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https://doi.org/10.15760/etd.7789

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Spatial and Seasonal Variations of Microplastic Concentrations in Portland's Freshwater Ecosystems

by

Rebecca Talbot

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Geography

Thesis Committee:
Heejun Chang, Chair
Elise Granek
Nancee Hunter

Portland State University
2022

Abstract

Microplastics are a pollutant of growing concern and are ubiquitous in a variety of environmental compartments. The majority of microplastics research to date has been conducted in marine waters, and less is known regarding the sources and delivery pathways of microplastics in urban rivers. The first chapter is comprised of a review of the scientific literature regarding the spatial and temporal factors affecting global freshwater microplastic distributions and abundances. Microplastic spatial distributions are heavily influenced by anthropogenic factors, with higher concentrations reported in regions characterized by urban land cover, high population density, and wastewater treatment plant effluent. Temporal variables of influence include precipitation and stormwater runoff and water flow/discharge. Despite these overarching trends, variations in study results may be due to differing scales or contributing area delineations.

In the second chapter, two watersheds in the Portland metropolitan area representing an urban-rural gradient were selected to assess microplastic concentrations and potential links with a variety of spatiotemporal factors (e.g., land use, arterial road length, water velocity, precipitation). Samples were collected from four sites in the Clackamas River watershed and from six sites in the Johnson Creek watershed, with one sampling event in the dry season, one in the early wet season, and a third in the mid-wet season. Samples were analyzed for total microplastic count and type, and nonparametric statistics were run to evaluate potential relationships with the explanatory variables, with spatial analyses conducted at both the subwatershed and nearstream scale.

Microplastic concentrations in August (dry season) were significantly higher than in February (mid-wet season). August concentrations also negatively correlated with flow

rate, suggesting that lower flow rates present in the dry season may have facilitated the accumulation of microplastics. Only one correlation was noted regarding antecedent precipitation amount and microplastics, and included a positive correlation between microplastic concentrations and 24-hour antecedent precipitation in February. Additionally, negative correlations were found between wet season microplastic concentrations and agricultural lands at the nearstream level.

While additional research is needed, results indicate that the presence and abundance of microplastics in Portland's waterways may be more strongly influenced by nearstream variables as opposed to subwatershed-scale variables. Fragments were the most commonly observed microplastic morphology, with a dominance of gray particles and the polymer polyethylene. The findings of this research can be used to inform management decisions regarding microplastic waste and identify hotspots of microplastic pollution that may benefit from remediation.

Acknowledgments

A big thank you to my advisor Dr. Heejun Chang for his continuous support, guidance, and invaluable insights throughout the course of this project. A special thank you to my committee members: Dr. Elise Granek for her encouragement, guidance, and for sharing her expertise in microplastics, and Dr. Nancee Hunter for her support, expertise, and feedback. I would like to thank Dr. Yangdong Pan for kindly answering my numerous questions regarding statistical analyses. I would also like to thank Portland State University for awarding a Faculty Enhancement Grant to Drs. Chang and Granek in support of this research, as well as the agencies and organizations that provided funding and support: the City of Portland, Clackamas River Water Providers, the City of Gresham, the East Multnomah County Soil and Water District, and Sigma Xi.

A huge thank you to Rosie Wood for her help both in the field and in the lab, and for her encouragement and kind words. Thank you so much to Will Long and Geoff Szafranski for their help with sample collection, to Kurt Carpenter for loaning me equipment critical to sample collection, and to Matt Yates, Will Milligan, Kim Swan, and Suzie Cloutier for their help with site selection/access. I would also like to thank the members of both the WISE and ACE labs for their advice, input, and encouragement.

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Chapter 1

Microplastics in Freshwater: A global review of factors affecting spatial and temporal variations

Rebecca Talbot and Heejun Chang

Rebecca Talbot: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing - original draft, Writing – review & editing; Heejun Chang: Conceptualization, Supervision, Visualization, Writing - original draft, Writing – review & editing.

