TREE SPECIES' EFFECTS ON THE DEPOSITION AND TRANSPORT OF NUTRIENTS AND POLLUTANTS IN URBAN AND RURAL MIDWESTERN FORESTS

By

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ABSTRACT

The interaction of precipitation with tree canopies is an important process that affects ecosystem-scale nutrient cycling, plant nutrition, and spatial patterns of deposition of pollutants. Precipitation that falls on a tree canopy, interacts with foliar and woody components of the canopy and passes through to the soil below is termed throughfall (TF). Throughfall is a pathway for nutrients from atmospheric deposition to enter the soil system, a pathway by which plants can take up or lose essential nutrients, and it is a process that can concentrate atmospheric pollutants scrubbed from the air by tree canopies. By investigating canopy leaching we can understand how throughfall processes influence forest nutrient cycling and nutrient use efficiency, as well as how trees intercept particulate matter and gaseous pollutants and funnel them to the soil below. For my dissertation, I investigated the transport, exchange, and deposition of nutrients and pollutants via throughfall across Michigan's urban, suburban, and rural forests.

In Chapter 2, I studied the influence of adjacent land use, local point sources, and woodlot stand structure on subcanopy N transport and enrichment via throughfall in three woodlot fragments in southern Lower Michigan, USA. I found that one site had markedly higher TF N concentrations compared to the other two; however, my data indicate that elevated TF concentrations resulted from differences in tree species composition, rather than differences in surrounding land use. In Chapter 3 I investigated the role of TF in driving the transfer of nutrients from trees to soil, and in determining the NUE across a range of forest stands in the Manistee National Forest in the northwestern lower peninsula of Michigan, USA. The results of Chapter 3, demonstrate that TF losses of nutrients and litterfall losses both increase from low to high-fertility soils; however, the relative contribution of TF to total losses is greater in high-fertility soils.

Microplastics are an anthropogenic contaminant of emerging concern due to their durability and persistence in the environment. Microplastics are particles with a size range of < 5 mm and have been detected in aquatic ecosystems, soil, and airborne particles. There are data gaps on the effect of rainfall and the fate and transport of microplastics in urbanized areas. In Chapter 4, I seek to understand the role of individual tree species in the removal of microplastics in a medium-to-low-density metropolitan area in East Lansing, Michigan, USA. The study focuses on the quantification of throughfall concentration and fluxes to determine the interspecific and temporal variability of throughfall microplastics under urban tree species during fully-leafed and partially-leafed periods. Throughfall samples were collected from four species: red maple (*Acer rubrum),* eastern white pine *(Pinus strobus),* honeylocust *(Gleditsia triacanthos*), London planetree (*Platanus x acerifolia*). A fluorescence microscope was used to identify and quantify microplastics in throughfall. While the morphology and origin of microplastic particles a scanning electron microscopy (SEM) combined with an energy-dispersive X-ray (EDS) was used. In my study I was able to characterize polystyrene (PS), polyethylene (PE), and fiberglass in throughfall samples. Throughfall microplastic concentration and fluxes trended higher under London planetree, when compared to other commonly occurring urban tree species, but the species effect was not statistically significant. Understanding the fate and transport of microplastics in urbanized areas can help us determine the role of tree species in removing microplastics or other airborne pollutants that could negatively impact ecosystems and human health.

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CHAPTER 1

INTRODUCTION

1.1 Background

Forest ecosystems cover one-third of the world’s land area (Matyssek et al. 2012). Forest ecosystems provide extensive ecosystem services which include carbon sequestration, water regulation, and erosion control (Thompson et al., 2011). Forest ecosystems can serve as a sink for pollutants and nutrients across the globe. Forest ecosystems have been impacted by anthropogenic gases and aerosol which are produced by combustion and industrial activities (Fowler et al.,2019). The input of nutrients to the forest ecosystem has been shown to be an important factor in forest growth and forest health (Hansen et al., 2013).

Trees are like natural air filters and provide ecosystem services such as mitigation of urban air pollutants, reducing energy use, improving air and water quality, lowering rainfall runoff, and flooding, and reducing noise levels in urban areas (Nowak and Dwyer 2000). These intercepted particles are often resuspended to the atmosphere, washed off by rain, or dropped to the ground when the leaves and twigs fall (Nowak et al., 2014). Trees help to reduce air pollution by absorbing greenhouse gases through the leaves. The tree canopy has been shown to intercept suspended particles and gases, as well as favor the entry of leached nutrients into the soil (Clark et al., 1998; Lilienfein and Wilcke, 2001; Zimmerman et al., 2008).

Rainfall partitioning has been shown to affect both the hydrological and biogeochemical fluxes between vegetation and soil (Dunkerley,2000; Llorens and Domingo, 2007) which then can lead to spatial variability in soil. Rainfall partitioning in trees depends on meteorological factors (intensity, duration, wind speed, wind direction) and canopy structure (e.g., crown shape, branching patterns, stand age, and height) (Xiao et al.,2000; Fleischbein et al., 2005; Toba and Ohta 2005; Deguchi et al., 2006; Staelens et al., 2008; Holder 2012; Muzylo et al. 2012a, b; Levia and Germer 2015). Elements or compounds deposited in these three forms can add to soil element pools and are taken up by the forest vegetation (Weather and Ponette-Gonzalez, 2011). The composition and dominant trees as well as location, and distribution, these characteristics play a significant role in hydrological processes and help reduce flood problems. (Guevarra-Escobar et al., 2007). The interception of pollutants in the forest ecosystem by the foliage is determined by the leaf area index, leaf shape, leaf surface roughness, and stomata size.

The increase in population in urban areas on the local, regional, and global scales has led to environmental consequences. The environmental impacts of the population increase in urbanized areas include climate change, freshwater scarcity, water pollution, and deforestation (Uttara et al., 2012). These neighborhoods lack access to green spaces due to the development of cities and the fragmentation of forests. Urban forests have been shown to possess environmental, social, and economic value. By providing information to communities, we can integrate them into learning how urban forests can improve the quality of life.

1.2 Sources of Nutrients and Pollutants

Nutrients and pollutants in the atmosphere can be deposited in forest ecosystems from natural and anthropogenic sources. Nutrients are elements essential for the growth and survival of plants across the globe (Foster and Bhatti 2006). Plants require large amounts of macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), but only a small number of micronutrients such as boron (B), copper (Cu), Chlorine (Cl), iron (Fe), manganese (Mn), molybdenum (Mb), and zinc (Zn). These nutrients are important for growth, life cycle, and biological functions. Atmospheric precipitation is an essential source of nutrients in forest ecosystems (Su et al., 2019). While Nitrogen (N), phosphorus (P), magnesium, (Mg), calcium (Ca), potassium (K), and iron (Fe) are some examples of nutrients that do not exist in gaseous form and are largely derived from mineral weathering (Weathers and Ponette-Gonzalez,

2011) and enter the atmosphere via wind erosion. Sea salt spray is one of the major sources of ions in which elements such as Chloride (Cl), sodium (Na), sulfate (SO4 $^{2-}$), and Ca and Mg, this is due to wave action, particles are then suspended in air and being deposited downwind in forest ecosystems (Art et al., 1974). Additional natural sources of gases and particles can be presented in dust particles (e.g., organic chemicals, metals), volcanoes which can emit a significant amount of carbon dioxide sulfur dioxide, hydrogen sulfide, fire which is a central component of the earth system (Bowman et al., 2009; Keywood et al., 2012) and the activity depends on the ignition source available in the region, which can be natural (lightning) or anthropogenic (accidental or deliberate) (Bistinas et al., 2014). For example, N a growth-limiting nutrient, is one of the most significant nutrients in all ecosystems (Vitousek and Howarth 1991), its deposition from air pollution has the potential to cause a wide range of ecological impacts such as eutrophication or cause the growth of some species while causing others to be outcompeted**.**

Nutrients and pollutants are also sourced by anthropogenic activities, with key point sources including industrial plants, power plants, and vehicles with internal combustion engines that produce nitrogen oxides, VOCs, sulfur dioxide, and particles. Much of the anthropogenic sources have their origin in industries and conventional energies (mining industries, fossil fuels, chemical and metallurgical industry, noise, industrial waste, etc.), agriculture, transportation, and urbanization (Gheorghe and Ion 2011). Another anthropogenic source that has caught the eye of the scientific community is the global increase of plastic in the environment. Their impact on the environment is concerning due to polymers being vectors to polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals (e.g., Cu, Zn, Pb). Microplastics are small-sized particles driven by fragmentation due to ultraviolet radiation and mechanical abrasion, where they have dimensions of < 5 mm. Wang et al. (2020) indicated that once the microplastics are in the atmosphere, they can be transported passively by a complex of two- and three-dimensional physical winds, resulting in a large variability over the land and surface ocean. Air pollutants can be classified as either primary or secondary pollutants. Primary pollutants are emitted from a direct source to the atmosphere, whereas a secondary pollutant is an air pollutant that forms as a result of the interaction between primary air pollutants and other atmospheric components. Primary pollutants can be found in the form of sulfur dioxide (SOx), nitrous oxide (NOx), fluorides, and carbon monoxide (CO), which have a direct effect on humans through the effects on the ecosystems. Other important primary pollutants are particulate matter which includes Particulate Matter10 and Particulate Matter2.5, heavy metals (e.g., Pb, Cd, As), and volatile organic compounds which can give rise to photochemical oxidants such as ozone. Secondary pollutants are the result of interaction with primary pollutants once they are emitted into the atmosphere. They result in known substances such as ozone and acid rain which are formed due to interactions with VOC, CO, NOx, and SOx in the presence of sunlight.

1.3 Transport, dispersion, and deposition of nutrients and pollutants

The atmosphere can transport, disperse, and ultimately deposit different elements and compounds that influence plants, animals, and humans. The atmosphere is a medium in which atmospheric reactions serve to remove contaminants. Transport and dispersion of elements can be affected by atmospheric dynamics, fluid, and physical laws that govern the atmosphere. Dispersion relies mostly on wind systems that transport and dilute air pollutants at different scales. Dispersion refers to the distribution of air pollution in the atmosphere. Regional wind fields, including vertical layering, affect air pollutants on a larger scale, while fluid dynamics often describe air flows around obstacles in street canyons and inside vegetation barriers (Janhall, 2015).

Atmospheric pollutants impact trees via wet and dry deposition. These potential impacts can produce acidifying soils as well as impact the availability of nutrients (Bytnerowicz et al. 2007). Atmospheric deposition is shown to be a mechanism by which pollutants and nutrients can reach forest ecosystems and have a direct and indirect impact on soil, water, and plant health. This mechanism inputs macro and micronutrients, as well as pollutants, which can be deposited via (i) rain and snow (wet deposition) (ii) particles and gases (dry deposition), and (iii) cloud droplets (e.g., cloud, fog deposition) (Weathers and Ponette-Gonzalez, 2011). The interactions between vegetation and rainfall are of considerable significance from the physiological, ecological, and hydrological points of view (Aboal et al., 1999; Crockfrod and Richardson, 2000; Toba and Ohta 2005; Deguchi et al., 2006; Staelens et al., 2008) Dry deposition is the result of particles and gases being deposited through gravitational sedimentation, impaction, and interception (Hansen et al., 2013). Dry deposition consists more of gases, aerosols, and dust, and is largely influenced by physical and chemical properties of the receptor surface. During dry deposition, particles are constantly intercepted and resuspended and they depend highly upon wind speed (Nowak et al., 2014). Understanding the mechanism of atmospheric deposition such as wet, dry, and cloud can help us comprehend the impacts on nutrient cycling, soil health, and the potential benefits of trees in the mitigation of urban pollutants. Furthermore, dry deposition can be accumulated on the foliage and is then washed off by precipitation and enhanced deposition under the canopy. A study by De Schrijver et al. (2007) found that the effect of forest type on canopy exchange processes can be direct or indirect. A direct impact can be caused by differences in canopy characteristics such as aboveground biomass, nutrient amount, species-specific concentration in leaves (Lovett et al. 1989), and evergreen character (Smith 1981). A high roughness of the canopy can cause higher air turbulence and more intensive interaction between the air and the foliage. The interception of elements/ compounds by the foliage can be determined by factors such as leaf area index, leaf shape, leaf surface roughness, and stomata size. Zhang et al. (2017) found that the surface roughness of the leaves from the bread-leaved tree species is directly proportional to the capturing capacity of the leaves. The surface roughness is greater with broad-leaved tree species than the leaves of needle-leaved tree species, however, the dust retention is higher for needle leaves than for broad-leaved tree species.

1.4 Distribution of Nutrients and Pollutants in forest ecosystems

In areas where nutrients are limited, precipitation serves as a source of nutrient input and helps in the growth and progression of forests (Dawoe et al., 2018; Lu et al., 2017; Navar et al., 2009). Another method in which nutrients are introduced to forest ecosystems is via the dissolution of elements in throughfall (Van Langenhove et al., 2020). Precipitation washes off particles and gases that have been deposited via dry deposition on the vegetation surfaces and acts as a medium that transfers nutrients to the soil (Su et al., 2019). Large input of nutrients to forest ecosystems may result in detrimental effects on forest health and growth. Vegetation is a temporary retention of atmospheric particles, that are moved back to the atmosphere or to the soil (Nowak et al., 2014). Throughfall (TF) represents one of the largest components of rainfall partitioning which reaches the forest floor and accounts for more than 70% of the gross precipitation (Staelens et al., 2006; Levia et al., 2011; Carlyle-Moses and Lishman 2015). The spatial and temporal variability of throughfall is due to the interaction with precipitation and the forest canopy (Zimmerman et al., 2008; Sun et al., 2017). TF impacts forest ecosystems because of its relationship to the leaching and flushing of nutrients that are absorbed by branches and leaves. The variability of throughfall depends on a series of factors such as tree species, canopy cover, and distance from the nearest tree trunk (Carlyle-Moses et al., 2004; Kowalska et al., 2016; Zhang et al., 2017; Sun et al., 2017;

Cardil et al. 2018). TF is an important mechanism in biogeochemical cycles in forest ecosystems (Su et al., 2019). Canopy traits such as phenoseason, canopy cover, leaf area index, and crown length are some examples of how vegetative structure are an example of factors that can increase the difficulty of understanding throughfall distribution in forest ecosystems (Park and Cameron, 2008; Siles et al., 2010; Zabret, Rakoven and Sraj, 2018). The variation of sub-canopy fluxes could also be due to tree age and species-specific characteristics such as leaf shape and orientation (Carleton and Kavanagh, 1990; Crockford and Richardson, 2000). Variability between tree species can be attributed to leaf texture and the hydrophobicity of vegetative surfaces (Levia and Frost 2006). For example, Nanko et al., (2013) found that the throughfall drop size was small on trees with more hydrophobic foliar surfaces. This would mean that throughfall can be influenced by leaf traits such as roughness, inclination, and geometry (Goebes et al., 2015; Nanko et al., 2013; Wang et al., 2014).

1.5 Research Objectives

The interaction of precipitation with tree canopies is an important process that can affect plant nutrition, the spatial pattern of the deposition of air pollutants, and nutrient cycling. Investigating canopy leaching will help us comprehend how throughfall processes influence forest nutrient cycling, and how trees intercept airborne particles and pollutants return to the soil. The intended goal of this study was to utilize the data collected via in situ sampling from low to high-density metropolitan regions to expand our knowledge on the transport, exchange, and deposition of nutrients and pollutants via throughfall across Michigan’s urban, suburban, and rural forests. The first part of this manuscript looks at the impacts of N deposition in a midwestern fragmented forest and the role of tree species composition in TF chemistry. The second manuscript looks at how TF is a mechanism of transport of nutrients from trees to the soil and the potential importance of TF losses in determining tree nutrient use efficiency (NUE). The third and final manuscript seeks to comprehend the fate and transport of microplastics and the role of individual trees in intercepting airborne microplastics.

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CHAPTER 2 NITRATE AND AMMONIUM DEPOSITION IN THE MIDWESTERN FRAGMENTED FOREST

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2.1 Abstract

Whereas the impacts of N deposition on forest ecosystems have been well studied in remote areas in predominantly forested landscapes, we know relatively less about the impacts of N deposition on forests in heavily human-modified landscapes. We studied the influence of adjacent land use, local point sources, and woodlot stand structure on sub-canopy N transport and enrichment via throughfall in three woodlot fragments in southern Lower Michigan, USA. We found that one site had markedly higher TF N concentrations compared to the other two; however, our data indicate that elevated TF concentrations resulted from differences in tree species composition, rather than differences in surrounding land use. Specifically, we observed that the local abundance of basswood (Tilia americana) was positively associated, and the local abundance of northern red oak (Quercus rubra) was negatively associated with TF N concentrations. One site had markedly greater TF N fluxes compared to the other two, which was driven by a lack of understory vegetation, possibly due to higher deer browsing at this site. Together, the results of this study demonstrated that TF N concentrations and fluxes were more strongly influenced by the internal characteristics of fragmented woodlots, such as forest structure and species composition, than by the surrounding land use.

Keywords: nitrogen deposition; atmospheric deposition; edge effect; enrichment ratio; Tilia americana; Quercus rubra

2.2 Introduction

Anthropogenic nitrogen (N) emissions associated with motor vehicles, power plants, agriculture, and other human activities have resulted in elevated rates of N deposition to forest ecosystems on a global scale (Galloway 1998, 2002; Galloway et al., 2004). The effects of anthropogenic N deposition on forests can include changes in the composition of plant communities, disruptions in nutrient cycling, increased emission into the atmosphere of the greenhouse gas nitrous oxide (N_2O), accumulation of N compounds in the soil, increased availability of N to primary producers, soil acidification, and increased susceptibility of plants to other stress factors (Aber et al., 1989; Aber et al., 1998; Bobbink et al., 1998; Fenn et al., 1998; Driscoll et al., 2003). Whereas the impacts of N deposition on forest ecosystems have been well studied in remote areas in predominantly forested landscapes, we know relatively less about the impacts of N deposition on forests in heavily human-modified landscapes. Unique considerations in these landscapes include forest fragmentation, resulting edge-interior gradients, and the importance of local sources of N emissions.

Forests of the eastern United States are highly fragmented due to roads, suburban development, and agriculture (Heilman et al., 2002; Riitters et al., 2012). Forests of the Southern Great Lakes Ecoregion (southern Michigan, northern Indiana, and northern Ohio) are the most fragmented in the country, with most forest lands consisting of remnant patches surrounded by urban land, suburban development, or agriculture (Heilman et al., 2002). Fragmented forests have a greater proportion of "edge" habitat that is influenced by adjacent land uses and lower proportions of interior habitats that are less affected by surrounding land uses (Vallet et al., 2010). Another potential impact of fragmentation is that forest fragments may receive additional N deposition from local sources. In agricultural landscapes, adjacent croplands can heavily impact forest fragments through fertilizer drift. Honnay et al. (2002), found higher levels of ammonium (NH_4^+) in the edge habitat compared to the interior habitat in five Belgian forest fragments and attributed this to an influx of agricultural fertilizers from adjacent croplands. Livestock is also a major source of volatile ammonia (NH_3) in the atmosphere across the globe (Bittman and Robert, 2009). When NH_3 is emitted into the atmosphere, it can be converted to NH_4^+ aerosols and washed out by rain or by dry deposition. Ammonia can then be deposited within a few kilometers of the source due to its short atmospheric lifetime (Theobald et al., 2009). Thus, forests near livestock farms can experience very high levels of N deposition, leading to negative effects on the ecosystem (Lopez-Aizpún et al., 2018). Automobiles can also be major sources of atmospheric NH_3 since the introduction of catalytic converters, causing roadways and adjacent vegetative surfaces to serve as hotspots for N deposition in urban and suburban areas (Bettez et al., 2013; Fenn et al., 2018).

These numerous forms of land use in agricultural and urban areas lead to N deposition in forest fragments adjacent to or within proximity to the emissions sources. Once deposited in these fragmented forests, N in its various forms can be subsequently distributed below the canopy by several mechanisms, including throughfall (i.e., precipitation that interacts with foliar and woody surfaces in the canopy or passes through canopy gaps en route to the forest floor) (Dunkerley, 2000; Xiao et al., 2000; Zhang et al., 2016). Throughfall chemistry is often enriched relative to that of open precipitation and may vary spatially within a fragmented forest based on species composition and stand structure, among other factors (Levia and Frost, 2006; Limpert and Siegert, 2019). This spatial variability in throughfall chemistry impacts nutrient delivery to forest soils, creating the potential for biogeochemical hotspots. Rainfall volume and the length of a dry period (Lovett and Linberg, 1984) have also been shown to have an impact on throughfall chemistry and the contribution of deposition and exchange processes of deposition under the forest.

While these and other studies have helped elucidate our understanding of subcanopy N flux via throughfall, they have predominantly focused on rural forests. As higher proportions of forested lands are fragmented to accommodate expansive development, there remains a need to understand how these processes behave in highly developed areas. To determine the relative impacts of adjacent land use, local point sources, and woodlot stand structure on subcanopy N transport and enrichment via throughfall, we based our study on three woodlots in southern Lower Michigan. Within each woodlot, we monitored N deposition in throughfall along the edge to interior gradients to test the following hypotheses:

1. N deposition to forest fragments, as evidenced by throughfall N composition, will vary by adjacent land use.
2. Nitrogen enrichment of throughfall is highest at the edge and decreases with distance from the edge.
3. Spatial variability in throughfall N enrichment is impacted by species composition.
4. Throughfall N concentrations, flux, and enrichment ratios will be higher for storm events with a longer antecedent dry period.
5. Higher rainfall intensity will result in higher throughfall N concentration, flux, and enrichment ratios.

2.3 Materials and Methods

2.3.1 Study Area and Experimental Sites

We conducted this study in East Lansing, Michigan, USA (42.7370° N, 84.4839° W). East Lansing has a population of 47,741 (U.S. Census Bureau, 2023) and is within the larger Lansing capital metro area. The city is 226 m above sea level and has a cold and temperate climate (Köppen–Geiger climate classification Dfa). East Lansing is in the USDA Hardiness Zone 5. The mean annual rainfall for the region is 949 mm. September is the wettest month in the region ($\mu_{\text{precipitation}}$ = 88.9 mm), while February is the driest month ($\mu_{\text{precipitation}}$ = 48.3 mm). The average annual temperature is 9.4 °C in East Lansing, with July being the warmest month of the year ($\mu_{\text{temperature}}$ = 21.3 °C) and January typically the coldest month ($\mu_{\text{temperature}}$ = −6.2 °C).

We conducted this study in three woodlots on the East Lansing campus of Michigan State

University (MSU), all of which are within a fragmented landscape with a mix of urban, residential, and agricultural land use. We selected the three woodlots (Biebersheimer, Hudson, and Lott North) from a pool of 25 MSU Campus Natural Areas to be as similar as possible in their structure and species composition, but to vary in terms of landscape context relative to local sources of N emissions. All three woodlots were mature, mesic forests dominated by sugar maple (*Acer saccharum* Marsh.), with northern red oak (*Quercus rubra* Lobatae.), American beech (*Fagus grandifolia* Ehrh), and basswood (*Tilia americana* Linden) as the next most dominant to varying degrees (Table 1). Whereas the three woodlots were similar in species composition and stand structure, they were selected to vary in their location relative to landscape features likely to serve as local sources of N deposition (Figure 1). Biebersheimer woodlot is in one corner of a "cloverleaf" interchange of two interstate highways and is hereon referred to as the highway site. Lott North has varied surrounding landscape features, including agricultural fields (corn–soybean rotation), other woodlots, and a section of interstate highway. It is hereon referred to as the mixed land use site. Hudson is surrounded by agricultural fields (corn–soybean rotation), plus MSU's Dairy Cattle Facility is located 750 m due west of the edge of this woodlot. Hudson is hereon referenced as an agricultural site.

Figure 1. Study Area.

