grasslands store much higher percentages of carbon belowground in comparison to forest ecosystems. This could effect soil respiration values because total soil carbon is correlated with microbial activity. The Clair Road East site was covered more extensively with grass, in comparison to the other study sites. The Clair Road East site will eventually become an established urban forest, but what exists there now may not function like a forest ecosystem.
Finally, high respiration values measured 5 years after disturbance could be correlated with methods used to prepare and revegetate the site. The humus layer could have been mixed in during topsoil placement, which could contribute to a fertilization effect. Also, hydroseeding is a method of applying a seed mixture to bare soil areas. Hydroseeding is routinely used to revegetate and stabilize soils following construction or disturbance events (State of California, 2003). There is a large area covered by grass at the Clair Road East site, and it is possible hydroseeding was used to revegetate the area following the construction of the stormwater management pond. The hydroseeding mixture contains seed, wood fiber mulch, emulsifying agents, and fertilizer (State of California, 2003). The added fertilizer amendment could contribute to higher soil respiration levels, and could result in a legacy effect of increased microbial activity (Postma-Blaauw et al., 2010).

The site-by-site results are consistent with the original hypothesis. The sites that were disturbed 7 and 11 years ago adhere to a priori predictions that soil respiration values would show trends towards normalization to levels of undisturbed soil. The soil respiration values from the site disturbed 5 years ago were high relative to 7 and 11 year old forest patches, which is contrary to predictions. The high soil respiration values may be a result of changes to soil physical, chemical, and biological parameters upon which soil microbes respond in an uncharacteristic manner compared to what typically occurs after similar soil disturbances. The results of this study are primarily consistent with current hypotheses in soil disturbance literature. There are limitations to the methodology and results, which will be described in the following section.

5.4. Study Limitations

5.4.1 Methodology limitations. The site selection and soil sampling methods have limitations. Firstly, the methods used for site selection resulted in incomplete site disturbance histories, because of a small amount of available high resolution photographs. SWOOP aerial photographs are not taken every year, so compiling high resolution photos which correspond with the timeframes of disturbance was a challenge. Full disturbance histories would ideally be accompanied with site plans and/or direct observation or measurement of disturbances.
The soil testing method used also has limitations. The Solvita® 24-hour soil respiration test is useful for professionals such as landscape architects or urban forest managers because of its low cost and ease of use. Lab based soil respiration protocols are more precise, but come with higher costs and decreased ease of use. Lab based protocols could also be used to measure disturbance parameters that are correlated with soil respiration, which would allow for further data analysis. Also, the use of twelve soil samples is a limitation of this study. More samples would allow checking consistency of test results and would increase confidence in project findings.

5.4.2 Results & data limitations. Soil respiration testing is useful for determining overall levels of microbial functioning in soil. With a broad measure of soil microbial functioning, such as soil respiration, intricacies of why the soil microbiome is functioning at a particular level cannot be extrapolated. Soil respiration is correlated with other microbial function metrics, but we cannot draw conclusions on what type of microorganism is responsible for the measured CO₂ output. For example, with a soil respiration test it is impossible to distinguish between respiration from fungi, bacteria, and other soil microorganism populations. However, it is a reasonable surrogate measure for microbial function.

Microbial efficiency, which may have contributed to unexpected results found 5 years after disturbance, cannot be determined from a soil respiration test alone. The Solvita® 24-hour soil respiration test is appropriate for an exploratory study such as this one, but further explanation should use supplementary or more specific methodologies for soil testing. This could include additional controls and more data collection. The conclusion chapter discusses how these results and their meanings contribute to a more informed landscape architecture.
Chapter 6: Conclusion

This chapter builds on the discussion section. Section 6.1 revisits the initial research question and problem. Section 6.2 discusses the implications of this study for landscape architecture. Section 6.3 provides directions for future research and practice.

6.1. Research Question and Problem

The initial research question was: do mechanical soil disturbances associated with urban land development affect soil respiration levels in urban forests 5-11 years after disturbance? In short, yes. Lower soil respiration levels were found in areas of disturbed soil 5, 7, and 11 years after mechanical soil disturbance, which is consistent with the hypothesis. The results can be used to reject the null hypothesis, which is that soil disturbances would not affect soil respiration levels. The specific research question evolved from a discrepancy between research and practice.

Typical urban land development practices can be detrimental to soil conditions. A body of research is growing on the detriments of mechanical, and other abiotic disturbances for the soil microbiome. Forest ecosystems benefit from many soil microbiological processes, and alterations to the microbial population structure, composition, and function may result in a less resilient ecosystem. Urban soils can be disturbed more frequently and to a greater extent, especially after land development. Understanding possible effects on the microbiome, such as those found in this study, could be beneficial for urban forest planning and management practices.