Talbot, R., and H. Chang. 2022. Microplastics in freshwater: A global review of factors affecting spatial and temporal variations. *Environmental Pollution* 292:118393.

https://doi.org/10.1016/j.envpol.2021.118393

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1. Introduction

Plastic production has increased dramatically in recent years, with some estimates of production rates topping 330 million tons per year (Jiang et al. 2019). While plastics such as microbeads are manufactured at very small sizes, larger plastics can degrade over time due to a host of environmental variables (Eerkes-Medrano et al. 2015), often becoming categorized as microplastics. While a standard definition of microplastics has yet to be agreed upon, many studies have included an upper and lower limit of five millimeters and one micron, respectively (Horton et al. 2017).

Microplastics are a growing concern in aquatic environments, impairing water quality and damaging organisms that ingest them (Eerkes-Medrano et al. 2015, Li et al. 2020a). The majority of early microplastics research focused on their abundance in marine environments, with the earliest studies published in the 1970s (Carpenter and Smith 1972, Colton et al. 1974). The focus on microplastics in freshwater environments is a relatively recent phenomenon, with the first studies published only within approximately the last fifteen years. Microplastics have quickly become a ubiquitous pollutant; indeed, it is not uncommon for freshwater research to observe and report microplastics at all sampling sites, and often in all collected samples (Liu et al. 2020, Shruti et al. 2019, Yin et al. 2020).

This expansion of the research focus to include freshwater is a critical one, as rivers are now known to play a key role in the transportation of microplastics (Hu et al. 2020, Klein et al. 2015, Rodrigues et al. 2018), particularly to marine environments (Jiang et al. 2019, Zhao et al. 2019). It was recently estimated that the Nakdong River in South Korea contained an annual load of between 53.3 and 118 tons of microplastics in

2017 (Eo et al. 2019), many of which wind up in ocean environments. In fact, recent riverine microplastic flux calculations indicate that marine microplastic concentrations may even exceed previous estimates (Hurley et al. 2018). We cannot fully comprehend the existence and abundance of microplastics in ocean waters if we do not also understand their transportation pathways and land-based sources.

In addition, the majority of microplastics are generated by land-based anthropogenic activities, and can be flushed into freshwater environments through runoff processes (Horton et al. 2017). In periods of dry weather, these plastics can have extended residence times in rivers and continually degrade over time (Li et al. 2020a). In wet seasons, more extreme flows can exacerbate microplastic pollution in these water bodies and resuspend particles that had previously been trapped in sediment (Hurley et al. 2018).

While many research studies address microplastics in major rivers, there is no indication that lower order streams are less at risk for microplastic pollution. Indeed, recent findings suggest that microplastic abundances in tributaries and streams are comparable to river mainstems and other larger freshwater bodies (Dikareva and Simon 2019, Hurley et al. 2018, Sankoda and Yamada 2021), and may thus serve as critical transportation pathways for microplastics (Hurley et al. 2018). Freshwater microplastics research has focused on evaluating trends in quieter waters as well, including lakes, ponds, and wetlands (e.g., Bertoldi et al. 2021, Su et al. 2016, Wang et al. 2017). These still waters can be substantially affected by microplastic pollution present in contributing streams and rivers (Migwi et al. 2020). As shown in Figure 1, a greater number of the reviewed studies collected samples in running water bodies (e.g., streams, rivers) rather than in still waters (e.g., lakes, ponds). Few studies sampled both types of water bodies.