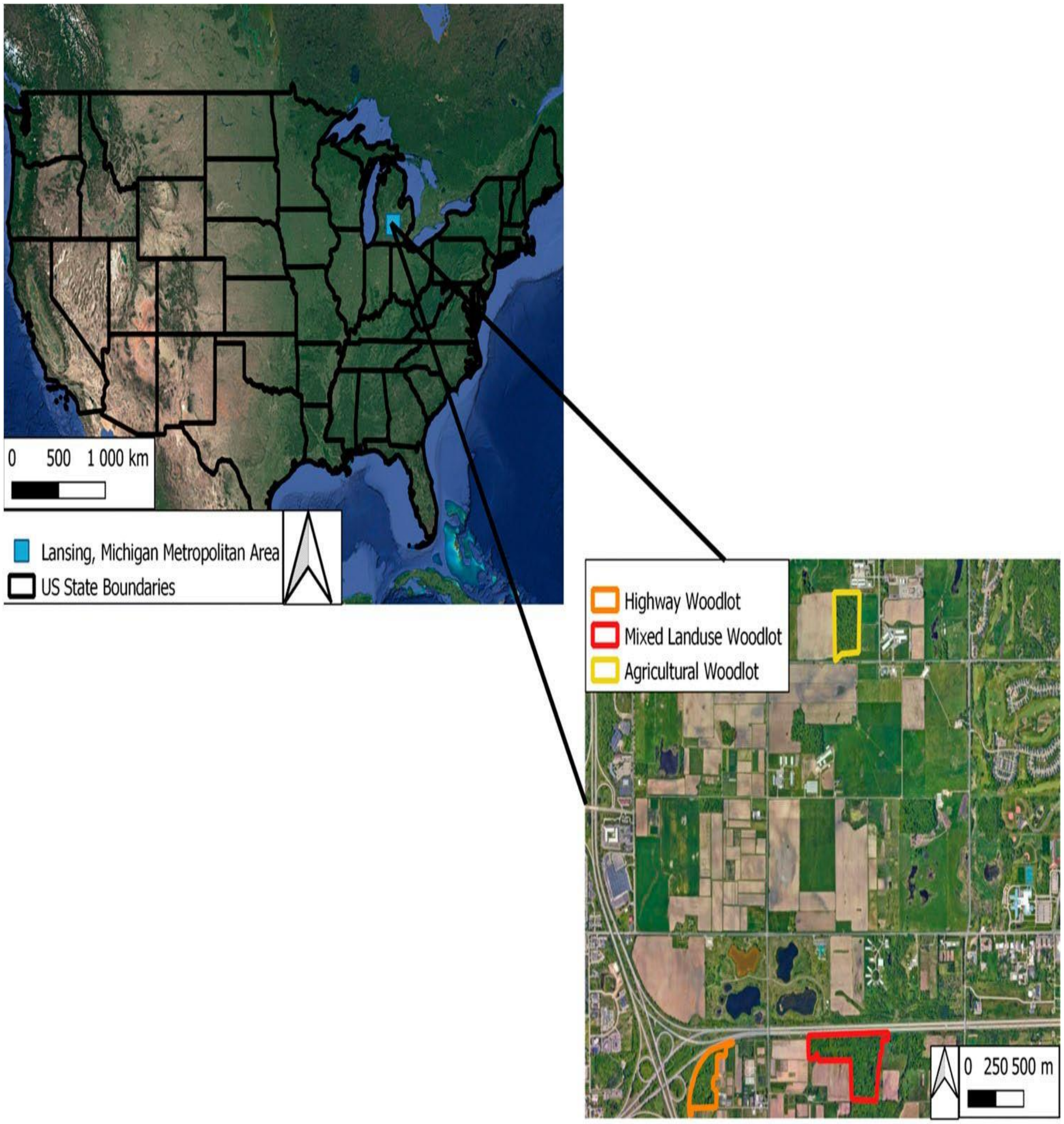
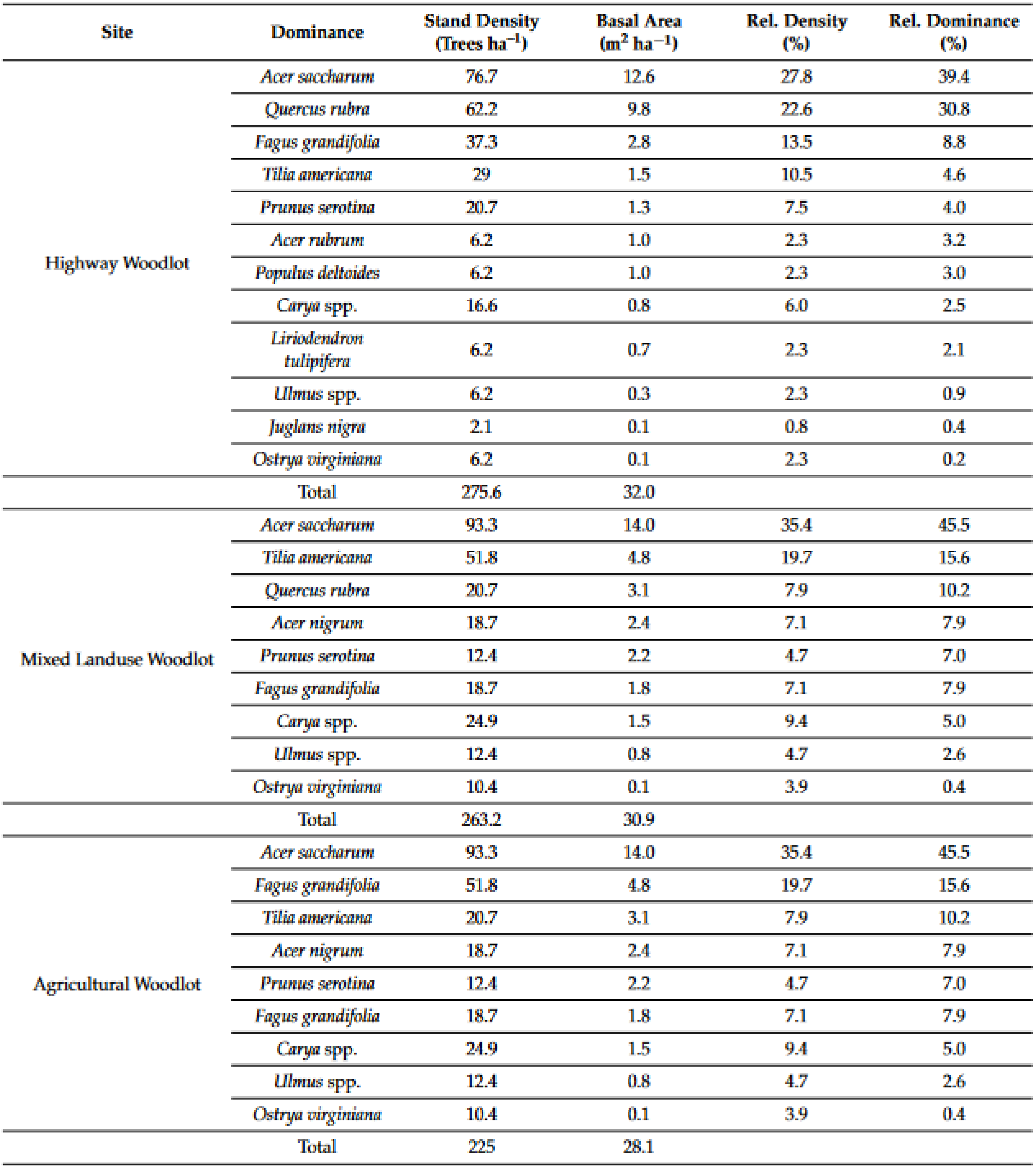


Table 1. Overstory species composition and structure of the three woodlots. Data are for all trees ≥ 10 cm dbh. Relative density is the percentage contribution of each species to total trees per hectare, whereas relative dominance is the percentage contribution of each species to the total stand basal area.



Site	Dominance	Stand Density (Trees ha^{-1})	Basal Area (m^2 ha^{-1})	Rel. Density (%)	Rel. Dominance (%)
Highway Woodlot	*Acer saccharum*	76.7	12.6	27.8	39.4
	Quercus rubra	62.2	9.8	22.6	30.8
	Fagus grandifolia	37.3	2.8	13.5	8.8
	Tilia americana	29	1.5	10.5	4.6
	Prunus serotina	20.7	1.3	7.5	4.0
	Acer rubrum	6.2	1.0	2.3	3.2
	Populus deltoides	6.2	1.0	2.3	3.0
	Carya spp.	16.6	0.8	6.0	2.5
	Liriodendron tulipifera	6.2	0.7	2.3	2.1
	Ulmus spp.	6.2	0.3	2.3	0.9
	Juglans nigra	2.1	0.1	0.8	0.4
	Ostrya virginiana	6.2	0.1	2.3	0.2
	Total	275.6	32.0		
Mixed Landuse Woodlot	*Acer saccharum*	93.3	14.0	35.4	45.5
	Tilia americana	51.8	4.8	19.7	15.6
	Quercus rubra	20.7	3.1	7.9	10.2
	Acer nigrum	18.7	2.4	7.1	7.9
	Prunus serotina	12.4	2.2	4.7	7.0
	Fagus grandifolia	18.7	1.8	7.1	7.9
	Carya spp.	24.9	1.5	9.4	5.0
	Ulmus spp.	12.4	0.8	4.7	2.6
	Ostrya virginiana	10.4	0.1	3.9	0.4
	Total	263.2	30.9		
Agricultural Woodlot	*Acer saccharum*	93.3	14.0	35.4	45.5
	Fagus grandifolia	51.8	4.8	19.7	15.6
	Tilia americana	20.7	3.1	7.9	10.2
	Acer nigrum	18.7	2.4	7.1	7.9
	Prunus serotina	12.4	2.2	4.7	7.0
	Fagus grandifolia	18.7	1.8	7.1	7.9
	Carya spp.	24.9	1.5	9.4	5.0
	Ulmus spp.	12.4	0.8	4.7	2.6
	Ostrya virginiana	10.4	0.1	3.9	0.4
	Total	225	28.1		

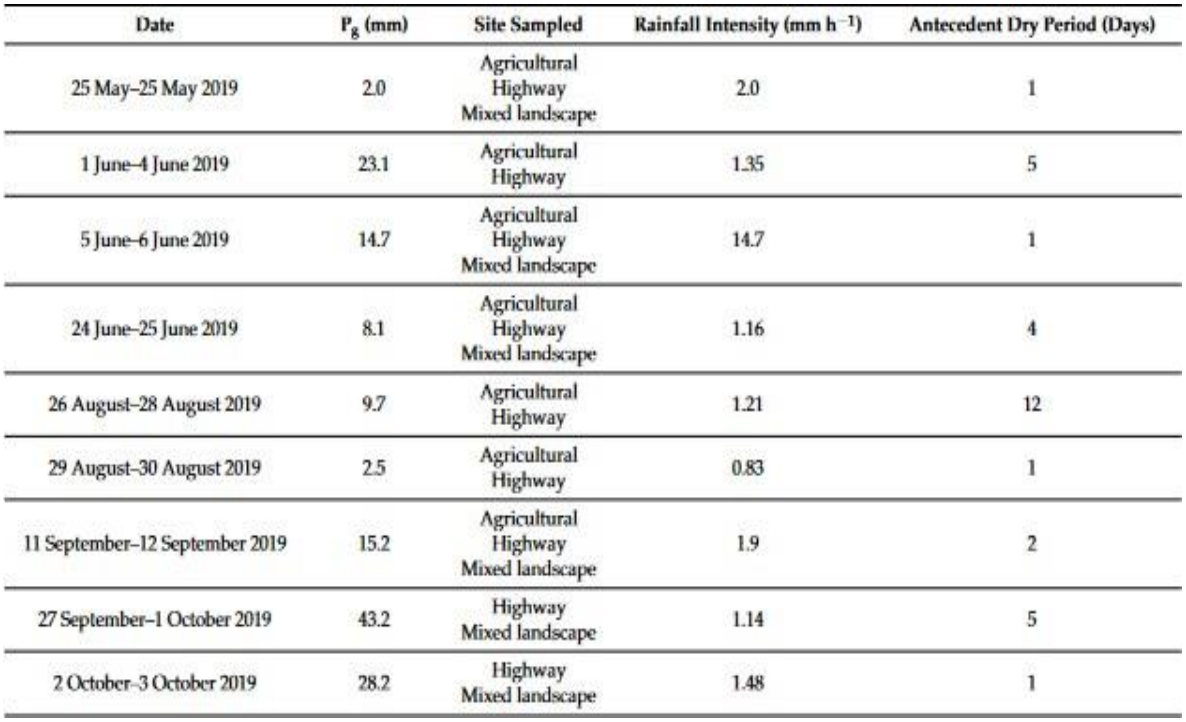
2.3.2 Sampling Design

At each experimental site, three transects were established perpendicular to the western forest edge, since the prevailing weather systems move from west to east. Along each transect, eight throughfall collectors were placed at distances of 0, 10, 20, 30, 40, 50, 75, and 100 m from the windward edge, for a total of 24 throughfall collectors within each site and 1 located outside the woodlot for open precipitation. Outside of each woodlot, a single collector was placed in an adjacent open field to measure and collect samples of open precipitation. Throughfall collectors consisted of 219 cm^2 high-density polyethylene (HDPE) funnels that were each attached to 3.78L HDPE containers. Collectors were suspended on posts ca. 1 m above ground to prevent interference by wild animals and rain droplets from splashing back into the collectors. Inside each funnel, we placed a small piece of glass wool to prevent any debris from contaminating samples. The glass wool was replaced periodically throughout the study.

2.3.3 Sample Collection and Analysis

Sample collection was conducted on an event basis; we visited the sites and emptied collectors within 48 h after each storm event. For this study, we defined an event as a storm that produced a minimum of 1 cm of precipitation, followed by a dry period of 12 h or more. We conducted sampling between 23 May and 4 October 2019. To identify storm events during our study period, we utilized precipitation data from the MSU Enviroweather *MSU Hort* weather station (42.634° N, 84. 4870° S, https://enviroweather.msu.edu, accessed on 20 January 2023). This weather station is located near all our sites (0.6 km from the highway site, 0.8 km from the mixed land use site, and 3.2 km from the agricultural site). Actual sampling dates, precipitation amounts, and antecedent dry periods are provided in Table 2.

Table 2. Total precipitation (Pg), rainfall intensity, and antecedent dry period for the study period.



Date	P_g (mm)	Site Sampled	Rainfall Intensity (mm h^{-1})	Antecedent Dry Period (Days)
25 May–25 May 2019	2.0	Agricultural Highway Mixed landscape	2.0	1
1 June–4 June 2019	23.1	Agricultural Highway	1.35	5
5 June–6 June 2019	14.7	Agricultural Highway Mixed landscape	14.7	1
24 June–25 June 2019	8.1	Agricultural Highway Mixed landscape	1.16	4
26 August–28 August 2019	9.7	Agricultural Highway	1.21	12
29 August–30 August 2019	2.5	Agricultural Highway	0.83	1
11 September–12 September 2019	15.2	Agricultural Highway Mixed landscape	1.9	2
27 September–1 October 2019	43.2	Highway Mixed landscape	1.14	5
2 October–3 October 2019	28.2	Highway Mixed landscape	1.48	1

At each collection date, we made volumetric measurements for throughfall for each collector. We also collected a 50 mL subsample of throughfall, storing these samples in clean HDPE vials for later laboratory analysis. Collectively, throughfall volume and hydrochemical data were used to determine the total throughfall depth and solute flux, respectively. To analyze our samples, we conducted colorimetric analyses for NH_4^+, concentration according to (Sinsabaugh et al., 2000) and for NO_3^- according to (Doane and Horwath, 2003). Throughfall fluxes (TF) of NO_3^- and NH_4^+, as well as total inorganic N, were determined using Equation (1):

$$\text{TF Flux} = \text{Volume (L)} \times \text{Concentration (mg L}-1\text{)/Funnel Area (m}^2\text{)} \quad (1)$$

For throughfall, we also calculated the flux-based enrichment ratio, a metric used to quantify the degree to which the chemical composition of precipitation (e.g., throughfall) is modified due to its interaction with foliar and woody components of the canopy (Levia and Herwitz, 2000; Levia and Frost, 2003; Siegert et al., 2017). Flux-based enrichment ratios for our study were calculated following (Limpert and Siegert, 2019; Levia and Herwitz, 2000; Levia and Frost, 2003; Siegert et al., 2017):

$$Et = (CtT)/(CpPg) \quad (2)$$

where Et is the flux-based enrichment ratio of throughfall (unitless), Ct is the solute concentration in throughfall (mg L^{-1}), T is the depth equivalent of throughfall (cm), Cp is the solute concentration of open collector rainfall (mg L^{-1}), and Pg is the depth equivalent of open collector rainfall (cm). The enrichment ratio determines the degree to which the forest canopy serves as a source or a sink for NH_4^+ and NO_3^-. While all enrichment ratio values are positive, Et observations > 1 indicate a net contribution of N from the canopy to throughfall, whereas Et values < 1 indicate net uptake of N by the canopy from the incident precipitation.

2.3.4 Tree Species Influence

To quantify the potential influence of local variability in tree species composition within woodlots on throughfall concentrations, fluxes, and enrichment ratios, we measured the size and species of trees surrounding each collector. We used circular plots (8 m), centered on each collector, and measured the distance and diameter at breast height (dbh; 1.37 m) of every overstory tree (≥10 cm dbh) within an 8 m radius of the collector and measured the distance and dbh for every understory tree (<10 cm dbh) within a 3 m radius of the collector. For the three edge collectors (0 m on transect) at each woodlot, we sampled half-circles on the interior side. To assess the local influence of different species on each collector, we adapted the simple competition index of (Hegyi, 1974) as an "influence index", as indicated in Equation (3):

$$Di/\ Disti \quad (3)$$

where D_i = dbh (cm) of tree i and $Dist_i$ = distance (m) from the collector to tree i. This index assigns greater influence to trees that are larger and closer to the collector and a smaller influence to trees that are smaller and more distant. Sugar maple, American beech, northern red oak, and basswood were the only species that occurred frequently enough for analysis.

2.3.5 Statistical Analysis

We used Kruskal–Wallis tests to determine differences among the three woodlots by hypothesis 1 using GraphPad Prism 9.3.1. Statistical outliers were identified by the ROUT test (Q = 0.1%) and removed (Motulsky and Brown, 2006). For hypotheses 2 and 3, we analyzed throughfall concentrations, fluxes, and enrichment ratios using a mixed effects model, with date treated as a random effect. The following terms were treated as fixed effects: Site, Distance, Site x Distance interaction, Maple Influence, Basswood Influence, Beech Influence, and Red Oak Influence. The mixed-effects model was conducted with RStudio version 1.2.5033. The following packages were used for running the linear models with random effects: lme4, lmerTest, and the ggpairs functions for the graphs with GGally and ggplot2. The significance level was set at ($p <$ 0.05). We also investigated the influence of abiotic and biotic factors on the throughfall concentration, fluxes, and enrichment ratio to evaluate hypotheses 4 and 5. Accordingly, for the model, the following terms were treated as fixed effects: site, antecedent dry period, and rainfall intensity, with date treated as a random effect. The antecedent dry period was defined as the amount of time (days) that had elapsed between the end of one storm event and the beginning of a new one. Rainfall intensity was calculated by dividing precipitation depth by storm duration, as outlined in Equation (4):

$$\text{Rainfall intensity} = \text{Precipitation depth (cm)}/\text{duration (hrs)} \quad (4)$$

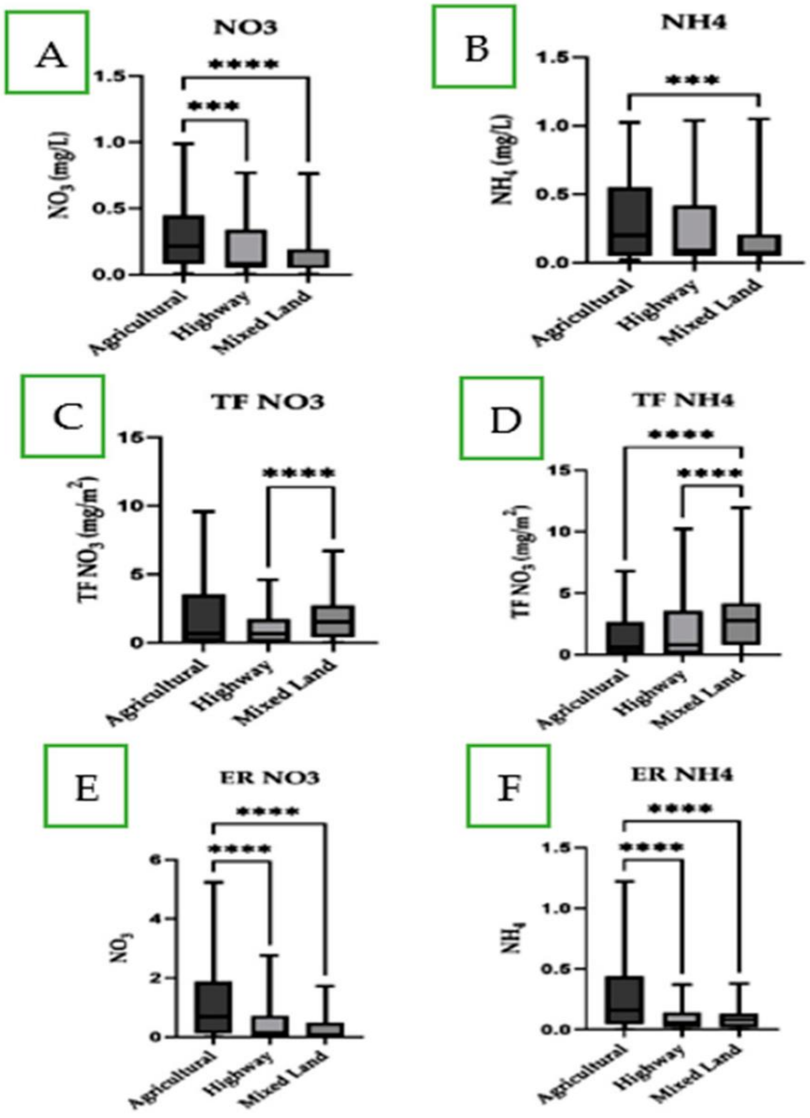
2.4 Results

2.4.1 Patterns in Throughfall Concentration, Fluxes, and Enrichment Ratios among Sites

The mixed land use had the highest volumes of TF due to lower canopy interception; across the study period, TF volumes averaged 53.4% of the gross precipitation at the agricultural site, 60.7% at the highway site, and 87.5% at the mixed land use site. Across all sampling dates, the throughfall NO_3^- concentrations were highest at agricultural and highway and lowest at mixed land use (Figure 2). The agricultural throughfall NO_3^- concentrations were significantly higher than those at both mixed land use ($p < 0.0001$) and highway ($p < 0.001$; Figure 2A). The highway NO_3^- concentrations trended higher than those at mixed land use, though this difference was not statistically significant ($p = 0.065$; Figure 2A).

However, the NO_3^- fluxes at the mixed land use site were significantly higher than those at the highway site ($p < 0.0001$; Figure 2C). The nitrate enrichment ratios were highest at the agricultural site compared to both the highway ($p < 0.001$) and mixed land use ($p < 0.001$; Figure 2E) sites. There was not a significant difference in the NO_3^- enrichment ratios between the highway and mixed landscape ($p = 0.498$; Figure 2E). While the enrichment ratio ranges did vary considerably in the agricultural (0.40–6.75), highway (0.1–4.02), and mixed land use (0.01–4.00) sites, the median enrichment ratio for NO_3^- was less than 1.0 for all the sites, indicating that the tree canopies at all 3 sites tended to serve as a sink, rather than a source for incoming NO_3^-. The median enrichment ratios were much lower for highway (0.15) and mixed land use (0.08), compared to agricultural (0.68), implying that the forest canopy at agricultural was a notably weaker sink for NO_3^-.

Figure 2. Box-whiskers plots showing differences among sites in the concentration (A,B), fluxes (C,D), and enrichment ratios (E,F) of NO_3^- and NH_4^+. Lines connect sites that were significantly different from each other, and the asterisks show the level of statistical significance. ($p = 0.05$, $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).