Currently, a small amount of research exists regarding the effects of disturbance on urban forest soils. This exploratory study aimed to contribute to a growing body of research on how disturbance affects the soil microbiome. Using a simple soil testing methodology, such as the Solvita® 24-hour soil respiration test, this study was able to analyze functional microbial changes to forested areas following mechanical disturbance. Using a commercially available soil testing methodology was important for this particular study because of the applicability for landscape architects, which will be described in the next section.
6.2. Implications and Applications for Landscape Architecture

Landscape architects work on projects with varying spatial scales and objectives, which is reflected in the three sites analyzed for this study. The implications of the research findings are restricted to urban forests, since other ecosystems may respond differently to disturbance. However, the methods used in this study can be used to analyze disturbance in other ecosystem types. The empirical evidence gathered in this study can be used to further understand how disturbance affects the soil microbiome in urban forests. The methodology and results can also be applied to landscape architecture practice during different stages of urban forest planning, design, and management.

The soil respiration methodology can be used during site analysis, especially for sites with a history of disturbance. As previously mentioned, a 24-hour soil respiration test is easy to use and to interpret, which makes it ideal for site analysis protocols. This type of soil analysis would also be applicable to many different ecosystem types. The results of a soil respiration test can be used to determine the state of soil microbial functioning. Knowledge of soil biological properties can supplement physical and chemical analyses, and could influence how a forest is designed and managed.

The results of this study have implications for re-forestation planning following soil disturbances and land-use change. If heavy equipment is to be used for subsoil grading, which is typical practice, any forested areas that are intended to remain intact should ideally be protected from mechanical soil disturbance. For areas intended to be re-forested, a practitioner could benefit from anticipating the effects of soil disturbance and can proceed accordingly. Potential soil amendments and profile rebuilding could be beneficial after mechanical disturbance (Chen et al., 2013 & Larney et al., 2016). The soil microbiome supports ecosystem resilience and functioning, and understanding this could benefit the planning and design process of urban forests.

Testing for soil microbial functioning can benefit post-development monitoring of site performance. For any ecological design/restoration, the degree of biological soil recovery could be measured using a soil respiration testing protocol. With feedback on soil respiration,
future construction practices can be adjusted in order to prevent certain types of soil disturbance. Microbial soil function and integrity are good indicators of ecosystem resiliency. Therefore, an adaptive approach to managing soil microbiology can result in a better functioning ecosystem. Managing soil microbial populations can also help urban forest ecosystems become more resistant and resilient to disturbance.

6.3. Future Directions

Aspects of the soil microbiome can be studied with multiple soil microbiological methods. A broad measure of soil microbial function was applicable for this exploratory study. Moving forward, supplementing soil respiration with additional microbiological measurements could allow for further interpretation of results. Tests for soil bulk density, microbial biomass, and phospholipid fatty acid analyses could provide results that were absent in this study. However, using genomic testing for microbiome analysis is currently the pinnacle of soil testing.

Genomic testing would provide useful insights for understanding the soil microbiome and its response to disturbance. More specifically, metagenomic testing, which involves sequencing DNA from samples with a high abundance and diversity of genetic material could be used for ecosystem scale soil tests. The focus of metagenomic testing has only recently shifted from profiling the identities of soil microorganisms to analyzing functional genes. Certain functional genes are for specific ecosystem functions, such as nutrient and carbon cycling. Recent advances has also allowed for soil microbiological genomes to be profiled without the need to culture the samples before testing, which was prohibitive to effective profiling. Finally, the pricing of metagenomic testing has decreased exponentially in the last five years, making it more accessible as a research methodology.

Building on this exploratory study, a few methodological changes as well as experimenting with different research questions would be useful. A higher quantity of soil samples (>12), taken during different seasons would be ideal in order to increase confidence in results. Manipulating different experimental variables could further the understanding how of disturbance affects soil conditions. This could include investigating how disturbance affects
other ecosystem types, how different modalities of disturbance affect soil microbiological measures, how different durations since disturbance affect test results, and if amendment applications are suitable for recovery after disturbance. Understanding the practical problem in more depth will lead to changes in practice and in perceptions of the soil microbiome.

Similar research can also be conducted on low impact development practices and green infrastructure technologies. Ecosystem service evaluation is a focus of many of these emerging green technologies, such as green roofs, bioswales, and rain gardens. While a focus on provided ecosystem services can provide quantitative evaluation, such as removal of total suspended solids in water, these ecosystem services can be optimized if the constructed system functions as an ecosystem. Future research on emerging green infrastructure technologies should include analysis of the biological aspects of ecosystem functioning.

To conclude, the results of this study have implications for future research on this topic and for professional practice. The results are applicable to temperate urban forests of various sizes. The soil testing methodology is inexpensive, easy to use, and reliable, making it appropriate for studying soil microbial functioning in any type of ecosystem. With advancements in soil metagenomic testing, novel research questions can be answered with unparalleled precision. The composition and functioning of the soil microbiome is invisible to the naked eye, yet is important for ecosystem functioning. Understanding how the soil microbiome responds to disturbance can benefit urban forest planning, design and management, and other applications for landscape architecture.
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