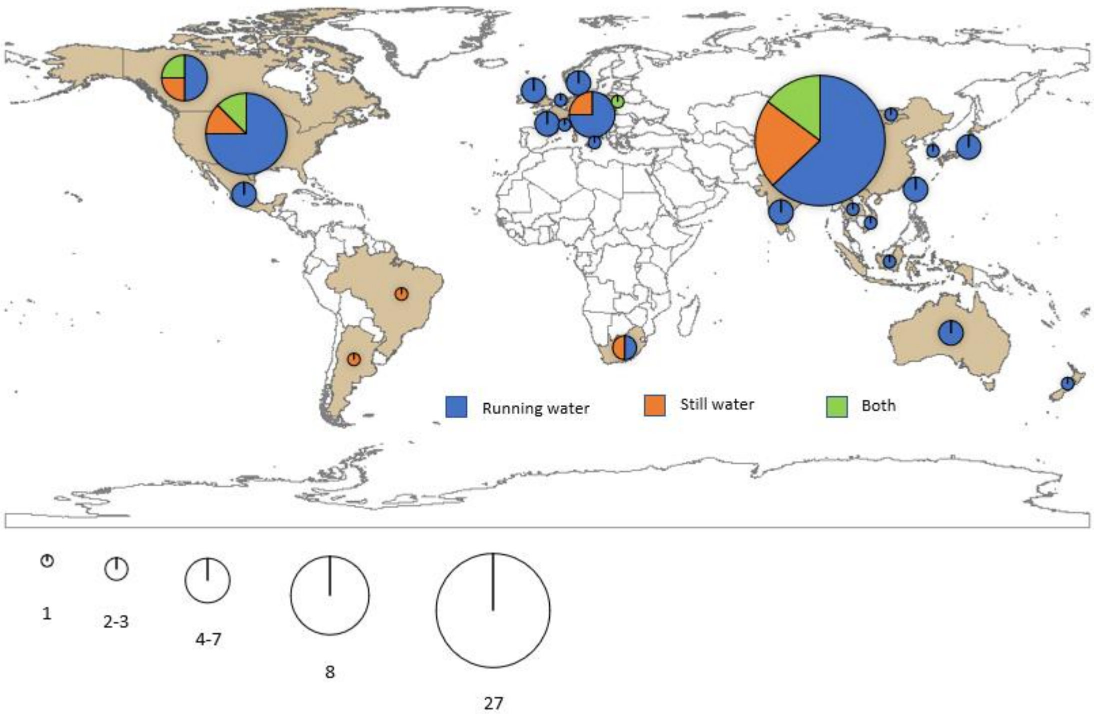


Figure 1. Global distribution of the selected freshwater microplastic publications as a function of whether samples were collected from running water (e.g., rivers, streams), still water (e.g., lakes), or both. The numbers shown refer to the number of publications in a particular size category.

It has become increasingly important to analyze microplastic pollution from both spatial and temporal standpoints, as these factors serve as the drivers of the distribution and abundance of microplastics in freshwater bodies (Stanton et al. 2020). In particular, land cover and proximity to anthropogenic activities are critical components of freshwater microplastic pollution, with microplastics originating from a broad range of terrestrial sources (Grbić et al. 2020). It is also necessary to examine how such land-based sources are transported to freshwater environments, and to understand the role of temporal factors such as the timing and volume of precipitation and runoff in these delivery pathways. Once in an aquatic environment, microplastics are subjected to hydrodynamic processes, which may influence their accumulation or deposition (de Carvalho et al. 2021, Mani and Burkhardt-Holm 2020). Figure 2 outlines these components of the microplastic cycle, with a particular focus on anthropogenic sources of microplastics and the processes that influence their introduction to and distributions within freshwater bodies. A thorough understanding of these components is crucial to the development of microplastic flux estimates of a water body (Eo et al. 2019, Xiong et al. 2019).