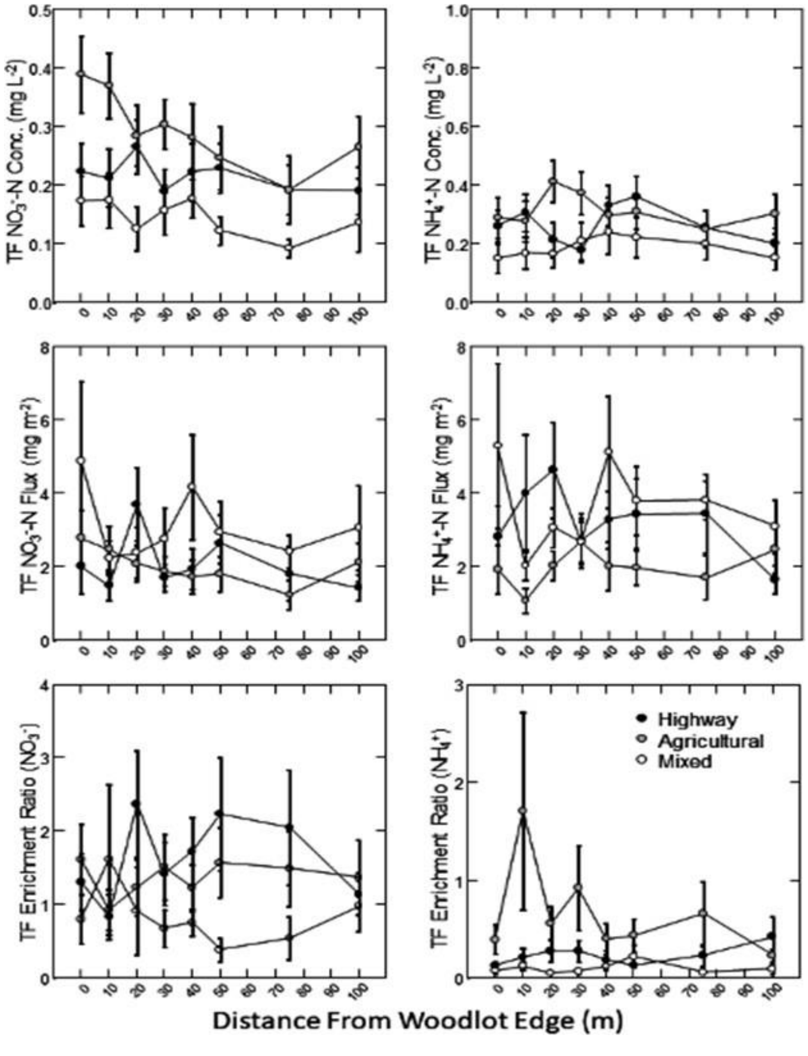
The concentration of NH_4^+ in throughfall was higher at the agricultural site (Figure 2) when compared to the mixed land use and highway sites with ($p = 0.0002$). The fluxes of NH_4^+ in the throughfall followed were higher for the mixed landscape site when compared to the agricultural and highway sites ($p < 0.0001$; Figure 2D). In our observations, we found that there was no difference in the NH_4^+ fluxes for the agricultural and highway sites ($p > 0.999$; Figure 2D). The NH_4^+ enrichment ratios were higher in the agricultural site compared to the highway ($p < 0.0001$) and mixed landscape sites ($p < 0.0001$; Figure 2F), with median values of 0.16 at the agricultural, 0.06 at the highway, and 0.08 at the mixed landscape sites. There was no statistically significant difference in the NH_4^+ enrichment ratios between the highway and mixed landscape sites ($p > 0.999$; Figure 2F). The ammonium fluxes and concentrations were higher in the open collectors than those found within the interior of the woodlots, indicating a net removal of NH_4^+ by forest canopies at all three sites.

For the open precipitation collectors, the median for the NO_3^- concentrations was 0.05 mg L^{-1} for all three sites, whereas the median NH_4^+concentration in the open precipitation was comparable at 0.08 mg L^{-1} at the agricultural, 0.20 mg L^{-1} at the highway, and 0.12 mg L^{-1} at the mixed landscape sites. For the open precipitation, there was no significant relationship for the NO_3^- concentrations in the agricultural ($p = 0.64$), highway ($p = 0.8204$), and mixed land use ($p = 0.95$) sites, and for the NO_3^- fluxes agricultural ($p = 0.57$), the highway ($p = 0.35$) and mixed land use ($p = 0.92$) sites. There was no significant difference in open precipitation NH_4^+ concentrations among the three sites, ($p > 0.05$, at all sites), nor were there significant differences in NH_4^+ fluxes among the three sites ($p > 0.05$).

2.4.2 Spatial Patterns in Throughfall N within Woodlots

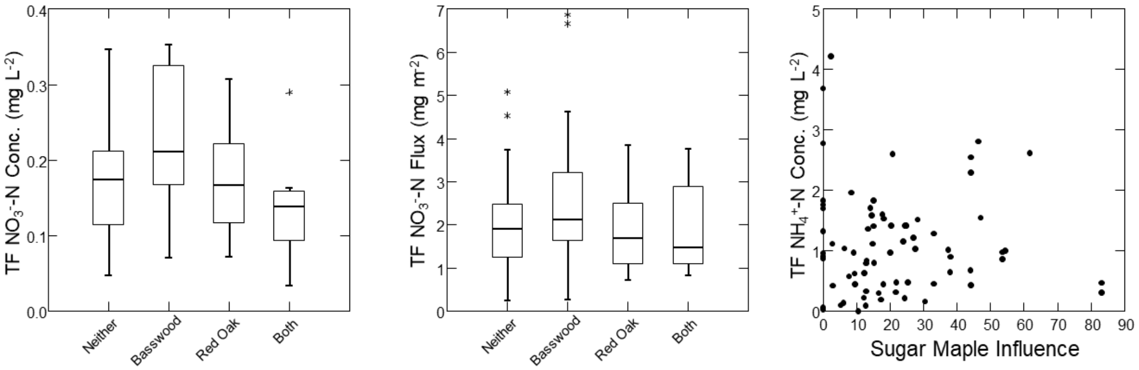
Within the study woodlots, the throughfall concentration, flux, and enrichment ratios of NO_3^- were influenced both by the position relative to the edge and by the local tree species composition around each collection point. At the agricultural site, the distance from the woodlot edge had a significant effect ($p = 0.002$) on the NO_3^- concentrations in the throughfall, with higher concentrations near the edge and lower concentrations in the interior, but these patterns were not observed for the mixed land use and agricultural sites (Figure 3). We found that there was no significant effect of distance from the woodlot edge on the NO_3^- flux (p = 0.20) or $NO3^-$ enrichment ratio (p = 0.73).

Figure 3. Average concentration, fluxes, and enrichment ratio of NO_3^- and NH_4^+ from distance from the edge during the sampling period (May–October 2019).



Local tree species composition had a significant effect on the NO_3^- concentrations in the throughfall, with NO_3^- concentrations increasing with increasing basswood influence ($p < 0.001$) and decreasing with increasing red oak influence (p = 0.03; Figure 4). It should be noted that basswood was present in all three woodlots; however, we did not encounter any red oak at the agricultural woodlot (Table 1). No other tree species had a significant effect on the NO_3^- concentrations, nor were there any significant interactions among species. The throughfall fluxes of NO_3^- were influenced by basswood ($p = 0.007$) and slightly by red oak ($p = 0.082$), with higher fluxes associated with greater basswood influence and lower fluxes associated with higher red oak influence. There were no effects of tree species' local abundance on the NO_3^- enrichment ratio.

Figure 4. Visualization of significant species' effects on throughfall N from the mixed-effects model. Asterisks (*) indicate outliers. Influence of basswood and red oak is shown with box-whisker plots showing TF NO_3^- concentration and fluxes for collectors that had a basswood tree within 8 m, red oak within 8 m, neither species within 8 m, nor both species within 8 m. The influence of sugar maple is visualized as a scatter plot of TF NH_4^+ concentrations against sugar maple influence.



For the study, we found that there was no significant effect of distance from the woodlot edge on the NH_4^+ concentrations ($p = 0.22$), NH_4^+ flux ($p = 0.67$), or NH_4^+ enrichment ratio ($p = 0.55$). For the NH_4^+ concentration analysis, the site ($p < 0.001$) and sugar maple abundance ($p = 0.021$, Figure 4) were the only significant effects. Sugar maple abundance was associated with higher concentrations of NH_4^+ in throughfall. Sugar maple was the most dominant and widely distributed species across all three of our woodlots, with a sugar maple tree occurring within 8 m of 66 of the 72 (92%) collectors deployed within the woodlots, with most collectors having multiple sugar maple trees within 8 m. In contrast, other tree species were much less abundant and generally occurred as scattered individuals. Overall, only 20 of 72 collectors (28%) had a basswood tree within 8 m, and only 15 of 72 (21%) had a red oak within 8 m. No other tree species had a significant effect on the NH_4^+ concentrations, flux, and enrichment ratio, nor were there any significant interactions among species. The enrichment ratio analysis for NH_4^+ was found to be significant for only one site ($p < 0.001$). There were no effects of tree species' local abundance on the NH_4^+ enrichment ratio.

2.4.3 Temporal Variability in Throughfall N

We investigated drivers of the temporal variability of the throughfall chemistry by assessing the relative impacts of rainfall intensity and antecedent dry periods. A detailed description of the rainfall patterns during the study period is presented in Table 2. Rainfall intensity had no apparent effect on NO_3^- concentration ($p = 0.69$), NO_3^- flux ($p = 0.72$), or enrichment ratio ($p = 0.963$). Similarly, there were no effects of rainfall intensity on NH_4^+ concentration ($p = 0.72$), NH_4^+ flux ($p = 0.77$), and NH_4^+ enrichment ratio ($p = 0.61$). For the antecedent dry period, there was no significant relationship found with NO_3^- concentration ($p = 0.62$), NO_3^- flux ($p = 0.77$), or enrichment ratio ($p = 0.31$). Similarly, there was no significant relationship between the length of the antecedent dry period and NH_4^+ concentration ($p = 0.56$), NH_4^+ flux ($p = 0.58$), or NH_4^+ enrichment ratio ($p = 0.59$).

2.5 Discussion

We hypothesized that N deposition to forest fragments would vary with adjacent land use and landscape context. In apparent support of this, we found that the agricultural woodlot, our site most strongly associated with row-crop agriculture and livestock operations, had the highest TF concentrations of NO_3^- and NH_4^+. However, examination of the net fluxes and enrichment ratios of NO_3^- and NH_4^+ suggests that internal characteristics of the woodlots—species composition and forest structure—may be additional drivers of TF N dynamics that are more important than the surrounding landscape context. Throughfall concentrations of NO_3^- and NH_4^+ were consistently higher at agricultural compared to the highway and mixed land use site; however, these were associated with consistently higher enrichment ratios at the agricultural site and not higher N concentrations in incoming precipitation (Figure 2) as would be expected if adjacent agricultural sources were driving this pattern. This suggests that the higher TF NO_3^- and NH_4^+ concentrations at agricultural woodlots were due to an effect of the forest canopy rather than an effect of the surrounding landscape. Individual values of enrichment ratios at agricultural sites spanned a broad range but were consistently higher than at the other woodlots. For example, enrichment ratios for NO_3^- ranged between 0.40 and 6.75 (median = 0.68) at the agricultural, 0.01 to 4.02 at highway (median = 0.15), and 0.01 to 4.00 at mixed land use (median = 0.08), indicating that the forest canopy at the agricultural consistently allowed a greater proportion of incoming NO_3^- to pass through in throughfall, and even contributed foliar NO_3^- to incoming precipitation to a greater degree than the other woodlots. Thus, differences in throughfall NO_3^- concentrations among the sites appear to be attributable to differences in rainwater interactions with the forest canopy. These interactions could include differences in foliar N uptake, foliar N leaching, dry deposition, and subsequent wash-off, or some combination of all three [Boerner, 1984; Potter et al., 1991; Lovett et al., 1996; Lohdi and Ruess, 1988).

Our model indicated that basswood abundance was positively associated, and northern red oak negatively associated, with TF NO_3^- concentrations and fluxes. Basswood was abundant at the agricultural woodlot, and red oak was absent (Table 1), suggesting that the higher TF N concentrations at agricultural woodlot may result from differences in tree species composition and abundance. This finding is likely attributable to differences in leaf chemistry, morphology, and physiology among the three most dominant tree species across our woodlots: sugar maple, basswood, and northern red oak. Basswood has twice the total leaf N concentration and 30 times higher leaf nitrate reductase activity than red oak and sugar maple (Tietema and Verstraten, 1991). Similarly, Lanning et al., (2020) documented that oaks typically have low foliar nitrate reductase activity levels. Nitrate reductase is the enzyme that catalyzes the first step in the process of NO_3^- assimilation, therefore very high leaf nitrate reductase activity in basswood indicates the presence of N in the form of NO_3^- in basswood leaves (Hasselrot and Grennfelt, 1987), which would be susceptible to leaching through contact with incoming precipitation. In contrast, low leaf nitrate reductase activity in northern red oak and sugar maple, suggests low levels of foliar NO_3^- susceptible to leaching.

In addition to higher leaf N and NO_3^- concentrations, basswood, and sugar maple also tend to have thinner leaves, with less of a waxy cuticle. Oaks, tend to have smaller crowns and waxier leaf surfaces that help shed intercepted rainfall (Doane and Horwáth, 2003). With oaks having thicker, waxier, less permeable leaves (Lanning et al., 2020), they are less likely to leach internal nutrients to incoming precipitation and less likely to collect dry deposition in dry periods between precipitation events. Therefore, these patterns suggest that high N fluxes at agricultural woodlot can be attributed to species composition rather than its surrounding land use. These findings aid in our understanding of how species composition might influence solute fluxes in the canopy by way of throughfall.

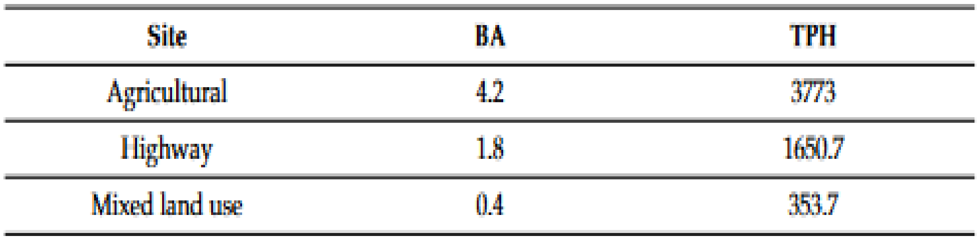
Another noteworthy difference among our woodlots was that the mixed land use consistently had the lowest concentrations of NO_3^- and NH_4^+ in TF yet had TF fluxes of NO_3^- and NH_4^+ that were equivalent to or higher than the other two woodlots (Figure 2). To have equivalent or higher fluxes with lower concentrations TF volumes must be higher. Indeed, this is what we observed with 87% of gross precipitation passing through the canopy as TF at the mixed land use, compared to 50%–60% at the agricultural and highway sites. There is unlikely to be any difference in the amount of incoming precipitation among the three sites because they are all so close together. Differences in overstory structure do not appear to explain the higher TF volumes at the mixed land use because basal area and tree density are all very similar among the woodlots (Table 1). In contrast, the understory structure and composition were markedly different at the mixed land use compared to the other two woodlots.

The mixed land use site had only 368 stems per hectare of understory trees compared to 988 and 1449 for highway and agricultural woodlots. In addition, understory trees were much smaller at mixed land use with a mean diameter of 3.4 cm compared to 4.15 cm and 5.12 cm at the agricultural and highway. Thus, it appears likely that a lack of interception by the understory leaf area at the mixed land use site explains the higher TF volumes and N flux at this site. Comparing our data on solute concentrations, fluxes, and enrichment ratios to other published studies indicates that our observations generally fall within the range of those found in the literature. In an oak–hickory forest, NO_3^- concentrations ranged from (0.10–0.25 mg L^{-1}) which overlaps well with the range of our observed values (0.14–0.26 mg L^{-1}) (Limpert and Siegert, 2019). In contrast, the oak–hickory forest in the southern United States found that TF NH_4^+ concentrations averaged (0.53.0 mg L^{-1}) depending on the species studied (Limpert and Siegert, 2019). Our study observed a range of TF NH_4^+ concentrations from 0.56 to 1 mg L^{-1}. The highest NH_4^+ values were associated with two species (*Quercus shumardii* and *Quercus stellata*), whereas NH_4^+ concentrations associated with other oak and hickory species were comparable to our results (Limpert and Siegert, 2019).

2.5.1 Edge Effect

In contrast to other studies, we observed little sign of edge effects on throughfall N in our woodlots. We observed a significant effect of distance from the woodlot edge for NO_3^- concentrations and fluxes at one of our three sites (Agricultural; Figure 3), and we found no significant effect of edge-to-interior distance on NH_4^+ in TF at any of our sites. For NO_3^- at the agricultural, the magnitude of the edge-to-interior effect was comparable to what has been observed in other studies. At the agricultural woodlot, we observed an approximately 50% decline in NO_3^- concentrations from the forest edge to the interior, and a 40% decline in TF fluxes of NO_3^-. In comparison, there was a 55% decline in fluxes of NO_3^- and NH_4^+ along a 60 m transect from the edge to the interior of a pine forest in southern Sweden (Spagenberg and Kolling, 2004). Similarly, there was a 60% decline in TF fluxes of NO_3^- along a longer 800 m transect from the edge to the interior of 6 spruce forests and 1 mixed spruce–beech in Southern Bavaria (Wuyts et al., 2008). It is unclear why we observed a distinct edge effect at the agricultural site and not at any of the other sites. Many factors can influence the penetration depth of TF deposition from the edge, including the ion under consideration (Wuyts et al., 2008; Devlaeminck et al., 2005; De Schrijver et al., 1998) meteorological conditions, such as wind speed and direction (Zhang et al., 2022; Zimmermann et al., 2008), and edge structural features, such as the leaf area index and stand density (Draaijers and Erisman, 1995; De Schrijver et al., 1998; Siegert et al., 2019). Of these factors, one that is suggested by our data is the differences in vegetation structure at the forest edge among our woodlots. Our stand inventories for the three sites indicated that while there were no significant differences in the density of overstory trees along the woodlot boundaries (i.e., trees located between 0–3 m from the edge), the density of understory vegetation along the boundary was higher at the agricultural site compared to the two other sites. To confirm the difference in vegetation structure, we looked at the basal area (BA) and trees per hectare (TPH) of the three sites (Table 3).

Table 3. Vegetation data from the tree plots per site that were located right along the woodlot's boundary.



Site	BA	TPH
Agricultural	4.2	3773
Highway	1.8	1650.7
Mixed land use	0.4	353.7

2.5.2 Temporal Variability

When evaluating the throughfall solute concentration and flux, we investigated the relationship between the antecedent dry period and rainfall intensity for NO_3^- and NH_4^+ concentrations, flux, and enrichment ratios. We found that neither the antecedent dry period nor rainfall intensity significantly impacted the throughfall chemistry at our three sites. This was surprising because many studies have found that TF concentrations of solutes are higher following longer antecedent dry periods (Levia and Frost, 2006; Zimmermann et al., 2008; Siegert et al., 2019) and/or during lower-intensity precipitation events (Levia and Frost, 2006; Levia et al., 2011). The antecedent dry periods from our study are short but are similar to those found in the literature (Siegert et al., 2019), and yet are shorter when they are compared to other studies (Zimmermann et al., 2008). One possible reason why we did not observe an effect of antecedent dry period is that the relationship between antecedent dry period (and other storm characteristics) and solute concentration and flux are easier to assess for those solutes that pass through the canopy with minimal or no stomatal uptake or other canopy exchange processes beyond wash-off (e.g., SO_4^{2-} and Na^+) (Rodrigo et al., 2003), NO_3^- and NH_4^+ are not such solutes, as their presence in throughfall reflects a series of canopy exchange processes. Another possible explanation is that we sampled a single time at the end of each storm event. Previous studies that have observed relationships between rainfall intensity and throughfall chemistry utilized intra-storm sampling to characterize these temporal patterns (Zimmermann et al., 2008; Rodrigo et al., 2003). Perhaps another reason why we did not observe temporal variability in our study is the fact that the quantity of rainfall varied between precipitation events, and we did not consider other meteorological factors (e.g., wind direction and direction, and duration). Past studies have found relationships between throughfall chemistry and the antecedent dry period, and the rainfall intensity utilized multiple intra-storm samplings of the throughfall (Levia and Frost, 2006; Zimmermann et al., 2008; Rodrigo et 2003; André et al., 2008; Zhang et al., 2019).

2.6 Conclusions

This study evaluated N deposition in fragmented forests, local tree species effects, and spatial and temporal variability in throughfall N composition. The results of this study suggest that TF N concentrations and fluxes were more strongly influenced by the internal characteristics of the woodlots, such as forest structure and species composition, than by the surrounding land use. Throughfall NO_3^- concentration and fluxes were higher under basswood and lower under red oak, whereas TF NH_4^+ was positively related to the local abundance of sugar maple. These results expand our understanding of how different tree species can affect nutrient fluxes in forest ecosystems. Stand density also impacted the spatial variability in throughfall, with NO_3^- and NH_4^+

tending to be higher in stands characterized by lower-density or absent understory vegetation, the latter of which may be attributed to deer browsing. We did not consider stemflow, which is an important factor in subcanopy nutrient flux, and variability in stemflow chemistry can be impacted by species heterogeneity. Incorporating stemflow in studies helps to investigate variability in subcanopy nutrient flux. Although we did not find a significant relationship between throughfall N composition and either antecedent dry period or rainfall intensity, future work would benefit from continued investigation into meteorological factors that impact subcanopy N fluxes. Our findings highlight the fact that even in highly human-modified environments where atmospheric deposition of N can be relatively high, stand structures have an important role in determining the subcanopy distribution of N via throughfall.

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CHAPTER 3 THE ROLE OF THROUGHFALL IN NUTRIENT LOSSES FROM TREES AND NUTRIENTS USE EFFICIENCY

3. 1 Abstract

Nutrient use efficiency is a concept that describes how plants use nutrients to fix carbon. However, decades of study on these topics have been largely focused on the exchange of nutrients from trees to the soil through litterfall; there has been almost no recognition of throughfall (TF) (e.g., precipitation that passes through the canopy and funnels down to the soil) as a component of NUE. This study is an investigation into the role of TF in driving the transfer of nutrients from trees to soil, and in determining the NUE across a range of forest stands in the Manistee National Forest in the northwestern lower peninsula of Michigan, USA. For this study, the findings of this work demonstrate nutrient losses via throughfall and litterfall both increase from low to high-fertility soils; however, the relative contribution of TF losses to nutrient total losses is greater in high-fertility soils. TF losses (g/ m^2) varied across the nutrients studied, which included K > Ca >Mg > N > P. While LF losses (g/ m^2) varied across the nutrients studied, which included Ca > N > Mg > K > P. TF and LF nutrient losses were attributed to were attributed to leaf characteristics, and high nutrient concentrations, to the wash-off of dry deposition. This work demonstrates how accounting for TF losses does decreases estimates of NUE across the fertility gradient.

3.2 Introduction

Nutrient losses from plants have been a central focus of study in the fields of plant ecology and ecosystem ecology for decades. Plant nutrient losses are central to the concept of nutrient use efficiency (NUE) which is key to understanding the adaptations of plants to soil resource availability (Vitousek 1982; Aerts and Chapin 1999). The nutrient use efficiency of perennial plants is a central concept in plant ecology that describes the efficiency by which plants utilize nutrients to fix carbon (Vitousek 1982; Aerts and Chapin 1999). Vitousek (1982) proposed that the NUE of perennial plants is best described not as the inverse of nutrient concentration, but rather is best described as organic matter lost from plants or stored within the plants per unit of nutrient lost or permanently stored.

A high NUE is advantageous in low-fertility sites (e.g., sites with low nutrients in the soil) because it allows for higher biomass production per unit of nutrient uptake (Aerts and Chapin 1999). Plant species within low fertility sites are more conservative of nutrients and limit their loss of nutrients in leaf senescence, which gives them a higher NUE (Aerts and Chapin 1999), limits the return of nutrients to the soil in litterfall, and produces litter that decomposes and releases nutrients slowly (Hobbie 1992). In contrast, plants within high fertility sites produce a litter with higher nutrient concentrations leading to lower NUE, greater nutrient returns to the soil, and faster decomposition and nutrient cycling (Hobbie 1992; Aerts and Chapin 1999).

Adaptations associated with low fertility sites such as low nutrient concentrations and high leaf defense (i.e., the protection the plants from losing nutrients) lead to a lower nutrient return to the soil and slower decomposition, which reinforces the low nutrient availability of a site (Flanagan et al., 1993; Chapin 1991; Hobbie 1992). The opposite effect can be observed in high nutrient availability sites in which species tend to have higher tissue nutrient contents and invest less in leaf defense, which leads to greater returns of nutrients to the soil and faster decomposition and nutrient mineralization (Chapin 1991; Hobbie 1992; Aerts and Chapin 1999).

Whereas virtually all the studies of NUE have focused on nutrient losses through leaf senescence, rainfall functions as a solvent as it interacts with plant canopies and can be another important mechanism by which nutrients are lost from plants and transported to the soil (Bruijnzeel,1989). Throughfall (TF) is defined as the fraction of gross precipitation that passes through the canopy en route to the soil while having contact with vegetation (Levia et al., 2019). Leaf characteristics (e.g., leaf nutrient concentration, cuticle thickness, leaf toughness) can affect the exchange of elements between trees and rainwater and thus the leaching of nutrients in throughfall. Higher leaf nutrient concentrations suggest a greater pool of nutrients in the canopy that can potentially be leachable to the soil (Tietema and Verstraten, 1991), however, thinner cuticles mean a more permeable leaf surface subsequently leading to higher leaching rates.