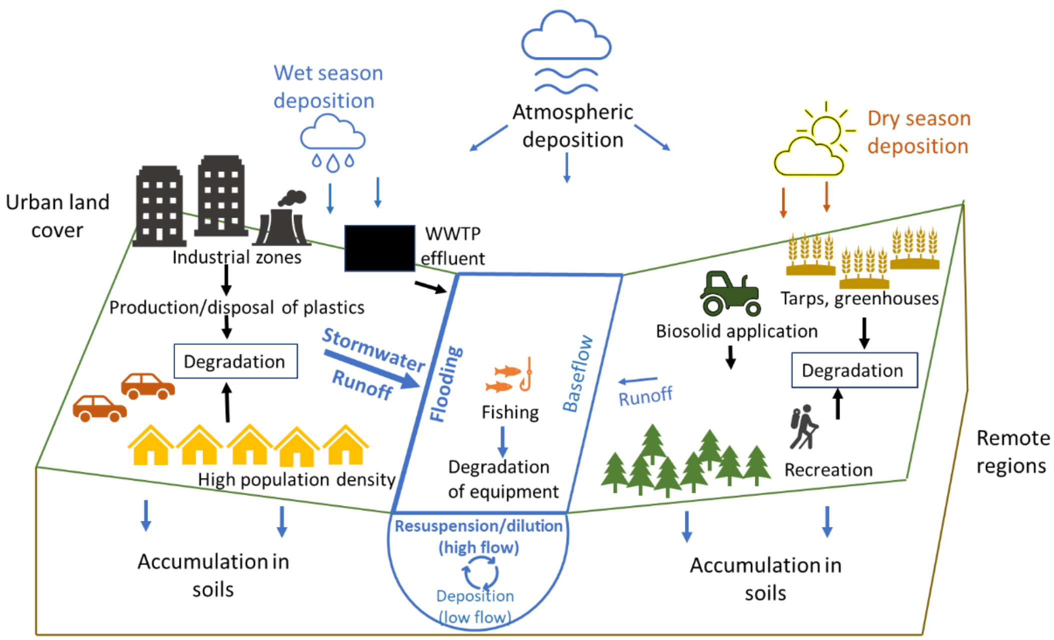


Figure 2. Spatial and temporal factors influencing the distribution and abundance of freshwater microplastics (adapted from Lintern et al. 2018 and Horton et al. 2017).

Recent reviews of freshwater microplastics have focused on topics including procedures for analyzing and detecting microplastics (Dris et al. 2015, Gong and Xie 2020, Koelmans et al. 2019, Zhang et al. 2020), impacts of microplastics on organisms (Li et al. 2020a, O'Connor et al. 2016), differing microplastic sampling procedures

(Eerkes-Medrano et al. 2015, Horton et al. 2017), microplastics in water versus sediment samples (Szymanska and Obolewski 2020) and primary versus secondary production (Akdogan and Guven 2019, Eerkes-Medrano et al. 2015). While some reviews have included discussions regarding microplastics and land-based sources, (Eerkes-Medrano et al. 2015, Horton et al. 2017), few have provided a more in-depth focus on the broad range of spatiotemporal factors affecting microplastic pollution. Thus, the current review aims to expand and build upon this knowledge base by providing an overview of the spatial and temporal factors affecting microplastic abundances in freshwater environments, and by evaluating watershed attributes and hydroclimatic variables that affect microplastic pollution.

Different studies use different scales of analyses, which may affect findings and conclusions drawn regarding potential microplastic sources or the microplastic cycle. For instance, a study focusing on a small local scale might capture only nearstream factors affecting microplastic pollution, which may differ from findings of a larger regional study that incorporates more distant and upstream regions (Grbić et al. 2020). From a temporal standpoint, microplastic concentrations may also vary between the event scale (e.g., a storm event and subsequent flooding) versus repeated samplings over the course of several seasons (Cheung et al. 2019, Stanton et al. 2020). In summation, these various scales of analyses include variations in spatial scale (e.g., river reach, full watershed scale) as well as temporal scale (e.g., sampling over the course of several hours, repeated seasonal samplings).

Given the above considerations, the main objectives of this review are to: (i) evaluate the influence of watershed attributes such as land cover, population density, and physical watershed/stream characteristics on microplastic abundances, (ii) examine the influence of seasonality, precipitation, and flow rate on microplastic abundances, and (iii) discuss the role of scale with regard to the distribution and identification of microplastics.

A literature search was conducted in the Web of Science database and included peer-reviewed journal articles published through June 2021. The search string was “microplastic*” and (“freshwater*” or “river*” or “stream*” or “lake*”). A total of 1,149 articles were produced, of which 75 were included for the purposes of this review paper. Papers were excluded for the following reasons: an exclusive focus on microplastics and organisms, laboratory studies, modeling studies, review papers, a general focus on plastics (not specifically microplastics), no apparent statistical analyses of spatial/temporal factors affecting microplastics, and no spectroscopic microplastic verification (e.g., μFTIR, Raman).

As previously mentioned, it is not uncommon for research publications to note a size range of 1μm-5mm for microplastic particles. However, not all of the reviewed studies included microplastics spanning this particular range. For instance, studies commonly varied with regard to the lower size boundary, which was often due to factors such as differing net mesh sizes during sample collection. Those using a larger mesh, such as the commonly used 333μm mesh plankton net, were unable to capture and quantify microplastics falling into smaller size ranges (Campanale et al. 2020, Constant et al. 2020, Hoellein et al. 2017, McCormick et al. 2014, Yonkos et al. 2014). Smaller classes of microplastics were captured with the employment of other methods, such as the use of smaller mesh nets or grab samples when collecting microplastics in surface waters (Stanton et al. 2020, Xia et al. 2020, Zhao et al. 2020), or by collecting sediment samples and using smaller mesh sieves (Corcoran et al. 2020a, Hurley et al. 2018, Sarkar et al. 2019). Thus, the lower size limit of observed microplastics differed among studies as a function of data collection methodologies.

2. Factors Affecting the Spatial Distribution of Microplastics

Spatial distributions of microplastics may be influenced by a variety of factors, including those relating to anthropogenic activities as well as physical watershed/stream characteristics. Previous empirical studies have focused on the impacts of anthropogenic activities such as land cover, wastewater treatment plants, and population density on microplastic abundances. While various physical watershed characteristics (e.g., elevation, slope) may also influence microplastic abundances, very few studies have directly addressed these links. Nevertheless, these will be included in the following discussion and highlight the need for additional research in this area. Table 1 shows positive and negative relationships between microplastics and both anthropogenic activities and physical watershed characteristics. A total of 35 publications reported significant results regarding such factors, and microplastic concentrations in these studies may thus be considered spatially dependent.

2.1 Urban land cover

Previous studies have shown strong links between microplastic pollution in freshwater bodies and specific land cover categories (Chen et al. 2020). In particular, urban land cover is closely correlated with microplastic abundance (de Carvalho et al. 2021, Feng et al. 2020, Su et al. 2020, Sang et al. 2021), potentially due to factors such as insufficient waste management strategies and littering (Battulga et al. 2019, Mani and Burkhardt-Holm 2020). Elevated levels of microplastics have been observed in watersheds characterized by a high proportion of urban land cover (Grbić et al. 2020, Nihei et al. 2020, Yonkos et al. 2014), and have been found in higher concentrations with increasing proximity to urban or industrial centers (Ding et al. 2019, Huang et al. 2021, Luo et al. 2019, Wang et al. 2017) (Table 1). Watersheds characterized by active industrial zones have been linked with elevated microplastic concentrations in their freshwater bodies (Chen et al. 2020, Corcoran et al. 2020b, Deng et al. 2020, Feng et al. 2020, Grbić et al. 2020, Lahens et al. 2018, Li et al. 2020b, Liu et al. 2020). Such results indicate that microplastic abundances are heavily influenced by proximity to anthropogenic activities.