Leaf traits that promote greater nutrient returns in litterfall and rapid decomposition (high nutrient concentrations, high surface area, thin cuticles), should also promote greater losses of nutrients in throughfall leaching (Boener 1984; Veneklaas 1990; Aerts and Chapin 1999; Zukswert and Prescott 2017). Once nutrients are lost via litterfall they are released into the soil via decomposition (Hobbie 1992, 2015). For example, oaks (genus *Quercus*) are known for having thicker, waxier leaves (Holder 2007, 2012, 2013) which should both reduce throughfall nutrient leaching and slow decomposition and nutrient turnover. In contrast, species with high leaf nutrient concentrations and thinner more permeable leaves such as sugar maple (*Acer saccharum*) and basswood (*Tilia americana*) should promote greater return of soluble nutrients to the soil and lower NUE. Only a few studies have looked at TF losses in the context of NUE (Boerner 1984; Kost and Boerner 1985, Gray 1983). Gray (1983) reported that TF leaching contributed to nitrogen (N) leaching 5.9 % substantial nutrient losses for N in chaparral and 7.5% of N losses in coastal sage scrub ecosystems in California, USA. In their study species with tougher, lower nutrient concentration leaves had lower leaching losses of N and phosphorus (P) which contributed to higher NUE when compared to nutrients such as potassium (K) , magnesium (Mg), and calcium (Ca). Boerner (1994) and Kost and Boerner (1985) studied NUE in relation to soil fertility in several midwestern forests. They did not actually measure TF leaching in the field but found that trees with higher NUE from higher nutrient availability sites lost more nutrients in a laboratory simulation of throughfall leaching.

Several studies have shown that TF loss rates vary greatly among different macronutrients, with K fluxes typically the highest, Ca and N fluxes intermediate, and lowest for Mg and P (Kopacek et al., 2009; Moslehi et al., 2019; Andre et al., 2008; Gray 1983; Tobon et al., 2004b). Differences among nutrients in TF loss rates are likely driven by both their concentrations in leaves as well as their relative susceptibility to leaching from leaf tissues. Nitrogen occurs in the highest concentrations in leaf tissues with concentrations typically about three times higher on average compared to K and Ca, and fifteen times higher than Mg and P (Bessaad et al., 2020). However, N is not easily leached from leaves due to it being a main constituent of proteins (Marschner, 2012). Potassium (K) has a high leachability due to being the most abundant cation in cells in ionic form (K) or in weak complexes which can be exchangeable (Edwards, 1982 a, b; Schroth et al., 2001; Carnol and Bazgir, 2013; Marschner, 2012). The leachability of K is due to the weak binding to structural tissue and enzymes located in the cytoplasm (Tukey et al., 1958). Calcium is found in high concentrations in the cell wall and thus has lower mobility (Marschner, 1995) and thus does not leach easily from the plant tissue (Habashi et al., 2019). Magnesium occurs at low concentrations in leaf tissue and is relatively immobile due to its strong bonding to the cell wall and occurrence in structural tissues (Tukey 1970, Marschner 1995; Willow, 2007). Phosphorus occurs at relatively low concentrations in leaf tissues but can be leached in both organic and inorganic forms (Bol et al., 2016; Hannapel et al., 1964a, 1964b). Zhang et al., (2022) reported how P can return to the soil via litterfall and throughfall and other studies have shown that the leachability of P in the forest is low due to its low availability (Dezzeo et al., 2004; Chacon and Dezzeo, 2004; Dezzeo and Chacon, 2005).

Throughfall and litterfall losses of nutrients from plant canopies is another potentially important pathway by which nutrients are lost from plants and returned to the soil; however, this pathway has been little studied in the context of either NUE in nutrient cycling (Gray, 1984; Kost and Boerner 1985; Boerner 1984). Not accounting throughfall and litterfall leachate leads to an underestimation of NUE across the fertility gradients. Furthermore, most studies of NUE on nutrient cycling focus solely on nitrogen (N), or less commonly N and phosphorus (P), whereas other macronutrients have been largely unstudied in this context. Including other macronutrients is vital in understanding how other nutrients are lost from plants and returned to the soil.

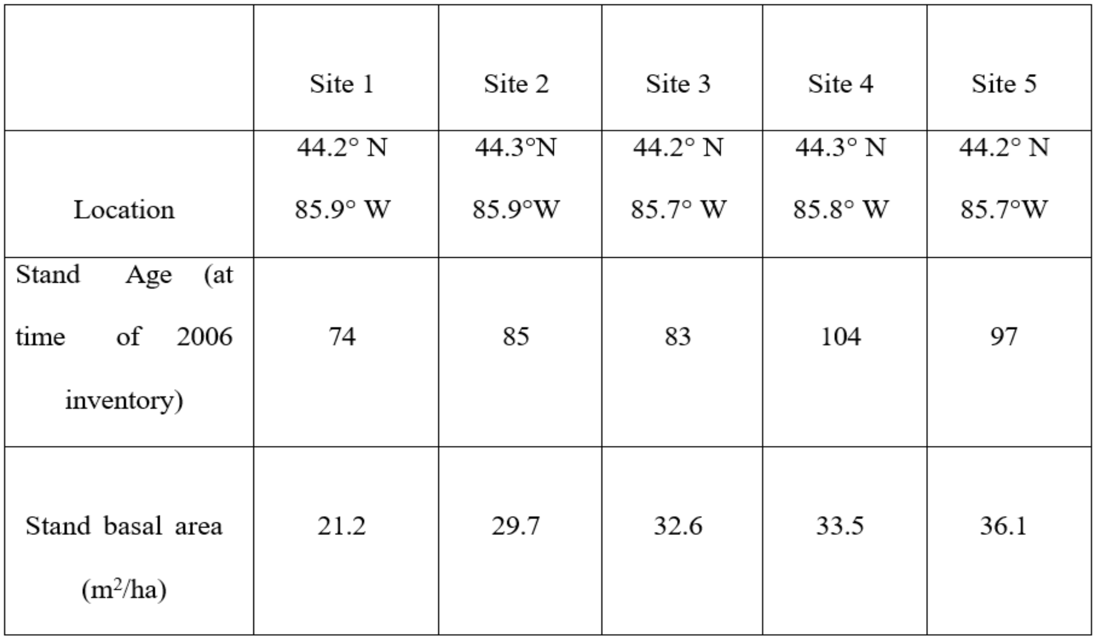
I investigated the importance of TF nutrient fluxes to NUE and nutrient returns to the soil using a naturally occurring gradient of soil fertility and tree species composition. This gradient spanned five forest ecosystems ranging from an oak (*Quercus*)-dominated forest on nutrient-poor sandy soil to a sugar maple (*Acer saccharum*) -basswood (*Tilia americana*) forest on nutrient-rich loamy soil. I used this gradient to test the following hypotheses:

1) Both litterfall and throughfall losses nutrient from the vegetation will increase across the gradient from low-fertility to high-fertility sites.
2) Throughfall losses will contribute an increasing proportion of nutrient total losses (throughfall and litterfall losses) across the gradient from low-fertility to high-fertility sites.
3) Accounting for nutrient throughfall losses will decrease estimates of NUE, and this effect will increase across the gradient from low-to-high fertility sites.
4) The significance of throughfall as a loss pathway will be greatest for K, intermediate for Ca and Mg, and lowest for N and P.

3.3 Materials and Methods

Data for this study was collected from five forest stands in the Manistee National Forest in the northwestern Lower Peninsula of Michigan, USA. The region averages roughly 810 mm of precipitation° per annum, which is evenly distributed throughout the year; the mean annual temperature is 7.2 C (Albert 1995). Rothstein (2009) established the five forest stands to span soil fertility and species composition gradient (Zak et al., 1986; Rothstein 2009; Scott and Rothstein 2017). All sites were within 32 km of each other (average distance = 14 km). The gradient ranges from low-fertility sandy soils dominated by white oak (*Quercus alba*) black oak (*Quercus velutina*) to high-fertility, loamy soils dominated by sugar maple (*Acer saccharum*) and basswood (*Tilia americana*) (Rothstein 2009). Within each site, a 100 x 40-meter study area was established with at least 100 meters from a forest edge and free of any canopy gaps or other disturbances.

Table 4. Stand and soil characteristics of the five study sites. Soil pH, carbon, and nitrogen data are for the 0-15 cm depth and soil texture for the 50-100 cm depth as reported by Rothstein (2009).



	Site 1	Site 2	Site 3	Site 4	Site 5
Location	44.2° N 85.9° W	44.3°N 85.9°W	44.2° N 85.7° W	44.3° N 85.8° W	44.2° N 85.7°W
Stand Age (at time of 2006 inventory)	74	85	83	104	97
Stand basal area (m^2/ha)	21.2	29.7	32.6	33.5	36.1

Table 4. (cont'd)

% basal area by species					
Quercus velutina	59 %	0 %	0 %	0 %	0 %
Quercus alba	40 %	19 %	0 %	0 %	0 %
Quercus rubra	0 %	63 %	53 %	15 %	0 %
Acer rubrum	0 %	19 %	0 %	0 %	0 %
Acer saccharum	0 %	0 %	46 %	40 %	66 %
Tilia americana	0 %	0 %	0 %	19 %	22 %
Fraxinus americana	0 %	0 %	0.4 %	18 %	4 %
Soil classification	Typic Udipsamment	Entic Haplorthod	Typic Haplorthod	Typic Haplorthod (Clay lamellae)	Typic Haplorthod (Clay lamellae)
Soil pH (Ca Cl2)	4.4	4.2	4.2	4.5	5.0
Silt + Clay content	2.5	5.5	5.0	5.0	8.5
Soil C:N ratio	23.3	25.3	20.6	18.6	14.5
N mineralization Rate	0.61	0.70	0.60	1.04	1.32
Nitrification rate	0.01	0.01	0.04	0.16	1.10
Fertility	Low	Low	Intermediate	High	High

All trees in each 100 x 40 m plot were inventoried for species identification, tagged, and marked with permanent paint at breast height (1.37 m). The diameter at breast height (DBH) of each tree in March of 2006, 2007, and 2008 was measured to estimate tree growth over the study period. The aboveground woody biomass (AWB) was estimated for each tree, at each sampling date, using species-specific allometric biomass equations from Ter-Mikaelian and Korzukhin (1997), and then summed across all trees in each 100 x 40 m plot. Because annual diameter growth was very small in these mature forests, I calculated an average annual woody biomass (kg m$^{-2)}$ increment across the study period using the initial (2006) DBH and final (2008) DBH measurements.

At each site, a total of five stations were selected for sampling canopy nutrient losses at using a stratified random approach. Sampling stations were located at a random point within each 20 x 40 m segment of the 100 x 40 m plot at each site. At each sample point, a single 0.37 m^2 litterfall trap was set out, and litterfall samples were collected every 2 weeks from August 15 through December 1 in 2006 and 2007. The litter from each collection date for each trap was composited. Then a subsample was pulverized in a ball mill and analyzed for N by dry-combustion on a Carlo-Erba NA 1500 elemental analyzer (Carlo-Erba, Milan, Italy) and for P, K, Ca, and Mg by nitric acid digestion followed by inductively coupled plasma-optical emission spectrometry (ICP-OES). For each nutrient, litterfall losses (in g m^2) at each sampling station were estimated as the product of the nutrient concentration of the composite litter sample and the total mass of litterfall (g/m^2) collected in each trap.

A single TF collector was installed at each sampling location. Throughfall collectors consisted of a 219 cm^2 high-density polyethylene (HDPE) funnel that was attached to a 2 L HDPE bottle. The collectors were strapped to a PVC post 1.5 m above the ground, and the collecting bottles were spray painted on the outside with silver metallic paint to block light transmission into the collector. In addition, a single TF collector was installed in an open field at 44.224 ° N, -84.819 ° W, which was centrally located relative to my five sites. In order to monitor the precipitation data from the Bear Lake Station the Enviroweather Automated Weather Station Network (55.5 mi) from Manistee National Forest (https://legacy.enviroweather.msu.edu/weather.php?stn=obl) was used and collected samples whenever cumulative precipitation exceeded 2 cm. Total TF volumes were measured with a graduated cylinder and 50-mL subsamples were collected, filtered through a 0.2 um Whatman Nuclepore Track-Etch membrane filter, and then stored frozen prior to analysis.

Samples were analyzed for concentrations of total dissolved N (TDN) by oxidative combustion-chemiluminescence (TOC/ TN analyzer, Shimadzu Corp., Kyoto, Japan) and for P, K, Ca, and Mg by ICP-OES The net exchange of nutrients in TF was calculated for each collector as the difference in nutrient concentration between the TF sample and the open precipitation collector, times of the volume of TF collected. Throughfall fluxes were expressed in units of g of nutrient per m^2 (eq 1):

TF flux: concentration in TF collector - concentration in open collector * TF volume/ funnel area (1)

To determine the total nutrient losses, the following equation was used:

Total losses: Site average TF/(Site Avg TF + Site Avg LF)* 100 (2)

3.3.1 Statistical Analysis and NUE Calculations

I analyzed patterns of total annual litterfall and TF nutrient fluxes using a two-way analysis of variance (ANOVA) with year and site as the main effects. Differences in mean values among sites, within years, and between years within sites were evaluated using Tukey’s HSD test. For this study, I calculated a Pearson correlation for each nutrient in order to determine significant correlation between annual NUE and the ANPP. All statistical analyses for this study were conducted using GraphPad Prism 9.3.1 and significance was accepted at $\alpha = 0.05$. I estimated aboveground net primary productivity (ANPP) for each site as the sum of aboveground woody biomass increment and annual litterfall mass. I then next calculated a site- level NUE for each macronutrient studied by dividing ANPP (kg m^2) by the amounts of each nutrient lost (g m^2). To assess the importance of accounting for TF nutrient losses in estimates of NUE, I calculated both a traditional estimate of NUE accounting only for nutrient losses in LF, as well as an estimate that accounts for total losses (LF + TF). I calculated a traditional metric of NUE (eq 1) using only litterfall nutrient losses (Vitousek,1982; Aerts and Chapin III 1999) and then separately calculated NUE including both litterfall and throughfall losses (eq 2) for each nutrient.

NUE = (kg AWP + kg litter production)/g nutrient lost in litterfall (2)

NUE = (kg AWP + kg litter production)/(g nutrient lost in litterfall + cumulative annual nutrient loss in (TF) (3)

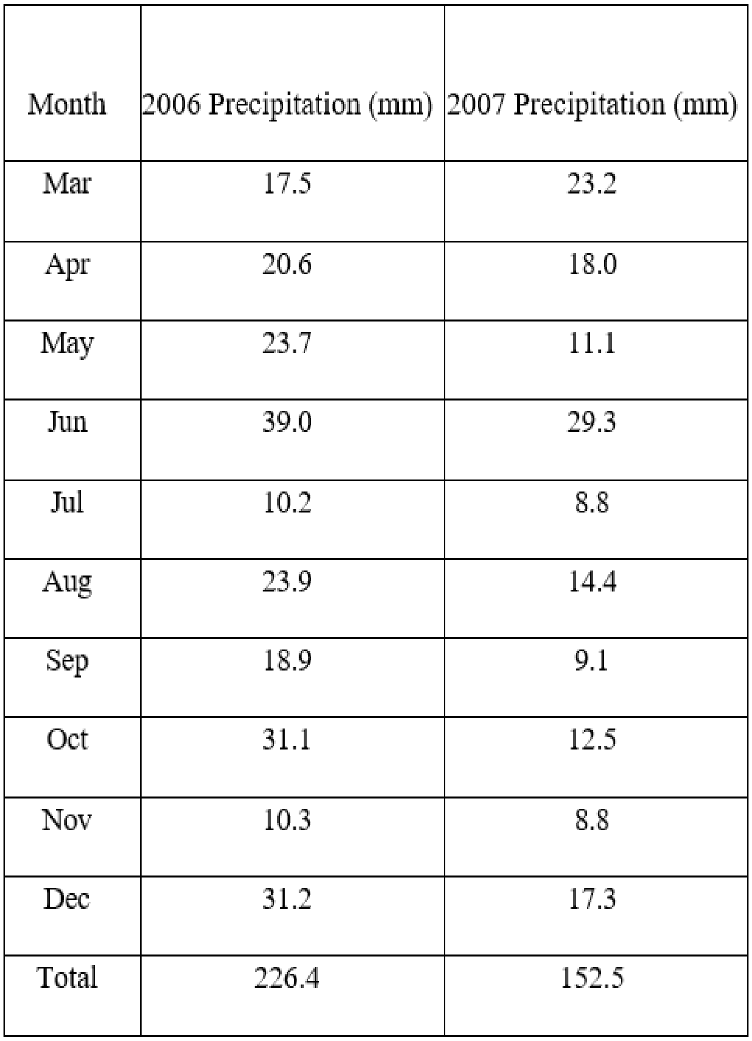
Because I only had an n=1 within each site for the aboveground woody biomass increment (single 100 x 40 m plot) we did not calculate any measure of variance or conduct statistical analyses to compare estimates of NUE among sites.

3.4 Results

3.4.1 Seasonal patterns

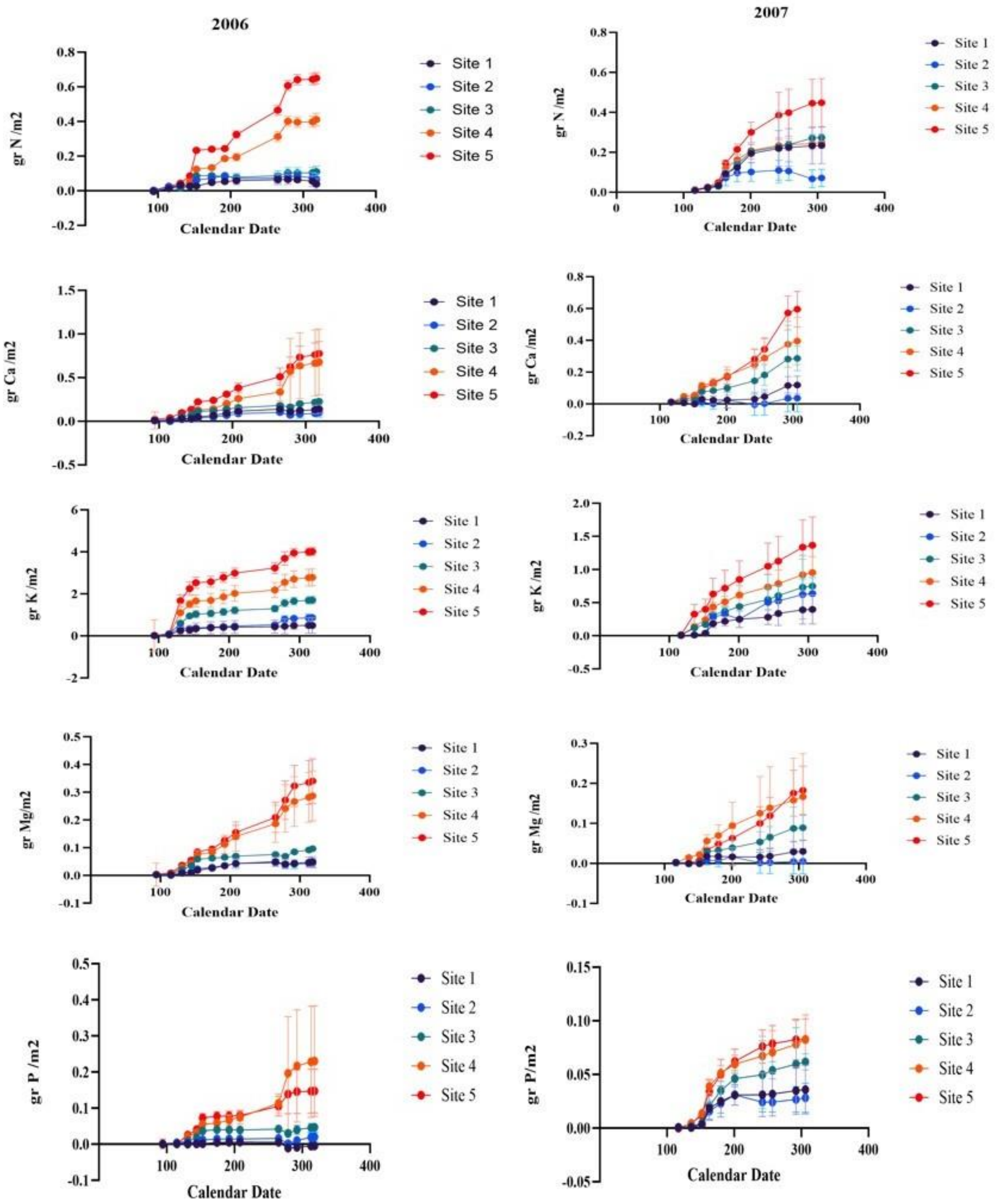
Precipitation patterns differed between the two study years. Overall, 2007 was drier with much lower precipitation than in 2006, particularly in May, June, August, September, and October (Table 5). Seasonal patterns of cumulative TF fluxes of N, Ca, K, Mg, and P across the five sites for 2006 and 2007 are shown in (Fig.5). Sites 4 and 5 consistently had the highest TF fluxes for all nutrients, whereas sites 1 and 2 were generally the lowest. Throughfall fluxes were highest for K, intermediate for N and Ca, and lowest for Mg and P. Throughfall fluxes of nutrients tended to be highest during the spring leaf out period and the autumn period of leaf senescence, with relatively low fluxes during the summer growing season.

Table 5. Precipitation patterns across the study period.



Month	2006 Precipitation (mm)	2007 Precipitation (mm)
Mar	17.5	23.2
Apr	20.6	18.0
May	23.7	11.1
Jun	39.0	29.3
Jul	10.2	8.8
Aug	23.9	14.4
Sep	18.9	9.1
Oct	31.1	12.5
Nov	10.3	8.8
Dec	31.2	17.3
Total	226.4	152.5

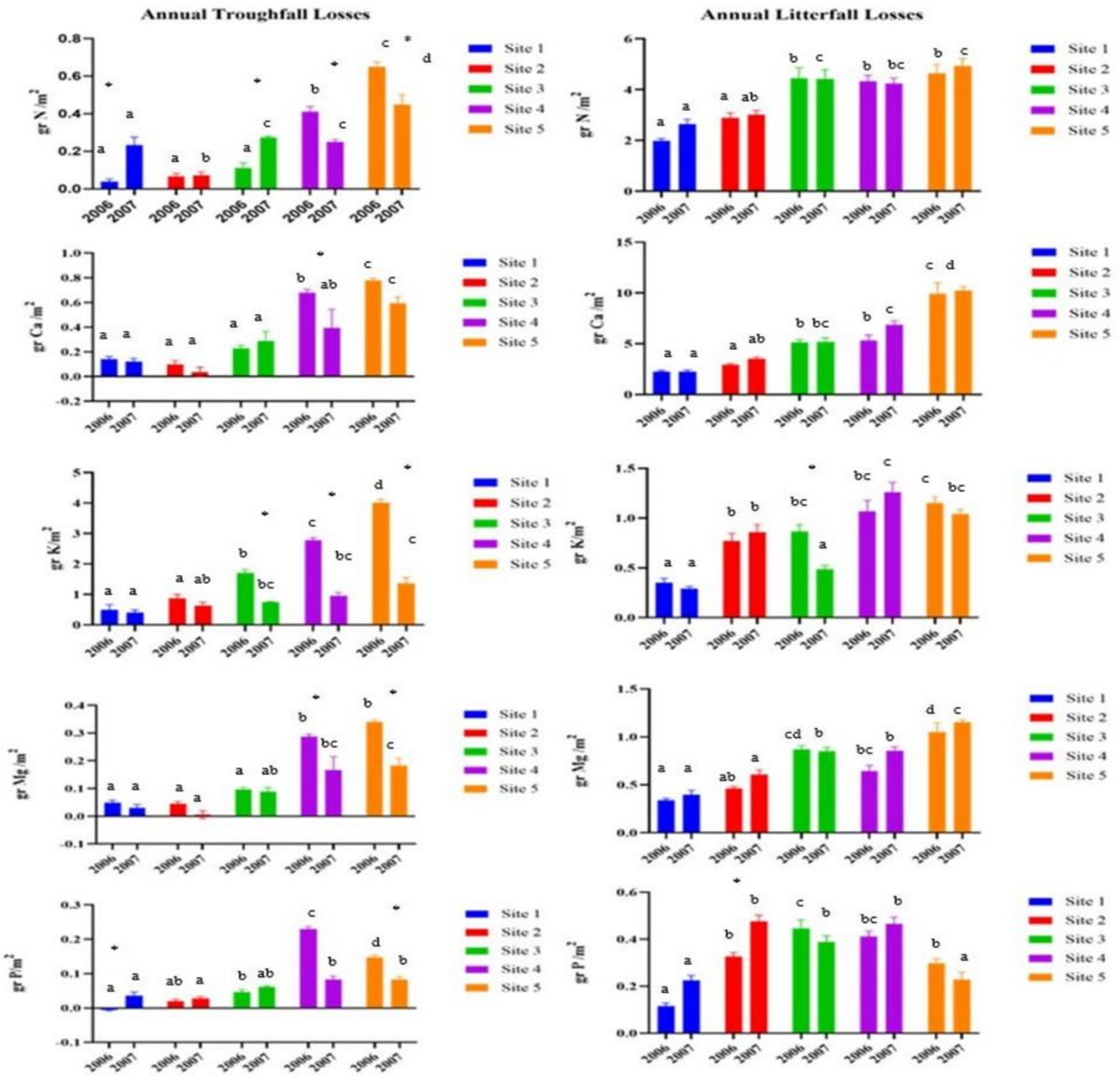
Figure 5. Trends of cumulative TF nutrient fluxes among sites and years for all nutrients. Values are means +/- 1 SE (n = 5).