It is less common for studies to report no significant correlation (Barrows et al. 2018, Belen Alfonso et al. 2020, Mai et al. 2021, Wang et al. 2020) or a negative relationship between microplastic concentrations and urban land cover (He et al. 2020b, Yin et al. 2020). Of the studies focusing on urban land cover, 33.3% of running water studies reported no significant relationship, with just one disclosing a negative relationship (He et al. 2020b). For still water studies, three reported no significant relationship (30%), and one reported a negative relationship (Yin et al. 2020) (Table 1). Negative correlations may potentially be due to strict local regulations regarding pollution (Liu et al. 2020) or to waste management strategies that greatly surpass those found at rural sites (Yin et al. 2020). Additionally, lack of a correlation could potentially be due to high rates of atmospheric deposition of microfibers over all land cover categories within a study region, thus obfuscating connections between urbanization and microplastics (Kaliszewicz et al. 2020). In certain instances, microplastic abundances may be higher in urban areas but the correlation is not significant, indicating the potential

Table 1. Spatial factors affecting MP concentrations in freshwater. Percentages in parentheses refer to the relative number of articles (as a function of either still water or running water) that assessed correlations with spatial factors.

	Lakes/reservoirs/wetlands			Running water		
Explana-tory factors	(+)	(-)	No relation	(+)	(-)	No relation
Human activity						
Urban land cover	Corcoran et al. 2020b, Deng et al. 2020, Di and Wang 2018, Feng et al. 2020, Liu et al. 2019a, Wang et al. 2017 (60%)	Yin et al. 2020 (10%)	Belen Alfonso et al. 2020, Kalis-zewicz et al. 2020, Liu et al. 2019b (30%)	Alam et al. 2019, Chen et al. 2020, de Carvalho et al. 2021, Ding et al. 2019, Feng et al. 2020, Grbić et al. 2020, Huang et al. 2021, Kataoka et al. 2019, Lahens et al. 2018, Li et al. 2020b, Liu et al. 2020, Luo et al. 2019, Nihei et al. 2020, Peng et al. 2018, Sang et al. 2021, Schmidt et al. 2018, Su et al. 2020, Tibbetts et al. 2018, Yonkos et al. 2014 (63.3%)	He et al. 2020b (3.3%)	Barrows et al. 2018, Battulga et al. 2019, Corcoran et al. 2020a, Huang et al. 2020, Jiang et al. 2019, Klein et al. 2015, Mai et al. 2021, Stanton et al. 2020, Wagner et al. 2019, Wang et al. 2020 (33.3%)
WWTP effluent	-	-	-	Grbić et al. 2020, Hoellein et al. 2017, Liu et al. 2020, McCormick et al. 2016, McCormick et al. 2014, Schmidt et al. 2018, Shruti et al. 2019 (58.3%)	-	Bujaczek et al. 2021, Klein et al. 2015, Peller et al. 2019, Stanton et al. 2020, Tibbetts et al. 2018 (41.7%)
Ag land cover	-	-	-	-	Grbić et al. 2020, Huang et al. 2020 (40%)	Barrows et al. 2018, He et al. 2020b, Nihei et al. 2020 (60%)
Popu-lation density	Bertoldi et al. 2021, Corcoran et al. 2020b	-	Belen Alfonso et al. 2020, Feng et al. 2020,	Battulga et al. 2019, Fan et al. 2019, Grbić et al. 2020, Huang et al. 2020, Kataoka et al. 2019, Mai et al. 2021, Nihei et al. 2020,	-	Dikareva and Simon 2019, Feng et al. 2020, Kapp and Yeatman 2018, Klein et al. 2015, Tibbetts et al. 2018, Zhou et

	(40%)		Mbedzi et al. 2020 (60%)	Yonkos et al. 2014 (57.1%)		al. 2020 (42.9%)
Physical						
Elevation	-	-	-	-	Su et al. 2020 (100%)	-
Slope	-	-	-	Grbić et al. 2020 (100%)	-	-
Water body width	-	-	-	-	-	de Carvalho et al. 2021 (100%)

for additional influential factors (Mai et al. 2021). Future research could incorporate a focus on relationships between land use and specific microplastic type, as correlations between these factors could potentially be stronger than those between land use and microplastic abundance (He et al. 2020b).