3.4.2 Throughfall, Litterfall and Total nutrient losses

TF losses were highest for K, intermediate for N and Ca, and lowest for Mg and P (Figure 6). Annual TF N fluxes varied significantly among the sites (P < 0.001), though interannual variability was not significant (P= 0.9930). TF N fluxed varied significantly only for the interactive effect of site x year on TF N fluxes (P <0.001) (Fig 6). Annual TF Ca fluxes varied significantly among sites (P<0.0001), however, there was nearly a significant effect of year (P= 0.0536) and a significantly interactive effect of site x year interaction (P=0.0137). There were significant effects of site (P < 0.001) year (P < 0.001), and site x year (P < 0.001) on total annual TF K fluxes. There were significant effects of site (P <0.0001), year (P<0.0001) and site x year interaction (P = 0.0012) for total annual TF Mg fluxes. There was a significant effect of site on total annual fluxes of P (P < 0.001). The main effect of the sampling year was not significant (P = 0.116), however, there was a significant interactive effect of site x year of TF Mg fluxes (P < 0.001). Nitrogen losses in TF increased nearly threefold across the gradient each year. Within sites, TF N fluxes were significantly lower in 2007 compared to 2006 at sites 4 and 5, whereas they were significantly higher in 2007 at sites 1 and 3 (Figure 6). The Ca TF losses significant increased across the gradient each year and varied significantly between 2007 and 2006 for site 3 (P < 0.0001). Potassium losses in TF increased across the gradient in each year, however, this increase was far stronger in 2006 (8-fold increase from sites 1-5) compared to 2007 (3-fold increase from sites 1-5). The TF K losses increased across the gradient each year, and TF K fluxes were significantly lower in 2007 compared to 2006 for site 3, 4 and 5 (P < 0.0001). The increase in TF Mg losses was stronger for 2006 (3-fold increase from sites 1-5) when compared to 2007 (2-fold increase from sites 1-5). TF Mg fluxes were significantly lower in 2007 for sites 4 and 5 (P< 0.0001). Phosphorus losses increased across the gradient each year, with a stronger increase in 2006 (3-fold increase from sites 1-5) compared to 2007 (2-fold increase from sites 1-5). Within sites, TF P fluxes were significantly lower in 2007 compared to 2006 for site 5, whereas they were significantly higher in 2007 at sites 1 and 3 (P<0.0001).

Figure 6. Annual TF and LF losses across the gradient of fertility soils. Bars within a year with the same letter are not significantly different, an asterisk indicates significant differences between the years 2006 and 2007, within a site.

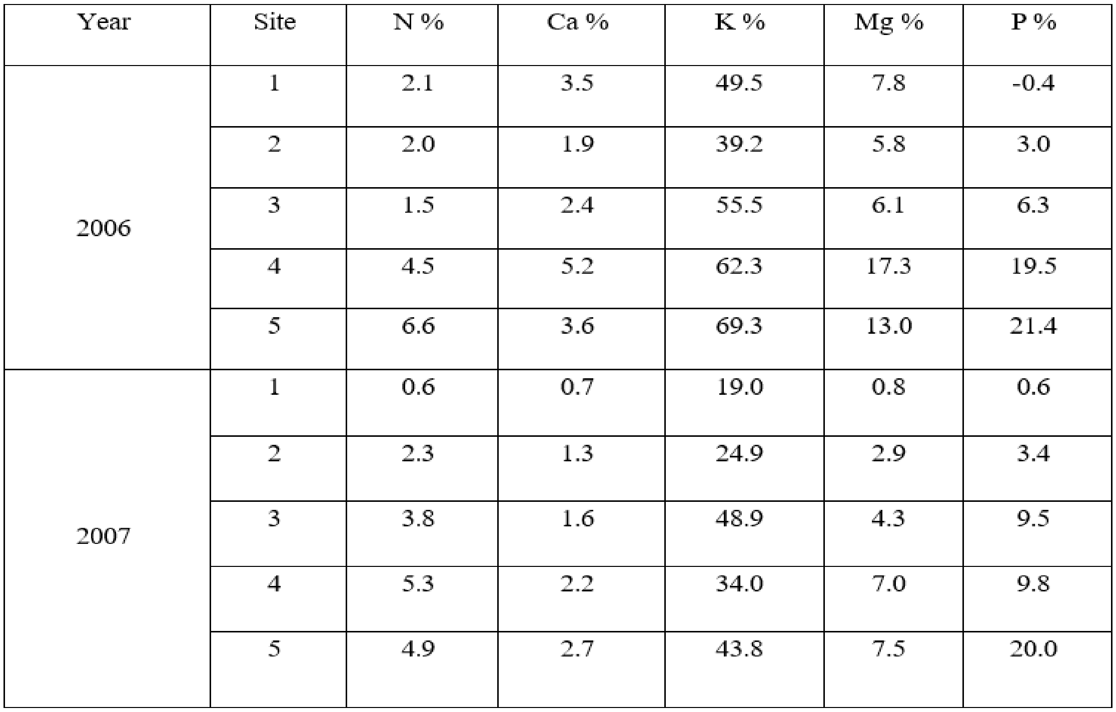


In contrast, nutrient losses in LF were highest for Ca, intermediate for N, Mg, and K, and lowest for P (Fig 6). Annual litterfall fluxes of N varied significantly among sites ($P < 0.001$), though interannual fluctuation ($P= 0.0900$) did not, however, there was a significant interactive effect of site x year on total annual LF N fluxes ($P <0.001$) (Fig 6). There was a significant effect of the site for the interannual LF fluxes of Ca (P<0.0001), however, there was not a significant effect on year (P = 0.0900) and interactive effect of site x year (P =0.4449).There was a significant effect of site on annual litterfall losses on K (P < 0.001) and an interactive effect of site x year on TF K fluxes (P < 0.001). Moreover, there was not a significant main effect for the year (P = 0.2328). There was a significant effect of the site for the annual LF Mg losses (P = 0.0023) and the main effect of year sampling (P<0.0001), however, there was not a significant interactive effect of site x year on annual LF Mg losses (P = 0.0026). For total annual LF losses for P, there was a significant effect for the main effects of the site (P = 0.001), year (P= 0.0251), and interaction (P = 0.001).

LF N losses increased threefold across the site gradient in each year, while exhibiting the strongest increase in 2007 compared to 2006. N LF losses did not exhibit significantly difference among the sites across the gradient. Ca LF losses increased across the gradient each year, with an increase stronger for 2007 (8-fold increase from sites 1-5) compared to 2006 (3-fold increase from sites 1-5). Ca LF losses did not exhibit significant difference among the sites across the gradient. For K LF losses were seen to increase threefold across the gradient for each year while having a stronger increase in 2006 compared to 2007. Within sites, TF K fluxes were significantly lower in 2007 compared to 2006 for site 5, whereas they were significantly higher in 2007 at site 3. LF Mg did not exhibit significance among the sites across the gradient. However, LF Mg was seen to increase threefold across the gradient each year, with an increase stronger for 2007 compared to 2006. P LF losses did not exhibit significantly among the sites across the gradient. Furthermore, we observed a unimodal distribution of LF P losses threefold increase across the gradient for the years, with a stronger increase in 2007 compared to 2006.

Table 6 summarizes the total nutrient losses for each macronutrient studied in this project, where nutrient loss is calculated as the sum of throughfall and litterfall losses for each nutrient. As expected, K was the macronutrient which accounted for the greatest proportion of TF losses accounting for 39-69% of total K losses in 2006 and for 19-44% of total K losses in 2007. Phosphorus and Mg were the next two nutrients for which TF losses made substantial contributions to total losses. For P, TF losses accounted for 0.5-21% of total losses across the gradient with little difference between sampling years. Throughfall losses of Mg ranged from 6-17% of total losses in 2006, but only from 1-8% in 2007. Throughfall losses of N and Ca generally made the least contribution to total losses throughout the study, generally ranging from ~1% to 6% of total losses with some variability from year to year. For all nutrients studied, the importance of TF losses to total nutrient losses increased across the gradient, particularly in the 2006 sampling year, which had higher precipitation totals. This pattern was particularly noteworthy for P, where TF losses contributed >1% to total losses at site 1 and up to >20% at site 5 in 2006.

Table 6. TF Fluxes as a % of total fluxes.

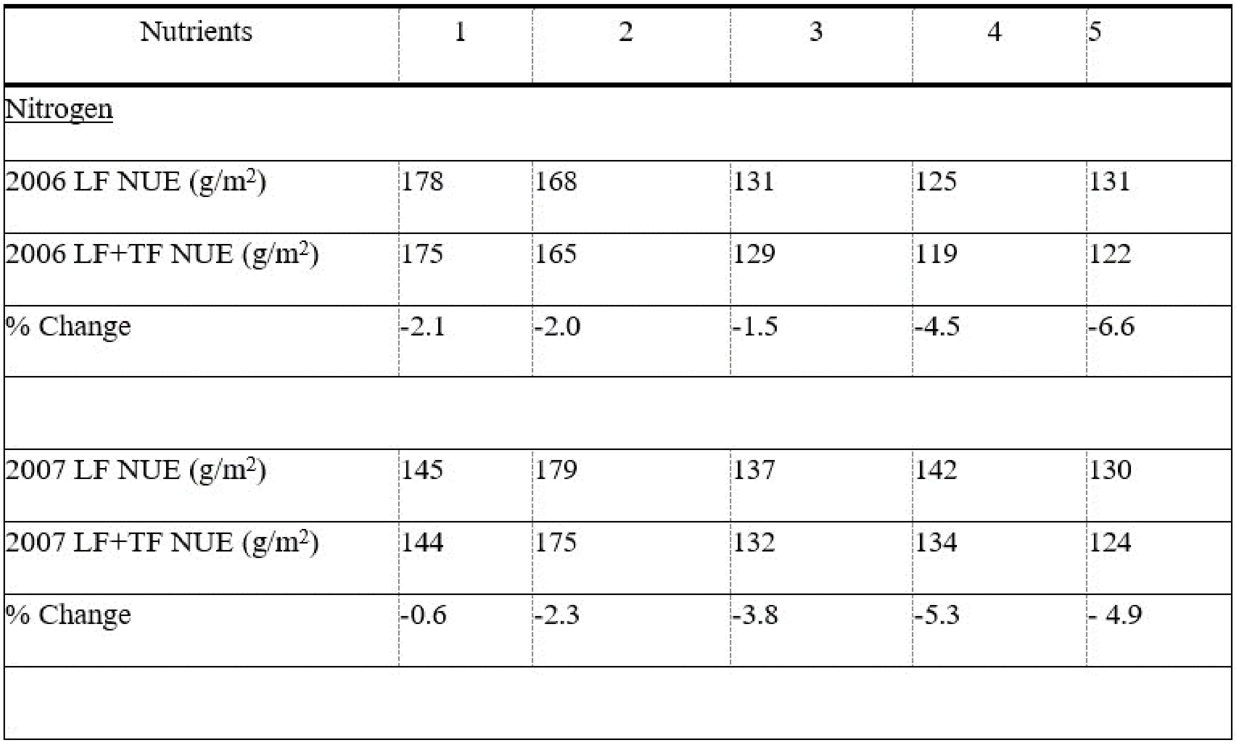


Year	Site	N %	Ca %	K %	Mg %	P %
2006	1	2.1	3.5	49.5	7.8	-0.4
	2	2.0	1.9	39.2	5.8	3.0
	3	1.5	2.4	55.5	6.1	6.3
	4	4.5	5.2	62.3	17.3	19.5
	5	6.6	3.6	69.3	13.0	21.4
2007	1	0.6	0.7	19.0	0.8	0.6
	2	2.3	1.3	24.9	2.9	3.4
	3	3.8	1.6	48.9	4.3	9.5
	4	5.3	2.2	34.0	7.0	9.8
	5	4.9	2.7	43.8	7.5	20.0

3.4.3 Estimates of Nutrient Use Efficiency (NUE)

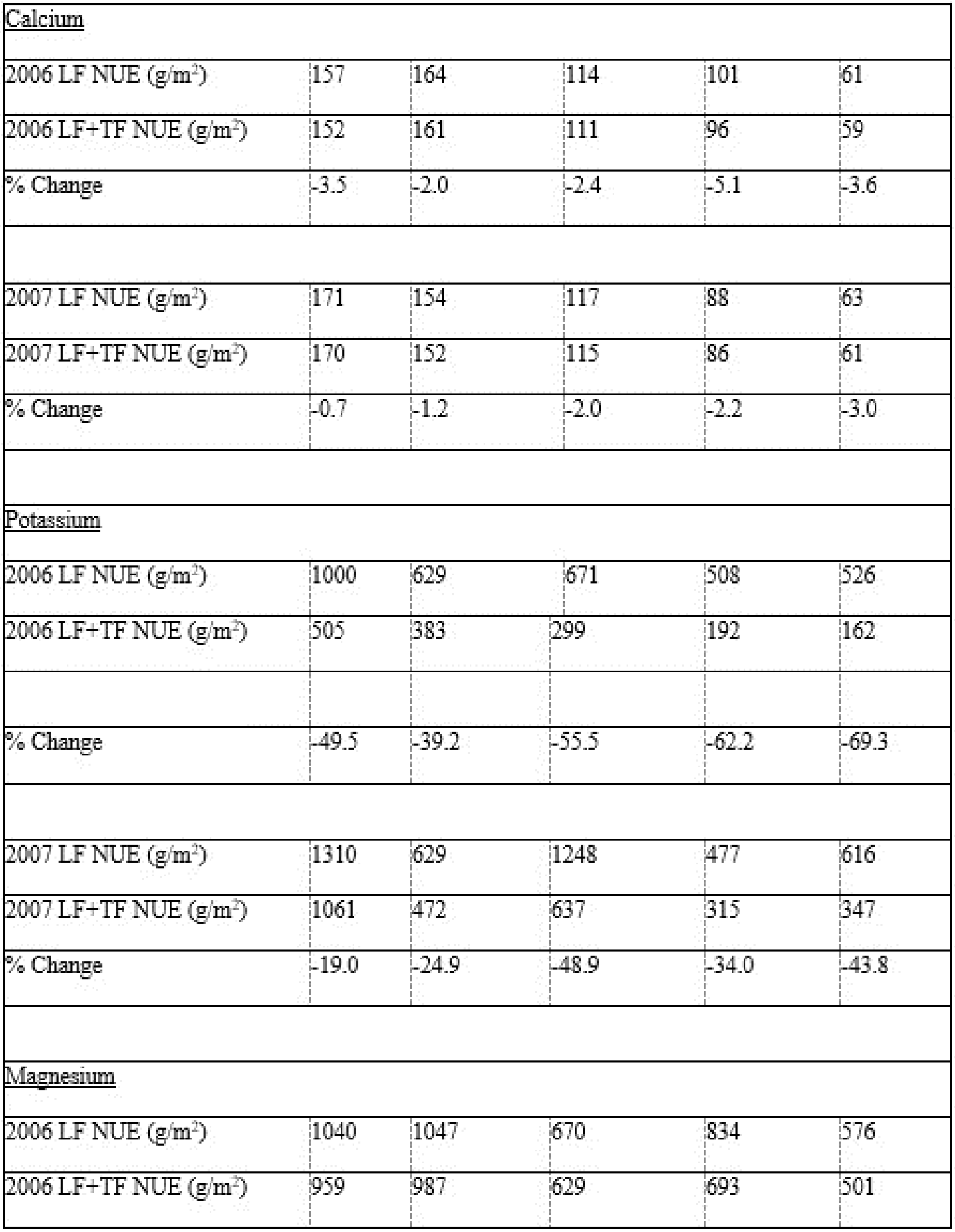
Table 7, below, presents estimates of aboveground NUE for each nutrient across all five sites. Exclusion of TF losses underestimated the actual NUE greatly for K (19-69.3 %), somewhat for Mg (0.8-13%) and P (0.3-20%), and for N (0.6-6.6%) and Ca (0.7-5.1%) makes little difference. NUE decreased with increasing nutrient availability. This relationship was predominantly stronger for mobile nutrients such as K, Mg, and P when compared to N and Ca which are nutrients that are not leached easily. NUE was highest in 2006 and lowest in 2007, with sites 3, 4, and 5. Across all the nutrients there was a reduction in NUE in the wetter of 2006 when compared to 2007. The results of the Pearson correlation comparing annual NUE and ANPP showed a strong negative correlation for Ca, Mg, and K (LF), while moderate for K (LF+TF), and lowest for P and N (Figure 7).

Table 7. Aboveground NUE for nutrients across the fertility gradient.



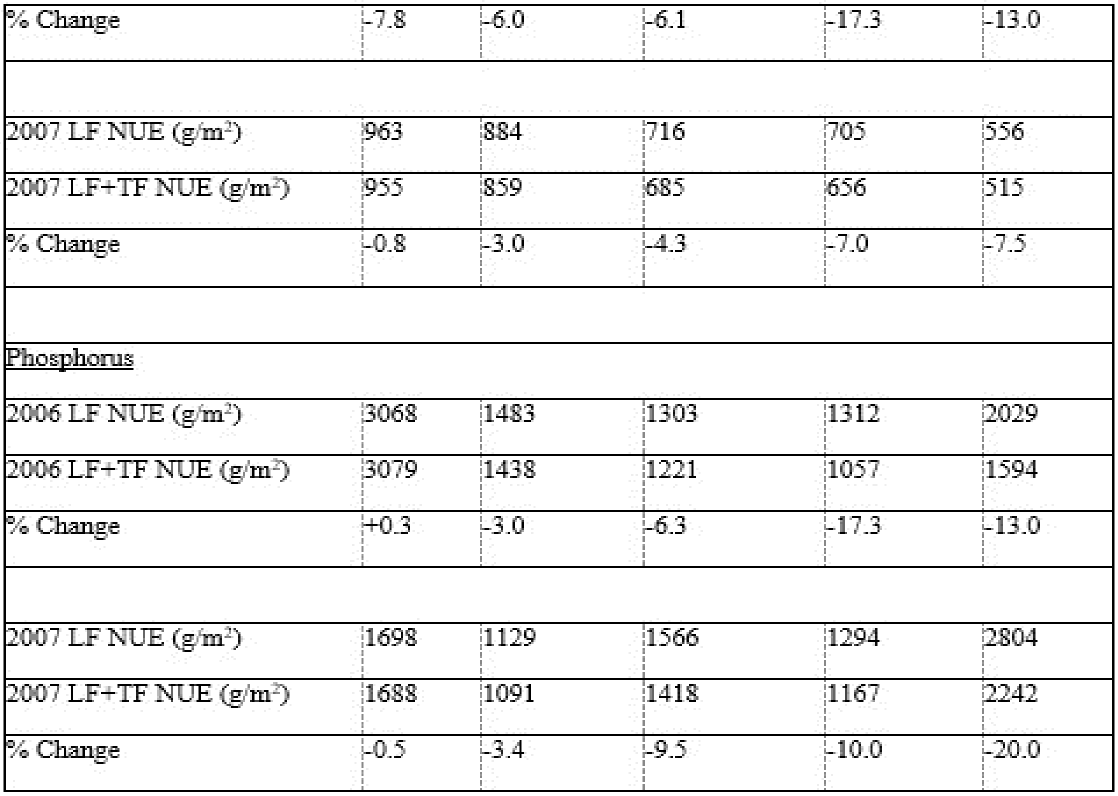
Nutrients	1	2	3	4	5
Nitrogen					
2006 LF NUE (g/m^2)	178	168	131	125	131
2006 LF+TF NUE (g/m^2)	175	165	129	119	122
% Change	-2.1	-2.0	-1.5	-4.5	-6.6
2007 LF NUE (g/m^2)	145	179	137	142	130
2007 LF+TF NUE (g/m^2)	144	175	132	134	124
% Change	-0.6	-2.3	-3.8	-5.3	- 4.9

Table 7. (cont'd)



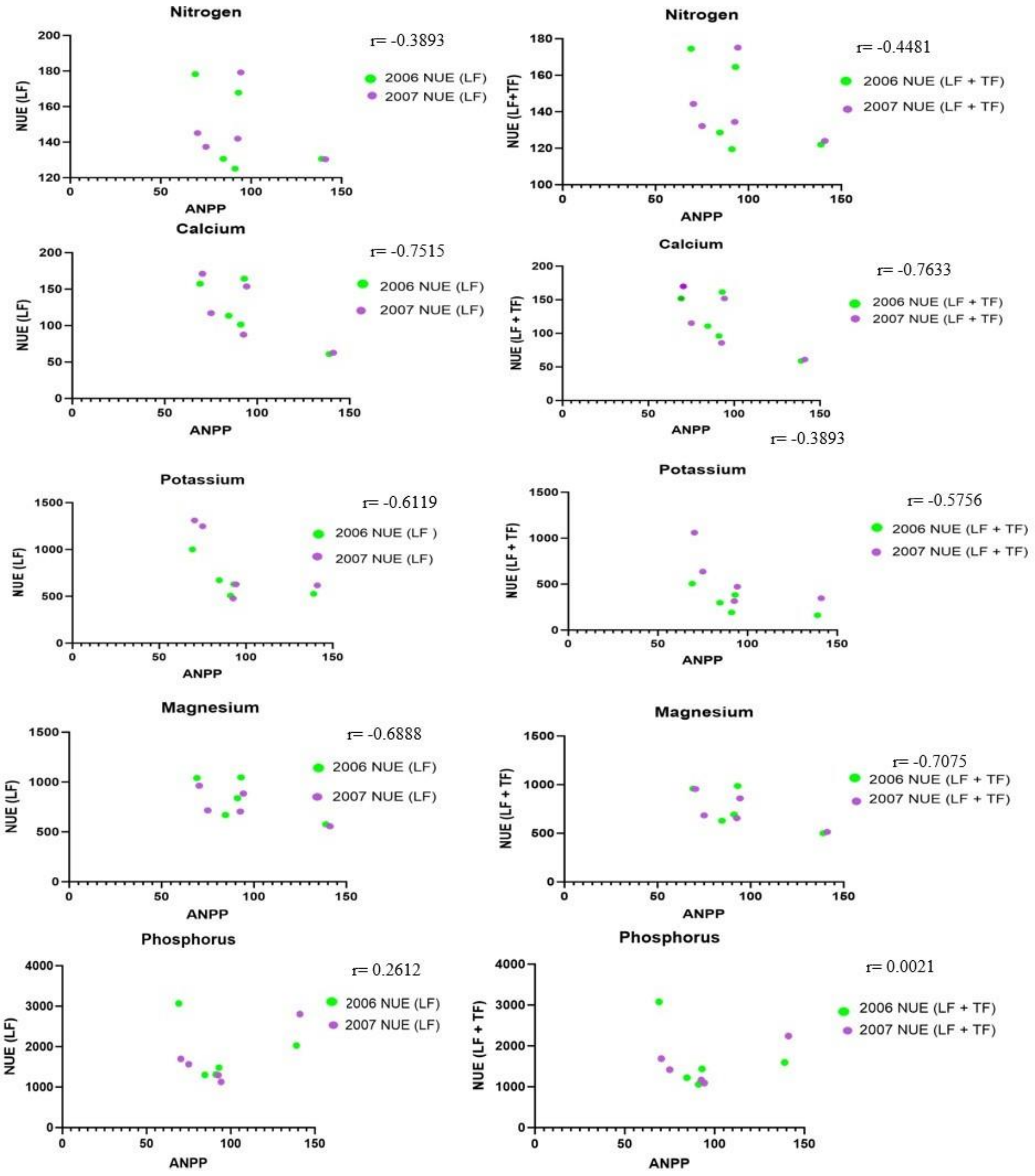
Calcium					
2006 LF NUE (g/m^2)	157	164	114	101	61
2006 LF+TF NUE (g/m^2)	152	161	111	96	59
% Change	-3.5	-2.0	-2.4	-5.1	-3.6
2007 LF NUE (g/m^2)	171	154	117	88	63
2007 LF+TF NUE (g/m^2)	170	152	115	86	61
% Change	-0.7	-1.2	-2.0	-2.2	-3.0
Potassium					
2006 LF NUE (g/m^2)	1000	629	671	508	526
2006 LF+TF NUE (g/m^2)	505	383	299	192	162
% Change	-49.5	-39.2	-55.5	-62.2	-69.3
2007 LF NUE (g/m^2)	1310	629	1248	477	616
2007 LF+TF NUE (g/m^2)	1061	472	637	315	347
% Change	-19.0	-24.9	-48.9	-34.0	-43.8
Magnesium					
2006 LF NUE (g/m^2)	1040	1047	670	834	576
2006 LF+TF NUE (g/m^2)	959	987	629	693	501

Table 7. (cont'd)



% Change	-7.8	-6.0	-6.1	-17.3	-13.0
2007 LF NUE (g/m^2)	963	884	716	705	556
2007 LF+TF NUE (g/m^2)	955	859	685	656	515
% Change	-0.8	-3.0	-4.3	-7.0	-7.5
<u>Phosphorus</u>					
2006 LF NUE (g/m^2)	3068	1483	1303	1312	2029
2006 LF+TF NUE (g/m^2)	3079	1438	1221	1057	1594
% Change	+0.3	-3.0	-6.3	-17.3	-13.0
2007 LF NUE (g/m^2)	1698	1129	1566	1294	2804
2007 LF+TF NUE (g/m^2)	1688	1091	1418	1167	2242
% Change	-0.5	-3.4	-9.5	-10.0	-20.0

Figure 7. Annual NUE and ANPP correlation for nutrient losses across the fertility gradient.



3.5 Discussion

I hypothesized that both litterfall and throughfall losses of nutrients from vegetation would increase across the gradient from low-fertility to high-fertility sites. Plants from low fertility sites have long been known to be more nutrient conservative, losing low amounts of nutrients in litterfall

(Hobbie 1992, 2015). In contrast, plants from high-fertility sites are less conservative of nutrients and lose high amounts of nutrients via litterfall (Hobbie 1992, 2015). I also observed this well-established pattern of increasing losses of nutrients in litterfall across the gradient from low to high soil fertility. In addition, I found that TF followed the same pattern with low TF losses of all nutrients at low fertility sites and increasing TF losses with increasing soil fertility across the gradient. The observed variability in throughfall losses across the gradient can be likely attributed to the variability in species composition across sites. Previous studies have found that throughfall losses can be higher for trees with thin cuticles foliar, and lower for trees with thicker cuticles (Fujinuma et al., 2005; Tietema and Verstraten, 1991; Holder 2007, 2012, 2013). By having these leaf characteristics, they are less likely to leach nutrients to incoming precipitation or from dry depositions in the dry period between rainfall events (Rivera-Cubero et al., 2023). Stand composition varied among the five sites in this study, with oak dominance at sites 1 and 2 and sugar maple and basswood dominance at sites 4 and 5 (Table 5). The parallel increase in LF and TF losses supports the idea that leaf characteristics associated with high litterfall losses also promote increased leaching of nutrients in throughfall (Boerner,1984; Kost and Boerner 1985; Gray 1983). This is likely attributed to the patterns of leaf characteristics, which change across the gradient. Higher foliar nutrient concentrations are an additional factor in addition to thin cuticles which could promote leaching losses of nutrients.

I hypothesized that the importance of throughfall as a loss pathway would be greatest for K, intermediate for Ca and Mg, and lowest for N and P. Study findings confirmed that relative throughfall losses were highest for K, intermediate for N and Ca, and lowest for Mg and P (Table 6). Potassium TF losses were greater because this element is more susceptible to canopy leaching compared to Ca (Adedeji & Gbadegesin, 2012, Staelens et al., 2006; Habashi et al., 2019). This is due to K being highly mobile and susceptibility to leaching in cell near the leaf surface in a very soluble form (Schlesinger, 1997, Tobon). On the other hand, Ca leaching is low from plants foliage due to its relative immobilization on membranes (Habashi et al., 2018). $NO3^-$ and $NH4^+$ can be either leached or retained from the forest canopy (Filoso, Williams, and Melack, 1999). Precipitation interactions with the forest canopy has been shown to be an important driver on how $NO3^-$ concentrations and can be attributed to dry deposition, wash-off or even foliar N leaching. Foliar concentrations of Mg have been found to be relatively low relative compared to other nutrients, which often results in comparably less leaching (Parker, 1983). The inputs of P are reported to be very small and its availability in the soil is in low concentrations (Tobon 1999). Phosphorus leaching from the canopy is attributed to the reduction in leaf functionality during the senescence, this results in leaves becoming more permeable.

Litterfall losses were highest for Ca, intermediate for N, Mg, and K, and lowest for P. For LF losses, increases in N, Ca, Mg, and P, across the fertility gradient were higher in 2007 compared to 2006. However, Ca LF losses were the highest compared to K due to Ca being absorbed by the leaves and exhibiting higher leaching rates. The highest concentration of Ca and Mg is due to the high concentration in the leaves due to Ca being an important constituent of the plant cell wall and Mg being bound to organic molecules (e.g, Chlorophyll) (Krammer and Kozlowski 1979, Moshlei et al., 2019). Magnesium and Ca are not easily leached since they are an important component of plants cells, they are only released once foliage drops from the canopy, becoming litterfall and decomposes over time. Since Ca and Mg is tightly bound in plant cells during the decomposition process, they can accumulate in the leaf litter and released slowly afterwards (Yavitt and Fahey 1986). Nitrogen and P are known to be cycled mainly through litterfall (Vitousek, 1982). While N is lost through other mechanisms (throughfall, stemflow), highest losses from the system are observed via litterfall. Nitrogen is found in low concentrations in living tissues and has greater resorption (Vitousek 1982; 1984; Aerts and Chapin 2000). Phosphorus and Mg are the lowest for TF and LF losses because their low concentrations in foliage are in accordance with the literature due to their net leachability being low, which can be attributed to their low availability in forest ecosystems (Duivenvoorden and Lips 1995).

In my study, I combined TF and LF losses to show the contribution of TF losses to total losses and I found that K was the highest contributor to TF losses, intermediate for P and Mg, and lowest for N and Ca (Table 7). For P I observed a little difference between sampling years and in my observation, I found that TF losses contributed >1 % to total losses at site 1 and >20% for site 5 in 2006. For N I observed that TF losses contributed > 2% to total losses at site 1, and > 5% for site 5 for 2006 and 2007. Ca TF losses contributed > 2% to total losses at site 2 and > 5 % for site 4 for 2006. For Mg, I found that TF losses contributed > 8 % to total losses in site 1 and > 13 % to total losses in sites 4 and 5 in 2006. For 2007 they contributed >1 % to total losses for site 1 and > 7 % to total losses. However, I found that K TF losses contributed to >50 % of total losses at site 1 and > 69 % of total losses for site 5 for 2006, while 2007 TF losses contributed > 20 % to total losses at site 1 and > 44% to total losses at site 5.

Across most nutrients and sites, TF losses were greater in 2006 compared to 2007. The most likely explanation for this is that the 2006 growing season experienced 33% more precipitation compared to 2007. The sampling year of 2007 was the driest and had the lowest rainfall for the months of May, June, August, September, and October. This means with more precipitation at the sites in 2006, there will be a likelihood of leaching which consequently results in more nutrient losses (Wood, Lawrence and Clark, 2005; Suescún et al., 2017). Another potential contributing factor for why TF losses were greater in 2006 than in 2007 may be due to flowering cycles and reproduction. Minor and Kobe (2017), conducted a study of flowering and reproduction in the Manistee National Forest (including at some of the same study sites) during the 2006 sampling period, and their key findings for the study were that 2006 was a peak year for flowering of sugar maples whereas 2007 was very low. In Fig 5 it is clear that the difference in nutrient losses between years in the sugar-maple-dominated sites can be largely attributed to differences associated with the spring flowering period. Reproduction requires a large investment of mineral nutrients (Minor and Kobe, 2017), which requires plants to invest more nitrogen in their reproductive parts compared to any other structure (Bazzaz et al., 1987). In contrast, the oak-dominated sites (1 and 2) tended to have the opposite pattern for N and P, with higher losses in 2007 compared to 2006. It is possible that oaks had a peak flowering year in 2007.

I hypothesized that accounting for losses of nutrients in throughfall would decrease estimates of NUE and that this effect would increase across the gradient from low-to-high fertility sites. Indeed, I observed that accounting for TF losses does decrease estimated NUE, and the degree of underestimation increased consistently across the gradient from low-to high-fertility sites. Many studies over the years (e.g., Vitousek 1982,1984; Birk and Vitousek, 1986) have demonstrated decreasing NUE based on litterfall nutrient losses alone; my findings demonstrate that accounting for TF losses amplifies the trend of decreasing NUE based on LF alone (Table 7). However, the degree to which TF losses affect estimates of NUE varied strongly among nutrients. For this work, I found that the correlation between the ANPP and the annual NUE for each macronutrient was found not to be statistically significant. In my findings the Pearson correlation comparing annual NUE and ANPP showed a strong negative correlation for Ca, Mg, and K (LF), while moderate for K (LF+TF), and lowest for P and N. These findings show how there is no relationship of annual NUE and ANPP for each nutrient across the fertility gradient.

In my study, I demonstrate that the NUE of the most mobile nutrient, K, cannot be understood without accounting for TF losses. For example, NUE for K in my study has an 8-fold increase across the gradient (Table 6). Interestingly, estimates of NUE for P and Mg were also greatly underestimated at high fertility sites by not accounting for TF losses. Although TF losses of P and Mg were relatively unimportant at oak-dominated low fertility sites, they accounted for approximately 1/5 of total losses at the high fertility sites (Table 4). In contrast, accounting for TF losses made little difference in my estimates of NUE for the immobile nutrients N and Ca. These findings were in accordance with the study of a leaching experiment conducted on a mixed oak stand and had similar results (Kost and Boerner, 1995). One important caveat to consider in this study is the possibility that wash-off of nutrients that accumulate on leaves through dry deposition could be a confounding factor in the study.

Throughfall chemical composition contains both wash-off of nutrients that have been deposited due to dry deposition as well as nutrients that are leached from plant tissues (Su et al., 2019). I have accounted for background wet deposition through the use of an open-site collector; however, it is possible that in between precipitation events, particles, and gases are deposited via dry deposition and washed off the surfaces of the leaf by the next precipitation events, inflating the estimates of TF leaching losses. In order to assess the potential for dry deposition to confound my estimates of TF leaching losses I accessed estimates of dry deposition for northern Lower Michigan for the period 2000-2015 compiled from National Atmospheric Deposition Program (NADP) data by Rothstein (2018). For K, the average annual dry deposition was 0.03 g m^2, which was one to two orders of magnitude lower than annual estimates of TF fluxes at the higher fertility sites along the gradient (2.6 g/m^2 for 2006 and 0.8 g/m^2 for 2007). The average annual dry deposition for Ca was 0.24 g/ m^2 which was lower than annual estimates of TF fluxes at the high fertility sites along the gradient (0.37 g/ m^2 for 2006 and 0.28 g/ m^2 for 2007). For N, the average annual dry deposition was 0.16 g/m^2, which is one or two orders of magnitude lower when compared with the estimates of TF fluxes at high fertility sites along the gradient (0.32 g/m^2 for 2006 and 0.25 g/m^2 for 2007). For Mg, the average annual dry deposition was 0.04 g/m^2 which was one or two magnitudes lower when compared to TF fluxes across the fertility gradient (0.13 g/m^2 for 2006 and 0.09 g/m^2 for 2007). However, the average annual dry deposition for P was 0.02 g/m^2 which was one to two orders of magnitude lower than annual estimates of TF fluxes at higher fertility sites along the gradient (0.1 g/m^2 for 2006 and 0.5 g/m^2 for 2007). This suggests that patterns of TF fluxes for K, Ca, Mg, N, and P losses across the gradient are overwhelmingly dominated by actual leaching losses and not wash-off of dry deposition.

3.6 Conclusion

This study focuses on the importance of TF fluxes on nutrient losses from trees and nutrient use efficiency (NUE) across the fertility gradient. In my study, I found a pattern in which TF losses of TF and LF losses low at low fertility sites and increase across the gradient the fertility gradient. The results of this study highlight how TF losses were greater for K, intermediate for N and Ca, and lowest for P. However, LF losses were greatest for Ca, intermediate for N, Mg, and K, and lowest for P. Accounting for total losses, I found that K was the highest contributor to TF losses, intermediate for P and Mg, and lowest for N and Ca. These patterns of TF and LF losses were attributed to leaf characteristics, and high nutrient concentrations, to the wash-off of dry deposition. Low fertility sites were dominated by oak species while high fertility sites were dominated by sugar maple and basswood. Oaks are known for their waxy and thicker cuticles, which are likely to leach nutrients from incoming precipitation compared to the tree species that were found in the high fertility sites in this study. While basswood and sugar maple are known for promoting higher leaching due to high nutrient concentrations, and thinner cuticles. In my findings, I observed that accounting for TF losses does decrease estimates of NUE, and the underestimation increases across the fertility gradient with increasing nutrient availability. The inflation of the estimates of TF leaching losses is likely attributable to precipitation and particles and gases deposited in the surfaces of the leaves via dry deposition and washed off the leaves by the next precipitation event. This study highlights the importance of canopy leaching as a pathway where plants lose nutrients. The increase in precipitation patterns due to climate change could likely increase canopy leaching, resulting in plants losing more mobile nutrients (e.g., K) leading to a change in nutrient balance in forest ecosystems.

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CHAPTER 4 THE ROLE OF INDIVIDUAL TREE SPECIES SCAVENGING AIRBORNE MICROPLASTICS FROM URBAN ENVIRONMENTS

4.1 Abstract

Microplastics are plastic particles, smaller than 5 mm and have been detected in aquatic ecosystems, soil, and air. When dispersed in the atmosphere, microplastics are a form of airborne pollutant. They have become a source of concern due to their durability and persistence in the environment and the related impacts they may have on wildlife and human health. Trees may play an important role in removing microplastics from the atmosphere, especially in urban areas. Understanding the fate and transport of microplastics in urbanized areas can help us determine the role of tree species in removing microplastics or other airborne pollutants (i.e., particulate matter) that can negatively impact the ecosystem health and function, in addition to human health. The purpose of this study is to understand the role of individual tree species in the removal of airborne microplastics in a medium-to-low-density metropolitan area in East Lansing, Michigan, USA. This work focuses on quantifying and characterizing the composition of throughfall microplastic concentration and fluxes to investigate the interspecific and temporal variability in throughfall microplastic under individual urban tree species during fully-leafed and partially-leafed periods for three deciduous tree species and one evergreen species. Throughfall samples were collected from four species: *Acer rubrum (*red maple), *Pinus strobus* (eastern white pine), *Gleditsia triacanthos* (honeylocust), and *Platanus x acerifolia* (London planetree). A fluorescence microscope was used to identify and subsequently quantify microplastics that were found in throughfall. In order to determine the morphology and origin of the observed microplastics, Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) was used. Findings from the study show that polystyrene (PS), polyethylene (PE), and fiberglass were present in throughfall samples. Throughfall microplastic concentrations and fluxes were highest under London planetree compared to other species, however, this effect was not statistically significant. This work provides

important findings on the role different tree species may play in helping to remove airborne microplastics, which may yield implications for a better understanding of the interspecific variability in air pollution removal by urban tree cover.

4.2 Introduction

Plastics are versatile, durable, and cost-efficient materials, that have been used by the private and public sector in packaging, electronics, and agricultural production (PlasticEurope, 2018). The biodegradation of plastic can take years. The progressive fragmentation driven by ultraviolet radiation and mechanical abrasion transforms the plastic debris into smaller-sized plastic particles which include microplastic (MPs), defined as plastic with diameters < 5 mm (Browne et al., 2011; Cole et al., 2011; Van Cauwenberghe et al., 2013; Wright et al., 2013; Rochman et al., 2015; Huffer et al., 2017; Zhang et al., 2017). Microplastics tend to accumulate in the environment as primary MPs (e.g., small pieces formed through the manufacturing of plastic goods, abrasives in personal care products and cleaners), or secondary MPs generated via fragmentation of large plastics (e.g., fiber from synthetic textiles or amorphous plastic particles) (Thompson et al., 2009). There has been an increasing concern about atmospheric microplastic and its effect on urban environments (Cai et al., 2017, Dris et al., 2015, 2016; Liu L. et al., 2019a, b, Wright et al., 2020). Most of the research on MPs has focused primarily on aquatic settings, especially in the marine environment (Auta et al., 2017; Rezania et al., 2018), but little is known about the impact of airborne MPs in urban areas and terrestrial ecosystems.

Microplastics are introduced to the environment through landfill, air transportation, textiles, and land-based sources (Lambert et al., 2017). Airborne MP consists, in part, of the wear and tear of synthetic textiles and synthetic rubber tires (Prata, 2018). Other sources include construction materials, roadway particles, resuspension of airborne particles (Devriese et al., 2015), and landfilling (Dris et al., 2016). The rate at which microplastics are fragmented in the environment is dependent on environmental conditions (Lamichhane et al., 2023). Studies have observed how microplastics are found in atmospheric fallout (sedimentation of particles and fine particles from the atmosphere) (Allen et al., 2019; Cai et al., 2017, Dris et al., 2015; 2016). However, our understanding of the fate, transport, and effect of microplastics is limited due to the lack of standardized sampling and identification methods (Gasperi et al., 2018; Zhang et al., 2020 a, b). Most studies of airborne microplastics focus on their horizontal transport, with wind speed and direction identified as key determining factors when considering movement and the eventual deposition of airborne microplastics. The transport of airborne microplastic includes short distances (e.g., bioturbation) and long distances (e.g., surface runoff, rainfall, and wind (Xiang et al., 2022). To this date, no studies focus on the vertical transport (atmosphere to terrestrial surface) of atmospheric MPs and their attempt researcher to reduce the pollution of airborne MPs.

The environmental impacts of MPS have been shown to impact a variety of ecosystems (e.g., aquatic, terrestrial). The main concern with these polymers is when they are broken down, plasticizers, flame retardants, bisphenol A (BPA), and antibacterial agents are released into the environment (Madeleine et al., 2018). The leaching of additive released from these polymers has a detrimental impact on flora and fauna (i.e., block the gastrointestinal (GI) track of small birds and fish). Furthermore, there have been studies in the literature that have addressed the risk of airborne MPs to public health through inhalation, ingestion, and skin contact (Chen et al., 2020; Choi et al., 2020; Fang et al., 2022; Huang et al., 2022). However, the impacts on human health will be dependent on polymer biopersistence (the ability of fibers to remain in the lung) and dosage, which ultimately can impact the severity of respiratory illnesses that may result from microplastic inhalation (Wright and Kelly, 2017). Potential effects of ingestion of MPs include inflammatory processes (e.g., intestinal inflammation), changes in the composition of the gut microbiota, and effects on the gastrointestinal system (Campanale et al., 2020). However, inhalation is one of the most dangerous routes due to the diffusion of the particles in the respiratory system (Mariano et al., 2021). Exposure to high concentrations of MPs can potentially cause lesions to the respiratory system which results in occupational diseases such as chronic bronchitis and asthma (Prata, 2018; Prata et al., 2020). Airborne MPs and particulate matter (PM) (particles of solid or liquid matter suspended in the air) (US EPA 2023) share similar properties (e.g., size, and aerodynamic characteristics) (Wright et al., 2019). Airborne MPs share similar aerodynamic properties, such as shape and size, with particles in the air smaller than 2.5 micrometers in diameter (PM 2.5) These particles can penetrate deep into the lungs and alveoli (Enyo et al., 2019b). Once these particles enter the respiratory system they can cause inflammation in the lungs, thus reducing the lung capacity (American lung association, 2022).

Trees have been found to play an important role in the fate of airborne MPs but have not been considered in previous studies on atmospheric fallout (Cai et al., 2017; Dris et al., 2015a; Allen et al., 2019). Plant leaves are reported to capture particulate matter and can act as a sink for atmospheric pollutants in urban ecosystems (Wright et al., 2019). Stand structure and foliar physical traits (e.g., surface texture, leaf area) are some examples of factors that can influence the capture efficiency of these airborne particles (Chiam et al., 2019; Clayuela et al., 2019). Trees with a higher leaf area result in a higher capture efficiency (Bi et al., 2020), while the lipophilic (I.e., ability of properties of chemical compound to dissolve oils, fats and non-polar solvents) surfaces MPs have a stronger affinity with waxy-leaf cover (Bi et al., 2020).

While there is some understanding of microplastic deposition to forest ecosystems and the structural characteristics that may impact this process, there remains a need to expand our understanding of the full array of factors that may control this deposition. This may include variability in species composition. There are limited studies on the interspecific variability of MPs captured and redistributed by trees (Ponette-Gonzalez et al., 2020). TF serves as a tool for understanding the deposition of MPs to the canopy and the composition of MPs that are deposited on the canopy. Consequently, studying MPs found in TF can offer insight into how different tree species can actively contribute to the capture and retention of these airborne particles. Looking at the interspecific variability of MPs under each individual tree species allows us to comprehend how TF MPs vary between urban tree species, and thus, how individual species may be utilized in efforts to mitigate airborne MPs in developed areas. Xiao and McPherson (2016) conducted a comprehensive study showing that the variation in the interspecific variability of trees in relation to rainfall interception can be ascribed to the unique characteristics of tree species, such as leaf morphology and surface roughness. This study helps us understand the interspecific variability of tree in the interception of rainfall as well help us look at the interspecific variability of tree in airborne MPs found in the atmosphere. Klein and Fischer (2019) recorded observations from one of the limited number of studies on throughfall measurement under a beech/oak forest. The study found a high concentration of MPs and attributed this to the comb-out method by trees which is the ability of plants to filter particles from dry deposition. Although this study looked at a beech-oak forest my study looks to integrate other urban tree species to understand the ability of trees to mitigate airborne MPs deposited via atmospheric deposition.

This work focuses on the interspecific variability in TF MPs composition and urban forests are comprised of diverse species. This study helps us determine how different tree species differ in rainfall interception and ecosystem services such as scrubbing atmospheric MPs from the atmosphere. Studying TF helps us understand how the canopy intercepts atmospheric MPs. Studies looking at atmospheric fallout only look at wet and dry deposition and have ignored throughfall, stemflow, and litterfall flux (Bi et al., 2020). Studying throughfall aids in understanding pathways of microplastic transport which could be helpful in understanding their fate and potential impact on various ecosystems, macro/micro fauna, etc. This study aims to:

1. Quantify the concentration and flux of throughfall microplastic concentration of four urban tree species.
2. Characterize throughfall microplastic composition by looking a size, shape and chemical composition.
3. Determine the interspecific variability in TF MPs composition for commonly occurring urban tree species.

4.3 Materials and Methods

4.3.1 Study Area

The study was conducted at the Spartan Village Apartment complex located at the southwest corner of the Michigan State University campus in East Lansing, Michigan, USA (42°42'57"N, °29'55"W). The mean annual rainfall for the region was 647 mm. August was the wettest month in the region (μprecipitation = 76.2 mm) and January was the month with the least precipitation (μprecipitation = 17.78 mm) in East Lansing. The study area is a medium-low density metropolitan area surrounded by the following land use: highway (west), local roads (east-south), and the East Lansing Train Station (north) at 0.4 miles (0.6 km). Located east of the apartment complex there was an active construction site.