Recent research has also evaluated the role that roads and the transportation industry may play in freshwater microplastic pollution, with initial results showing vehicle tire particles present in samples (Grbić et al. 2020). Additionally, positive relationships have been found between microplastics and total road length at both the catchment scale and the riparian zone scale (Grbić et al. 2020).

2.2 Wastewater treatment plants

Urban and industrial regions are often home to wastewater treatment plants (WWTPs), which have been closely linked to microplastic pollution (Grbić et al. 2020, Shruti et al. 2019) (Table 1). More specifically, microplastic abundances are often higher at sites downstream of WWTPs (Hoellein et al. 2017, Liu et al. 2020, Schmidt et al. 2018, Shruti et al. 2019), with one estimate showing microplastic abundances at sites downstream of WWTPs exceeding those at upstream sites by a factor greater than nine (McCormick et al. 2014). In these instances, smaller particles and fibers may not be captured by treatment processes and thus end up in effluent (McCormick et al. 2016). Because of this, high downstream concentrations of smaller microplastics in particular may indicate that WWTPs serve as a pathway for these plastics to freshwater environments.

While WWTPs are generally accepted as major delivery pathways of microplastics, the relationship between microplastics and effluent is not always so clearly defined. Some analyses (41.7%) have not found correlations between the two (Bujaczek et al. 2021, Klein et al. 2015, Peller et al. 2019, Stanton et al. 2020, Tibbetts et al. 2018) (Table 1). One potential explanation is that nets with larger mesh sizes do not capture smaller microplastics (Dris et al. 2015), and consequently may not produce evidence of a relationship between microplastics and effluent. Additionally, higher microplastic loads upstream of WWTPs may be due to downstream dilution resulting from the release of effluent (Tien et al. 2020). Lastly, the influence of WWTPs on downstream microplastic concentrations may also depend upon the specific wastewater treatment processes, with tertiary treatments typically more successful in removing microplastics (Bujaczek et al. 2021, McCormick et al. 2016). Such results may indicate that WWTPs should not necessarily be generalized as main sources or pathways of microplastics. While effluent may certainly exert an influence, microplastic sources in freshwater bodies are very diverse (Huang et al. 2020), and other attributes may overshadow the role of effluent in certain situations (Bujaczek et al. 2021, Tien et al. 2020). Indeed, the lack of a correlation between microplastics and effluent led Klein et al. (2015) to conclude that hydrodynamic processes may in fact play a more important role in the distribution of microplastics. In light of this theory, an important avenue for future research may include the influence of such microscale variations on microplastic pollution.

2.3 Agricultural land cover

Links between microplastic pollution and agricultural regions are also not clearly defined, with some studies (40%) reporting lower abundances in these zones than in other land use categories (Grbić et al. 2020, Huang et al. 2020) (Table 1). This negative relationship may be attributed to factors such as lower population densities in agricultural regions (Huang et al. 2020), or to the potential for agricultural soils to serve as a sink for plastic particles (Feng et al. 2020). Other studies (60%) report no significant correlations between microplastics and agricultural land use (Barrows et al. 2018, He et al. 2020b, Nihei et al. 2020), indicating that other factors may exert a stronger influence on microplastic pollution.

While negative or no relationships have been reported in studies examining links between microplastics and agricultural land use, more studies are needed to incorporate other variables related to agricultural practices. Microplastic-rich biosolids have been applied widely to agricultural lands as crop fertilizers, which can contaminate soils and runoff (Leslie et al. 2017). Additionally, plastic covers and tarps have been used to retain moisture and discourage weed growth in agricultural fields, which can break down and work their way into the environment if not collected immediately after harvest (Feng et al. 2020). Therefore, it is important to understand the transport pathways of such microplastics to soils and streams. Exploring these connections and focusing on the proper management of agricultural lands should be a high priority in future research (Ding et al. 2019).