4.3.2 Sampling Process

The study was conducted during late summer-early fall of 2021. For this study, I selected 3 replicate trees from each of four species: eastern white pine (*Pinus strobus*), London planetree (*Platanus x acerifolia*), red maple (*Acer rubrum*), and honeylocust (*Gleditsia triacanthos*). All the study trees were isolated and located in grassy areas between apartment buildings. For each trees, diameter at breast height (DBH) measurements were made, and geographic coordinates were collected. Throughfall (TF) samples were collected using 32 oz glass mason jars with stainless steel funnel (5.5 in) that were placed in the jar aperture, affixed with tape, and set up in the ground with stakes. TF collectors had a stainless-steel strainer to prevent organic material from getting into the samples. Collectors were set up in the sampling sites before a storm event. Glass collectors with a stainless-steel funnel were used in accordance with guidance from the literature, as throughfall collectors comprised of these materials would prevent plastic contamination (Fan et al., 2022, Dris et al., 2017, Cai et al., 2017). After the sample was collected, each throughfall collector was then rinsed and cleaned with deionized water to minimize the risk of contamination for the next rain event. For each tree, I used two TF collectors, one within 30 cm of the trunk of the tree and the other located under the dripline, or the outer circumference of the tree crown. To assess MPs composition of open precipitation (i.e., that which was not influenced by the presence of any trees), one open precipitation gauge was set up in an open field adjacent to the research site. For both the open collector and throughfall collectors, sample collection occurred on an event basis, with sampling taking place within 48 hours of rain events producing > 1 cm of precipitation. Storm precipitation totals were determined via the Michigan State University Enviroweather network (https://enviroweather.msu.edu/), utilizing data from the network weather station that is closest to the research site (i.e., MSUHort weather station which, is located approximately 6.4 km from the study site). At each collection date, a 25-mL subsample was collected in clean vials for later laboratory analysis, and the volume of the remaining water was measured with a volumetric cylinder, with volumetric measurements used to determine the total throughfall depth. Two sets of samples were collected over the study period. The first was collected the onset of autumn (September 24, 2021) when leaf drop was minimal among the study trees. The second set of samples was collected in late fall (November 1, 2021) once most of the leaves had dropped from the deciduous trees included in the study.

4.3.3 Laboratory analysis

For this study, I followed the methodological guidelines from Klein and Fischer, (2019). In the analysis of the samples, I considered 4 open collector samples (3 replicates from fully-leafed and 1 from partially-leafed) for the study sites and 4 laboratory blanks. The laboratory blank consisted of 25 ml of DI water and offered information on the background contamination of microplastic particles from my laboratory processes. Deionized water for the laboratory blanks was obtained from a GenPure Water Purification System which delivers 18.2 megohm per cm (used to classify water with fewer ions), which is categorized as Type 1 or ultrapure water. The collected samples were stored at 4 °C temperature after the collection. To further mitigate any risk of microplastic contamination of the samples, all lab-based work was conducted while wearing laboratory coats made from 100% cotton. Additionally, latex gloves were always worn to eliminate the risk of indoor particles in the samples.

To remove organic matter, samples were first treated with sodium hypochlorite solution ($NaClO$, 6-14%) in a volume ratio of 0.15:1 and left to stand for 24 hours (Tamminga et al., 2017; Enders et al., 2016; Collard et al., 2015). Sodium hypochlorite is an oxidizing agent which results in a high organic matter removal when compared to other digestion methods (Monteiro et al., 2022). Pfeiffer and Fischer (2020) showed that NaClO is able to remove efficiently vegetal material (e.g., leaves and wood). The samples were then filtered using a glass vacuum filtration unit with a 25 mm cellulose filter (Catalog number 09-719-2A, particle retention 0.2 um). The glass material used in the process of filtration such as beakers and glass vacuum filtration devices were rinsed with DI water to remove any possible adhering microplastic particles found indoors. The filter was transferred to a glass petri dish covered with aluminum foil and left to dry for 24 h at room temperature. Filters were then stained with the lipophilic dye Nile Red. Nile Red was mixed with Chloroform in a ratio of 1 mg/ ml. Each filter was dyed with 1 ml (two times 0.5 ml to ensure a thorough distribution) of Nile Red solution (Tamminga et al., 2017, 2022; Fischer et al., 2016). To complete the staining process, the filters were then covered with watch glasses and left to dry at room temperature under a fume hood.

4.3.4 Fluorescence microscopy

For the study, I selected a total of 39 subsamples of a mix of dripline and trunk adjacent samples processed under the fluorescence microscope for investigation. Of those 39 samples, four of them were from bulk precipitation and 4 procedural blanks. I analyzed two sample dates for my analysis for this study (09/24/22 and 11/02/2022). Dried filter samples were analyzed using conventional fluorescence images which were captured on a Nikon Eclipse NiU upright microscope using a 10x Plan Fluor objective (NA 0.30) at the Center for Advanced Microscopy located at Michigan State University. Fluorescence is a useful technique to identify polymers based on their ability to emit fluorescence (Noren, 2008), this reduces failure to identify MPs and can lower the size limit of the detected MPs (Mariano et al., 2021). For the microplastics study, I used the Nikon Texas Red/mCherry/Alexa Fluor 594 filter set which uses a 560/40nm band pass filter for fluorescence excitation and a 635/60nm band pass filter for fluorescence emission. Images were recorded using a Nikon DS Fi-2 color camera and Nikon NIS Elements software (version 5.41.02). For comparison, all filters from the sampling dates and blanks were photographed at 5 locations of the filter, with each of the corresponding 5 micrographs subsequently analyzed for microplastic detection, classification, and quantification. Before determining the abundance of microplastics I used Adobe Photoshop CS6 to count the particles and classify them into shapes such as fiber, fragment, spheres, and foam. I used yellow-red fluorescence for the detection of stained polymers as suggested by Shim et al. (2016). The MPs were then grouped according to their shape into fragments, spheres, fibers, and foam (Fig 8). Sphere-shaped particles are classified as primary microplastics due to being directly emitted into the atmosphere. This includes plastic pellets personal care products, clothing, plastic pelleted and synthetic textiles, and vehicle tire wear (An et al., 2020, Andrady 2011). However, fragments, foams, and fiber are products of the breakdown of large plastic and are classified as secondary microplastics. This includes plastic bags and bottles, fish nets, and large plastic (An et al., 2020; Thompson et al., 2009).

Once the particles were identified by shape, I then proceeded to measure them using ImageJ software and classified them in size ranges. To determine the abundance of MPs in the TF samples, I calculated the particle concentrations for each image location (5 per filter) by dividing the total particles by the area of the image (4.6 x 10-2 cm$^{2)}$. I then multiplied the average particle concentrations of all five filter locations times the total area of the filter (4.9 cm^{2}) in order to estimate the total number of particles captured by the filter.

Total number of particles = average of particle concentration/area of the filter (1)

I then calculated the MP particle concentration in TF samples by dividing the total particles of the filter by the volume of each subsample and then corrected for my laboratory blanks. The average MP particle concentration for my laboratory blanks was 49.6 particles per ml (5 %).

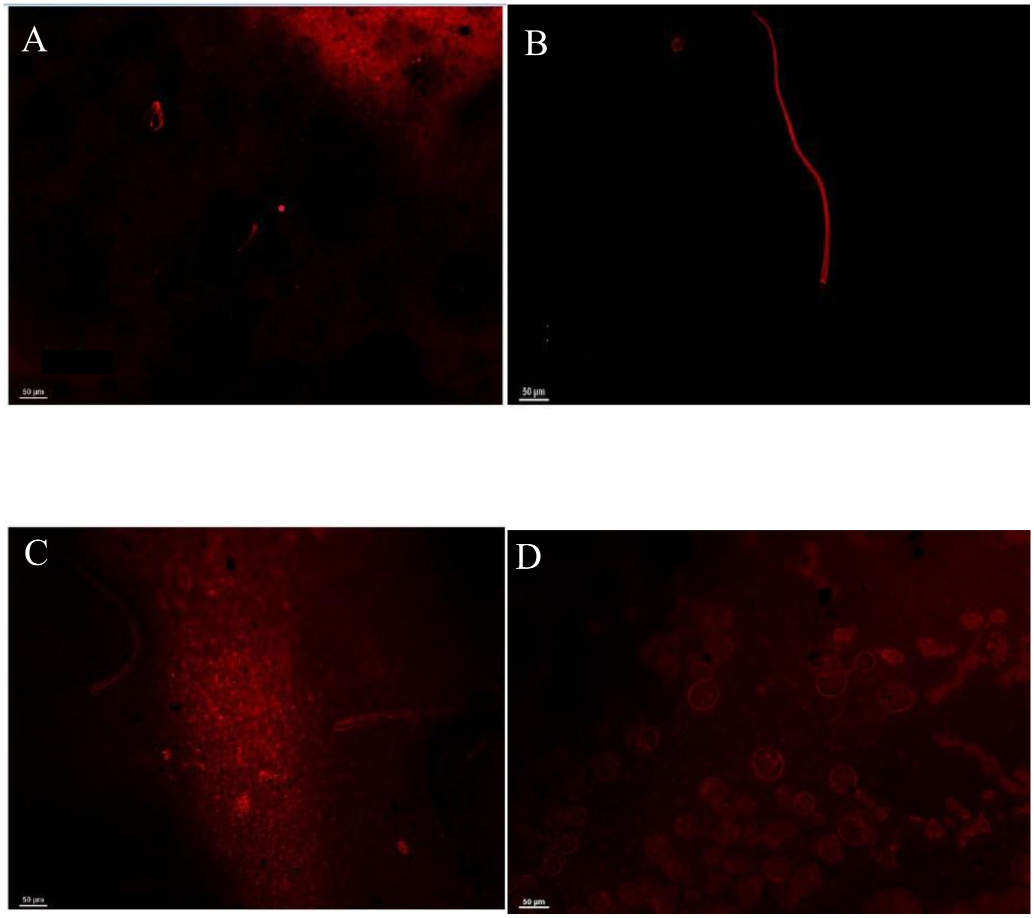
MPs particle concentration = Total particles / TF subsamples – avg MPs concentration of Blanks (2)

After the particle concentration in TF samples was obtained, I then calculated the total particles per collector by multiplying the particle density in the TF sample by the volume recorded from the volumetric cylinder. After obtaining this measurement, I then proceeded to calculate the TF flux by dividing the total particles per collector by the area of the funnel to obtain the number of particles that were entering the TF collector as # the of MP particles ^{m2}.

TF flux = total particles per collector/ area of the funnel (3)

Ideally, I would like to have a measurement of MP mass concentration and flux; however, this was not feasible in our study. As a proxy for MP mass, I measured the surface area (2D) of each particle in the TF samples. The surface area for each particle was determined by ImageJ. This should scale with mass and allow me to account for the size of particles. Once the surface area was determined, I then summed the area of all particles present in the samples and proceeded to calculate the surface-area based concentrations and fluxes of MPs in TF as described above for particle counts. The final measurement for the concentration was mm^2 per mL and for the flux analysis, the final measurement was mm^2 per m^2.

Figure 8. Fluorescence micrographs from TF samples (A) Fragments, spheres, foam (B) fibers, (C)fragments, fibers and spheres, (D) Spheres.



4.3.5 Scanning electron microscopy (SEM)

I further analyzed a subset of MP particles by SEM to determine the morphology and likely origins of the examined samples. The SEM provides high-resolution data of surface state and qualitative information about the chemical composition when combined with the energy-dispersive X-ray spectrometer (EDS). SEM-EDX is considered an expensive technique that requires substantial time and effort for sample preparation. After the abundance was calculated I then proceeded to select samples which exhibited the highest MPs flux in the TF collectors. This selection would allow us to increase the capacity of microplastic characterization in the TF samples. For this analysis, I selected a total of 10 filters that were taken to the advanced microscopy center: two subsamples of the filter for the 4 tree species for a total of 8, 1 open collector, and 1 blank. A piece of the filter was cut, and a gold grid/ nickel layer was then added on top of each piece of the filter for a total of 10 pieces by sample. Using a thin conducted layer of gold (Au)was used to obtain high-quality images which were imaged at 100x-2000x. The samples were viewed with a voltage of 15 kV. The SEM-EDX provided a high-resolution imaging of particle surface as well as elemental composition signatures. This information is important for screening MPs and ruling out non-plastics (Furfaro et al., 2022). The MPs present show degradation and abrasion signs, due to the exposure to the environment by the weathering processes. SEM/EDX analysis provided high-resolution pictures of the particle surface of the fibers, fragments, and spheres, as well as their elemental composition signatures. The literature has reported that the most common kind of plastics is Polypropylene (PP) and Polyethylene (PE), which shows a strong Carbon EDX peak (Wang et al., 2017).

4.3.6 Statistical Analysis

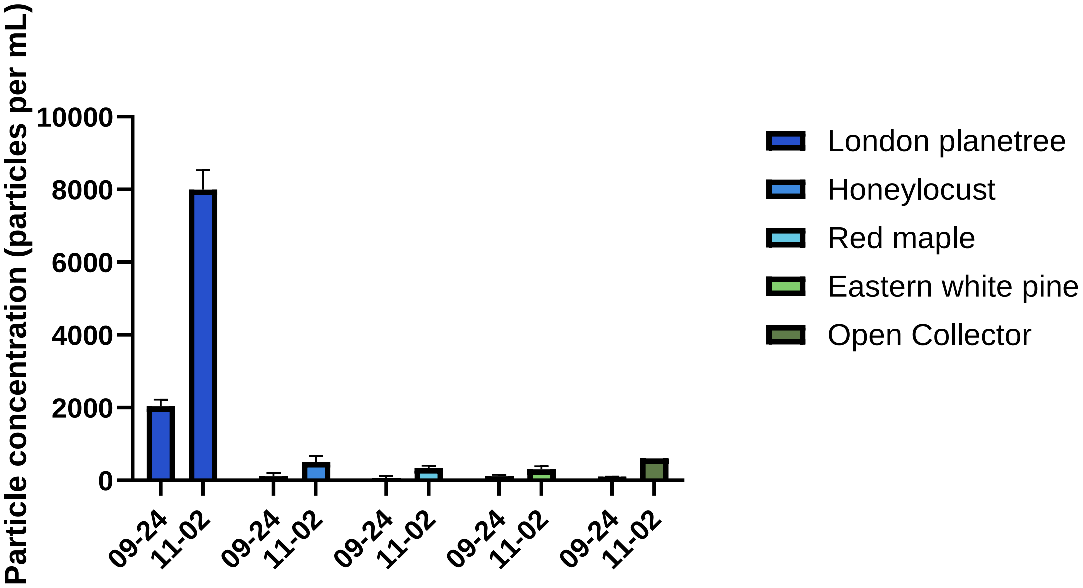
I conducted a mixed effect model and a model with a correlated error structure using the geospatial data (latitude and longitude) for MPs concentration and fluxes. For the model I treated species, tree ID, and dates as fixed random effect. The following terms were treated as fixed effects: species, location, date, species: location, species: date, location: date, species: location: date. The following packages were used for running the linear model for random effect: lme4, nlme, emmeans and ggpairs function for the graphs with GGally and ggplot2. The analysis was conducted using R-4.3.1 and significance was evaluated at α =0.05.

4.4 Results

4.4.1 MPs abundance in TF collectors

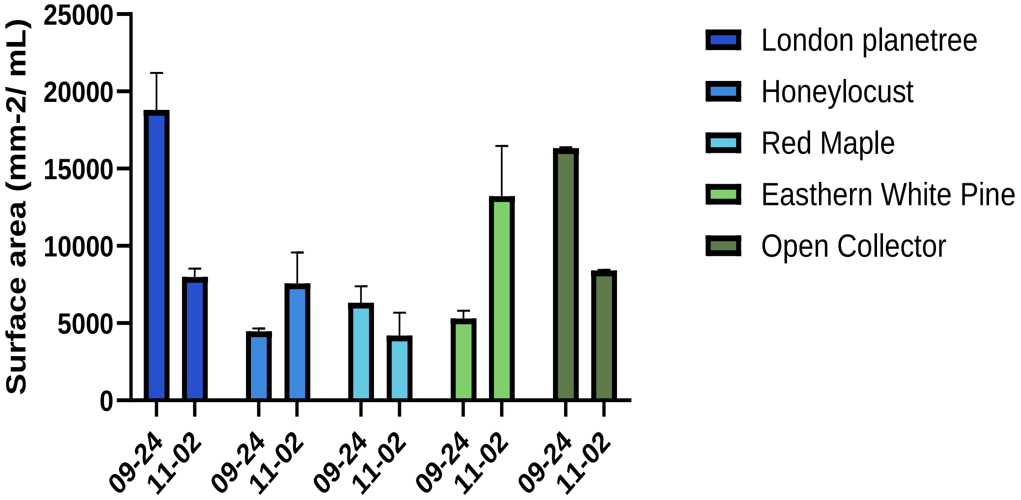
London planetree exhibited the highest MPs concentration compared to other tree species and the open collector (Fig. 9). Throughfall samples from underneath London planetree had average MP concentrations that were 3 times greater than the other three species during the fully leafed period (Figure 2). However, when accounting for spatial location there was not a significant effect of species (P=0.7584) on MPs concentration. For the effect of spatial location alone was not (P=0.5547); however, there was trend towards a species: location interaction (P=0.1134). Finally, neither sampling date (P=0.8405) location: date (P=0.7604), species: location: date (P=0.1439) effects were statistically significant.

Figure 9. Microplastic particle concentration in the throughfall solutions.



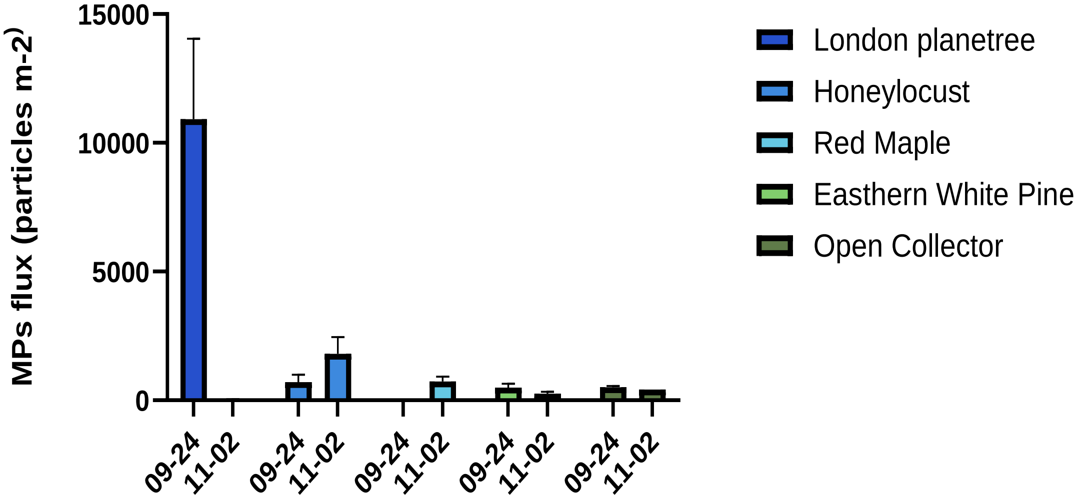
Microplastic surface area concentrations were also highest for London planetree during the fully- leafed period (Figure 10). Eastern white pine exhibited the highest surface area when compared to red maple (P < 0.0001) on the fully-leafed period. However, Eastern white pine had the highest MPs surface area when compared to honeylocust (P = 0.0095) during the partially-leafed period. When comparing the two-sampling period I found in my observations that the MPs surface area was highest during the partially-leafed when compared to the fully-leafed period for the Eastern white pine.

Figure 10. Surface area of microplastic concentrations in TF samples.



Throughfall samples from underneath London planetree had average MP fluxes that were 1000 times greater than the other three species during the fully-leafed period (Fig. 11). However, when accounting for spatial location there was not a significant effect of species (P=0.4789) on MPs concentration. There was a strong trend towards an effect of spatial location (P=0.0716); however, it did not reach the 0.05 threshold for statistical significance. When accounting for spatial location there was not a significant effect of location and date (P=0.7558) on MPs concentration. I also found that there were not significant effects of date (P=0.7558), species: location (P=0.6972), species: date (P=0.3258), location: date (P=0.2038), or species: location: date (P=0.3660).

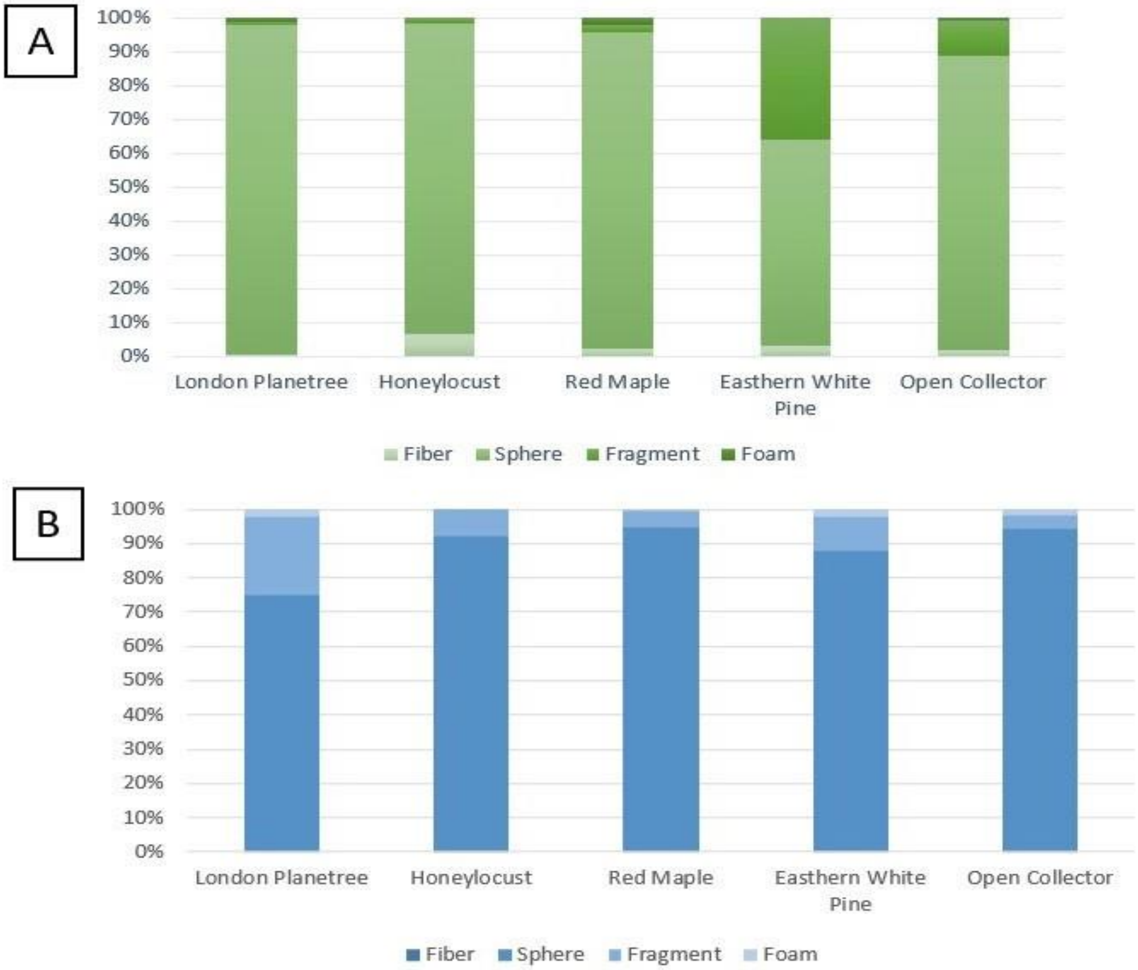
Figure 11. Microplastic flux in the throughfall collectors. Zeros in the analysis result in no MPs particles for London planetree (11/02) and for red maple (9/24).



4.4.2 Microplastic shape and size

In my observations, I found that the abundance of different MP forms followed the pattern of spheres > fragments > foam> fibers (Figure 5). In my observation, I found that the fully-leafed period exhibited the highest total of particles with 1162 total particles when compared to the light canopy period which exhibited a total of 1018 particles. The results show how spheres were the most abundant shape under London planetree > red maple > honeylocust > open collector > eastern white pine for the fully-leafed period (Figure 12). However, the fragment is the most abundant under eastern white pine > open collector > London planetree > red maple > honeylocust. Foams were shown to be the highest under London planetree > red maple and open collector, however, they were not present for honeylocust and eastern white pine. On the other hand, fibers were more abundant under honeylocust than London planetree, and equal for red maple, eastern white pine, and open collector. For the fully-leafed period, spheres were the most abundant shape under honeylocust > easter white pine > red maple > open collector > London planetree. Fragments were the most abundant under honeylocust > easter white pine > london planetree > red maple > open collector. Foam was abundant under an eastern white pine, equal for the open collector, London planetree, and red maple, and not present under honeylocust. Fibers were only present under honeylocust for the partially leafed period.

Figure 12. Distribution of particles by shape (i.e., fiber, sphere, fragment, and foam) (A) fully-leafed period (B) partially-leafed period.



4.4.3 SEM-EDX Analysis

The results of the spectra in our study were then compared with other published studies to establish a reference. The obtained spectra show carbon (C) peak and other element characteristics of plastic polymers. I classified microplastic particles as Polyethylene (PE), Polystyrene (PS), and Fiberglass based on EDX spectra as described by Furfarro et al. (2022), Kwak and An (2021) and Hwang et al. (2020). Particles were classed as fiberglass if they had strong peaks of C, O, and Si. Particles with strong C and O peaks, but no Si peak could be either PS or PE (Figure 13). To distinguish PS from PE I observed other EDX analyses and observed different Carbon and Oxygen magnitude peaks between PS and PE. High Carbon peaks (3,000 counts) could be observed on PE EDX compared to PS EDX less than 2000 counts.

Figure 13. SEM images and EDX analysis results for (A-B) fiberglass fiber and fragments showing the silicon peak and Carbon, Oxygen, and several other minor elements. (C) Primary MPs, spheres from a PS show strong Carbon and Oxygen peaks and several other minor elements. (D) Secondary MPs, Fiber showing weathering surfaces from a PE showing strong Carbon and Oxygen peaks and several minor elements.

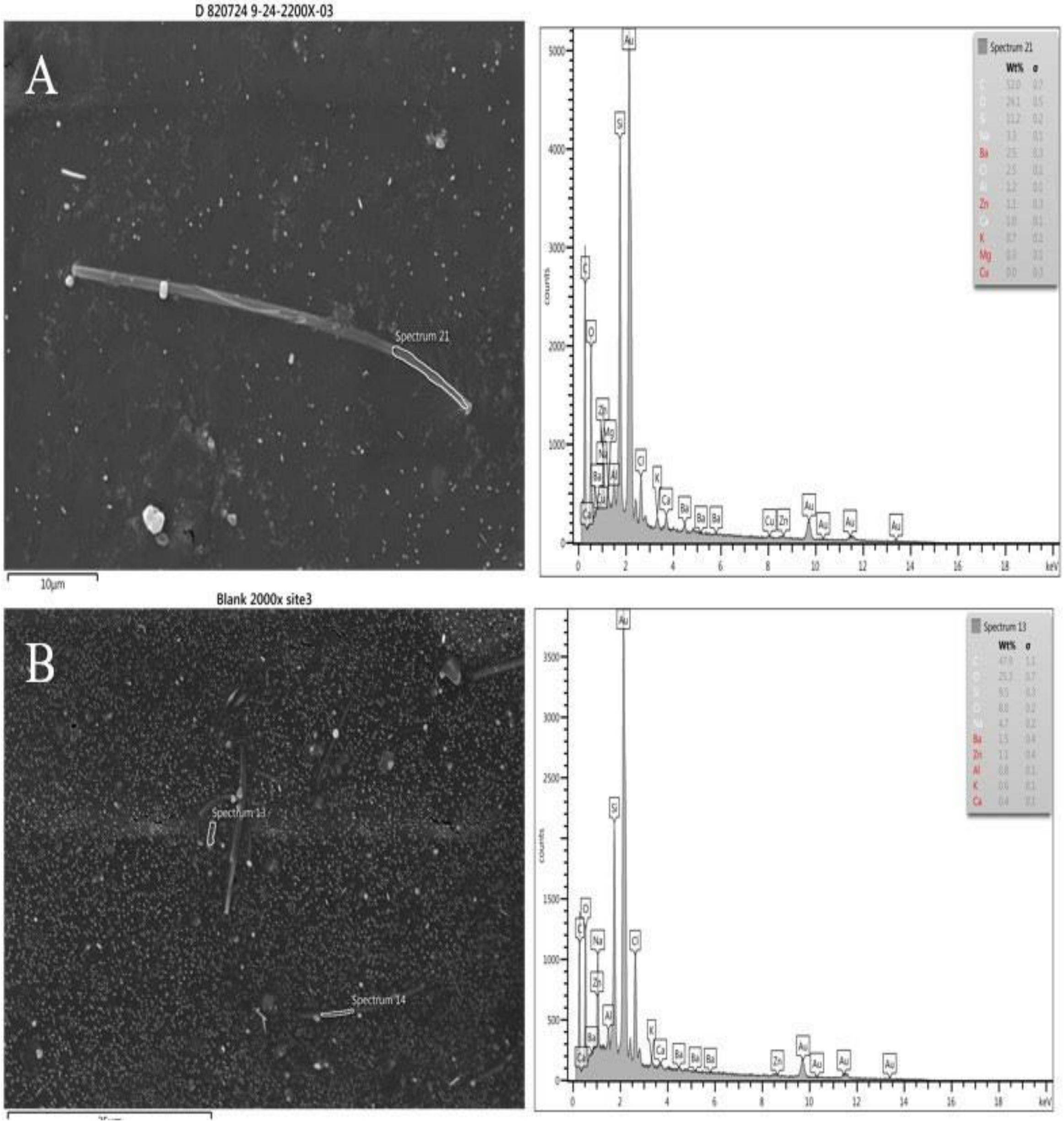
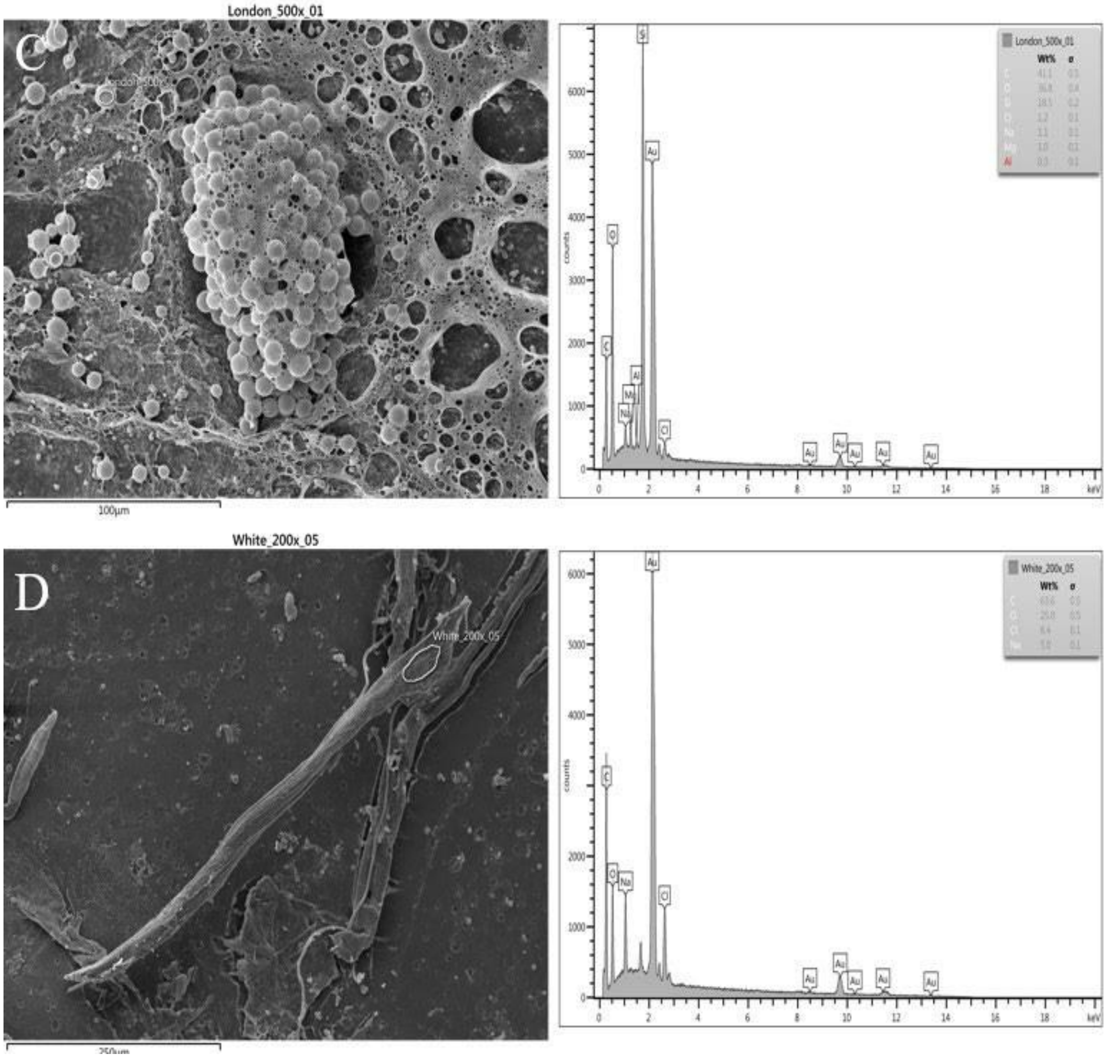


Figure 13. (cont'd)



4.5 Discussion

4.5.1 Microplastic shapes under individual tree species

One of the main objectives of this study was to identify and characterize types and shapes of microplastic under individual tree species. In my findings, I was able to identify dominant shapes such as spheres, fragments, fibers, and foam which have been identified in previous studies (Klein and Fischer, 2019; Cai et al., 2017, Dris et al., 2017; Allen et al., 2019; Lieu et al., 2019c; Wright et al., 2020). Klein and Fischer (2019) conducted their study in urban environments in

Hamburg, Germany, and found that the most dominant shape of MPs detected in their study was fragments (95%) and fibers (5%). Other studies looking at airborne MPs in cities such as Paris and Dongguan city found that the most dominant shape was fragments and fibers (Dris et al., 2016). Cai et al., (2017) found in their studies diverse shapes which included fibers, foam, and fragments, with fiber being the dominant shape. Wright et al., 2020 found in central London dominant shapes such as fragments, films, spheres, and foams, with fragments being the most dominant shape. In my study, I found that spheres were the dominant shape accounting for 92% of fully-leafed and partially-leafed periods, and fragments and fibers were the least abundant shape. Eastern white pine had the highest abundance of fragments and honeylocust for fibers during the fully-leafed period. During the partially-leafed period, honeylocust exhibited the highest abundance of particle shapes such as spheres, fragments, and fibers, while foam was found under eastern white pine. Potential sources from these particles in the samples of the studies can be attributed to wear and tear of tires and vehicles, active construction sites, and regional wind and storms from west to east.

4.5.2 Microplastic deposition

The study shows how TF MPs composition varies among four species common in a local urban forest. The particle concentration of MPs in TF samples was observed to be higher for individual London planetree compared with the other tree species. Location was found to be slightly significant for the MPs fluxes. Microplastics were the highest in collectors under London planetrees, but the effect on species was not statistically significant due to a combination of low sample size and spatial clustering of species. If the sample size increases and has a better spatial representation I might see a significantly greater scrubbing of airborne MPs by London planetrees. This analysis helps us to understand how by looking at the geospatial location individual tree species influence scavenging MPs from the atmosphere.

Once the MPs are deposited in the leaves and the canopy structure due to dry deposition they are then washed away by the precipitation and transported to the soil via a mechanism of rainfall partition such as TF. Leaf traits such as surface roughness, hydrophobicity, and the presence of waxy coats have been reported to determine how particulate matter can be intercepted from wind streams (Corada et al., 2021, Saebo et al., 2012). This relationship can be observed with London plantree which had a wide canopy structure compared to other tree species. Low MPs fluxes in our study were attributed to particles being lower than the blanks which I analyzed for background contamination. By understanding how a tree's leaf and canopy structure impacts atmospheric fallout I may have found a link to how trees could potentially serve for impacting the vertical transport of these particles. One possible explanation of how individual trees scrub airborne microplastics is likely due to leaf characteristics such as cuticle thickness, and surface roughness. Nowak et al., (2014) mentioned how the greater the three covers, the greater the pollution removal.

Trees removal rate will vary on location as well with the amount of tree cover, pollution concentration, length of the growing season, meteorological conditions, and the percentage of evergreen leaf area (Nowak et al., 2014). London plane is widely used in urban environments due to the large number of ecosystem services the trees provide (Cariñanos et al., 2020). The London planetree is known for growing fast, being tolerant to urban microclimate conditions, and being resistant to air pollution (Hull, 2009). London planetrees have a high capacity for capturing particulate matter and this is due to their significant leaf area (Lowicki, 2019; Cariñanos et al., 2020). The particle retention of London planetree can be likely attributed to a high leaf area, high roughness, and wettability. Li et al., (2021) reported that London planetree retains particulate from different sizes and attributed this retention ability to leaf surfaces with dense and narrow grooves, high roughness, and high wettability. This characteristic can potentially differentiate the London plane tree from other species and is a possible explanation for why I observed higher fluxes and concentrations of MPs in the TF collectors. It is likely that London planetree is more effective at scrubbing MPs than other tree species, but that would need a more robust experiment to demonstrate this conclusively.

Honeylocust during the partially-leafed period was found to be the tree species that scrubbed airborne MPs followed by red maple and eastern white pine. This is an example of how different urban tree species have their unique capturing capacity of atmospheric MPs even when their leaf had fallen during the senescence period. The findings of this study show the difference in tree scrubbing during leaf on and leaf off can be attributed to LAI. Even though the study did not conduct an analysis of microplastic on leaf surfaces or LAI these findings help us understand the role of canopy cover on the selected trees for the study. One of the major threats of microplastics is that once attached to the leaf surfaces, they act as an absorbent layer which enhances the accumulation of volatile organic components or heavy leaches on the leaf surfaces (Bi et al., 2020). Leonard et al., (2023), found in their study a poor correlation between microplastic and leaf hydrophobicity, but their study showed how the concentrations of microplastic in the leaves show the total amount of microplastic fallout from the atmosphere and could represent a risk of inhalation in the region. Canopy cover, leaf area, and leaf roughness are examples of tree characteristics mentioned previously in the literature that can help scavenge particles and gases from the atmosphere. Thus, the interspecific variability of MPs in TF will be dependent on canopy structure and leaf morphology and the behavior (e.g., density) of MPs in atmospheric deposition.

4.5.3 SEM-EDX Analysis

Fiberglass was found in filter samples from London plane trees and eastern white pine

(Figure 5 A-B). Glass fibers are added to the plastic for reinforcement of the polymer (Alsayed et al.,2000; Kavad et al., 2014). Following guidelines and comparing EDX analysis from Kwak and An (2021) and Hwang et al., (2020) I was able to characterize PE and PS in the filter from a honeylocust and eastern white pine filter (Figure 5 C and D). These are commonly known to be from sources of primary microplastic which have diameters ranging from 1 and 5 um and are found in spherical shapes in the literature (Hwang et al., 2020; Napper et al., 2015; Gregory, 1996; Carr, Liu, and Tesoro, 2016). In my findings, The EDX analysis of MPs had strong peaks of Carbon and Oxygen as main components while also present in their surface inorganic elements such as Al, Ca, K, Cu, Mn Mg, Si, Fe, and Na which are common additives of plastic polymers and debris on the surface of the microplastic. However, additives such as Ti, Ba, and Zn were found in our chemical characterization, these additives are commonly used for coloring plastic polymers. For example, Titanium dioxide is commonly used in the manufacturing industry to provide whiteness and opacity to coating and plastic products (Gázquez et al., 2014). For this study, there was a challenge in quantifying the number of MPs on a filter, and establishing a distinction between organic matter, inorganic matter, and the MPs of interest which aligns with other studies across the literature (Klein and Fischer, 2019; Tamminga et al., 2017; Erni-Cassola et al., 2017; Shim et al., 2016). This method uses chloroform as a solvent for Nile Red and one of the main disadvantages of chloroform is the dissolving PS (polystyrene) (Tamminga et al., 2017). PE is used for packaging, textiles, and fishing gear (Claessens et al., 2011; Hidalgo-Ruiz et al., 2012), while PS is used for food packaging, such as in-dinning and take-out food (Pan et al., 2019). The single use of a technique for characterization is not enough to identify and classify microplastics. Microplastic identification has been based on a two-step process (Gniadek and Dabrowska, 2019). SEM-EDX is a technique that can provide information on morphology, and aging, with high-resolution data on the surface and chemical composition. When compared to an optical microscope, SEM provides high magnified and resolution images which help to analyze the chemical composition of the surface (Gniadek and Dabrowska, 2019). I used an acceleration voltage of 15 kV to obtain high-quality images. One of the main advantages of a higher kV will excite higher energy ranges of x-rays in which we can observe elements that are not shown at low kV. Another advantage is that the higher the kV, the higher the energy beam is, thus the larger the volume of excitation from the collected data. Other studies have used the acceleration voltage from 7-10 kV and have obtained high-quality images and avoid covering other elements in the spectra (Gniadek and Dabrowska, 2019; Furfaro et al., 2022; Dehghani et al., 2017). I consider SEM-EDX to be an important method to identify form, shape, and types of additives in the microplastic when compared to an optical microscope, and combined with Fourier or Raman microscopy could provide more in-depth details on the chemical composition of these polymers.

4.5.4 Gaps and future research on microplastics

One of the challenges of quantifying microplastics in atmospheric deposition samples is the underestimation and overestimation of particles. Optical, stereoscopic, and anatomical microscopy have been widely used in the characterization of microplastics (Chen et al., 2020; Zhou et al., 2019). This leads to the use ~~ultimately~~ of the naked eye which is one of the biggest limitations for microplastic studies. The distribution of microplastic will vary depending on the proximity of the source. There has been a study made by Magnusson et al., (2016), in which they mentioned how the emission of road dust, and abrasion from tires and road surfaces which are known as secondary sources from the polymer It is important to assess that this study indicates the importance of throughfall which previous studies in the atmospheric deposition were not considered leading to an underestimating of MPs deposition fluxes and impacts to the ecosystem.

Contamination, overestimation, and underestimation of microplastic are some examples of challenges present in quantification due to the lack of optimized analytical methods (Silva et al.,2018). Including procedural blanks and replicated allows for method validation, internal quality control, and inter-laboratory studies (Silva et al., 2018). More guidelines are needed in order to improve the accuracy and validation of microplastics. One limitation of this study was that were able to analyze only two sampling dates due to low precipitation during the sampling period. Moving forward, Gasperi et al., (2018) and Zhang et al., (2020 a, b) recommended focusing on the occurrence, fate, transport, and effect of atmospheric MP due to the lack of standardized sampling and identification methods. Although our samples are very limited, this study provides information and a foundation for understanding the interspecific variability of microplastic in TF under urban tree species. Understanding how different tree species have capture capacities is essential for proper urban forest design and the mitigation of airborne microplastic pollution.

The literature has reported how MPs are vectors for polychlorinated biphenyl (PCBs) and polycyclic aromatic hydrocarbon (PAHs), which enhances their toxic effects in the environment (Liu et al., 2016; Beckingham and Ghosh, 2017; Hartmann et al., 2017; Koelmans et al., 2016, Koelmans et al., 2022). Heavy metals such as Cd, Zn, Ni, and Pb have been reported to be absorbed on the MPs surface (Feng et al., 2022; Shao et al., 2017). The chemical toxicity of the particles is attributed due to their large surface area and hydrophobicity (Teuten et al., 2007, 2009; Wang et al., 2016). The adsorption is believed to be due to the electrostatic charge on their large surface, biofilm growth, and additives or chemicals added to the polymer (Verla et al., 2019). A future direction could be conducting toxicity research on the impacts of microplastics on soil microorganisms and organisms.

4.6 Conclusion

In my study, I was able to demonstrate the types of airborne MPs present in TF with fiberglass, PS, and PE being characterized using an SEM-EDX. SEM-EDX combined with a fluorescence microscope can help to screen the microplastic particles and non-particles and provide high-resolution images of characteristics of surface cracks and pigments (e.g., Ti). SEM-EDX high magnification provides a direct observation of environmental weathering of airborne MP surface. Spheres were the most abundant shape found in both the fully-leafed and partially-leafed period followed by fragments, foams, and fibers. Only one species was effective at scavenging microplastic from the atmosphere when compared to other species. This study shows how London planetree intercepts airborne MPs via dry deposition while the other tree species', rainfall washes off particles that were deposited in the canopy and leaf surface. The finding of my study shows that despite a difference of particles found under London planetree It cannot be conclusively attributed to a species effect due to the tree species being spatially clustered and low sample size. Based on our results and analysis I would like to propose the following suggestion on the mitigation of airborne MPs. (a) Expand the study in terms of sample size, spatial extent, and length of time. (b) Integrate more tree species (e.g., birch) to assess their mitigation of MPs in urban, suburban, and rural environments. (c) Investigate the adsorption/ desorption of contaminants on the MPs to understand their role as vectors in forest ecosystems. (d) To develop guidelines and standard operational protocol (SOP) helps to assess a proper analysis for quantifying and characterizing airborne MPs.

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CHAPTER 5

CONCLUSION

The preceding chapters explore the impacts of tree species effect on the deposition and transport of nutrients and pollutants via throughfall in the urban, suburban, and rural forests across Michigan. Chapter 2 emphasizes the depositions of N deposition in fragmented forests, local tree species effect, and spatial and temporal variability in throughfall N deposition. The results of this study highlight how TF N concentrations are influenced due to the proximity of agricultural landscape while TF N fluxes and enrichment rations are dominated by the internal characteristics of the woodlot such as species composition and forest structure. These findings suggest that TF N dynamics are governed by the effect of the forest canopy rather than the surrounding landscape. Tree species abundance of basswood, northern red oak, and sugar maple had a significant effect on the subcanopy distribution of N species via throughfall. With the abundance of basswood, TF $NO3^-$ concentrations and fluxes increased while on the contrary, it decreased with the abundance of northern red oak. Sugar maple was the most abundant tree across our study sites the abundance relationship was only for $NH4^+$. Moreover, the study serves as a guide on how different species affect nutrient fluxes in forest ecosystems, and how stand structure impacts the distribution of N via TF. From a forestry perspective, it’s important to understand how sub-canopy flux changes temporal and spatial for TF for highly developed areas for other nutrients to determine the impact of adjacent land use, local point and non-point sources, and the role of fragmented forests.

Chapter 3 focuses on the feedback between trees and soil looking at the role of throughfall in nutrient losses from trees and nutrient use efficiency (NUE). The study highlights the importance of TF losses in the underestimating of NUE in forest ecosystems across the fertility gradient. Throughfall losses were greatest for K, intermediate for N and Ca, and lowest for Mg and P. However, LF losses were greatest for Ca, intermediate for N, Mg, and K, and lowest for P. These patterns of TF and LF losses increased across the fertility gradient through the two years of the study. This was attributed to leaf characteristics, high foliar concentrations, species composition, flowering cycles, reproduction, and wash-off of dry deposition. The findings of the study look at how the NUE of mobile nutrients cannot be understood without considering TF losses. TF losses decreased estimates of NUE and the degree of underestimation across the fertility gradient. Understanding the leaching of nutrients from plant canopies is essential in looking for pathways from which nutrients are lost from the plant and return to the soil. Leaching of nutrients has been looked at solely from the litterfall perspective, rather than considering TF, which means that they do not consider it for the NUE or plant-soil feedback on nutrient cycling. This study helps to understand how nutrients are being lost in plant-soil feedback and could help to estimate how much nutrients are being returned to the soil as well as how the plant can efficiently use nutrients in poor to high fertility sites.

Chapter 4 takes a look at filling data gaps of the fate and transport of microplastic deposition and abundance in urban environments. For this study, I was able to identify and characterize the types and shapes of MPs under individual tree species. The result of this research shows how the most abundant type of microplastic were spheres, which are a common source of primary microplastics. Fiber, fragments, and foams were also present in our study and with the use of a SEM-EDX, I was able to identify the morphology, aging, and elements present at the surface of the polymer. SEM-EDX combined with fluorescence microscopy helped to assess MPs particles and non-particles this in combination with Raman and Fourier helps with a stronger assessment and characterization of MPs. The key findings of this study were that vertical transport is dominated by both wet and dry deposition. Once particles are deposited on the canopy structure they are washed off by precipitation thus reaching the soil and via runoff can potentially affect other ecosystems, flora, fauna, and microorganisms in the soil. I was able to characterize three types of polymers, which were fiberglass, polyethylene (PE), and polystyrene (PS). Fiberglass is commonly used as a reinforced plastic in the construction industry and PPE, PE is used in the packaging, textiles, and fishing industries while PS is used in food containers. The results highlight how individual tree species intercept this polymer and can serve as a guide on how they can potentially be used for the mitigation of microplastic in urban, rural, and suburban regions across the country. London planetree intercepted particles deposited via dry deposition for the full canopy period, while the other trees wash off scrubbed particles from the leaf and canopy via rainfall. The existing literature's lack of data to assess the potential ecotoxicology risk is not known since risk assessments have yet to be developed. Research should be developed to understand the chemicals being absorbed on the polymer surfaces and the ecological impacts on the soil biota. Developing guidelines and SOPS to avoid underestimation of airborne MPs in the ecosystem. In conclusion, the results of this study emphasize the species-specific interactions with the deposition and interception of pollutants and biogeochemical cycling. Trees are able to capture both nutrients and pollutants. Once deposited on the canopy they are washed off by rainfall and transported to the urban, suburban, and rural ecosystems via TF. The chemical composition of the rainfall can then be modified resulting in high solute concentrations in TF. By investigating throughfall we can determine the impacts on nutrient cycling, nutrient use efficiency, and the fate and transport of solutes and pollutants within forest ecosystems.