2.4 Microplastics in remote regions

Additional research has supported the trend of decreased microplastic concentrations at sites located further in proximity from urban and industrial regions (Di and Wang 2018, Grbić et al. 2020, Huang et al. 2021, Peng et al. 2018, Su et al. 2020, Tibbetts et al. 2018, Yonkos et al. 2014). This may be the case particularly in forested regions (Grbić et al. 2020) and in water bodies located near nature preserves or natural areas (Huang et al. 2021). However, water bodies in these regions have still been found to contain microplastics. While microplastic concentrations generally decrease at sites far from anthropogenic activities, microplastics have been found in historically pristine regions as well, despite no nearby industrial or developed regions (Jiang et al. 2019).

High levels of microplastics in these regions may be due to heavy tourist activities, resulting in increased littering (Feng et al. 2020) and the transfer of plastic wastes to more remote downstream locations. Recreation and tourism may thus potentially serve as important sources of microplastics (Barrows et al. 2018, Feng et al. 2020), as can fishing and fishery activities, as nets and fishing lines degrade over time and remain in freshwater environments (Belen Alfonso et al. 2020, Di and Wang 2018, Xia et al. 2020). Wind may also serve as a critical large-scale transport mechanism by carrying microplastics from developed regions to more remote ones (Jiang et al. 2019), thus underscoring the importance of atmospheric deposition. These findings are pivotal to microplastics research, as they indicate that potentially no body of water is immune to microplastic pollution.

2.5 Population density

Population density is often tied to microplastic pollution in freshwater bodies, with numerous studies finding positive correlations between the two (Battulga et al. 2019, Bertoldi et al. 2021, Corcoran et al. 2020b, Fan et al. 2019, Grbić et al. 2020, Huang et al. 2020, Kataoka et al. 2019, Mai et al. 2021, Nihei et al. 2020, Yonkos et al. 2014) (Table 1). High microplastic concentrations may be found in waters adjacent to regions characterized by high population density for a number of reasons. Fibers in particular are produced by the laundering of synthetic materials, subsequently making their way into washing machine effluent (McCormick et al. 2016, Peller et al. 2019). Direct laundering of clothing in rivers can also be key in introducing microplastics to freshwater environments (Alam et al. 2019). Additionally, pellets found in personal care products such as exfoliants often show up in household sewage (McCormick et al. 2016). Links have been found between residential zones and microplastic concentrations (Sang et al. 2021), with domestic sewage, new residence construction, and roads contributing microplastics to aquatic environments (Dikareva and Simon 2019). Additionally, recent research has found positive links between microplastic pollution and gross domestic product (Fan et al. 2019, Huang et al. 2020, Zhou et al. 2020), highlighting the potential for socio-economic factors to play a role in the presence and prevalence of microplastics.

Other research has not shown clear connections between microplastics and population density (Belen Alfonso et al. 2020, Feng et al. 2020, Kapp and Yeatman 2018, Klein et al. 2015, Mbedzi et al. 2020, Tibbetts et al. 2018, Zhou et al. 2020) (Table 1). As a potential explanation, Dikareva and Simon (2019) suggested that previous reported links between the two may be due to study designs of a "coarse manner with a limited number of sites," or to designs that encompass sites representing only population density extremes. Thus, the degree to which a broad population density gradient is represented may exert an influence on observed microplastic concentrations, in addition to factors such as the total number of study sites and number of samplings (Belen Alfonso et al. 2020, Dikareva and Simon 2019). Additionally, population density may serve as a stronger driving force for microplastic pollution when considered in tandem with other factors, such as seasonality. For instance, activities conducted in a populous region may change across seasons, resulting in a significant interaction effect between seasonality and population density (Mbedzi et al. 2020).

2.6 Physical watershed/stream characteristics

While many studies have addressed links between microplastic pollution and the influence of anthropogenic activities, very few have examined the role of physical watershed characteristics and geomorphology (Table 1). For instance, increased slope of the riparian zone can lead to elevated microplastic abundances in surface water samples (Grbić et al. 2020). In addition, Su et al. (2020) found higher microplastic concentrations in Australian water bodies located at lower elevations (Table 1). Very little data exist regarding whether water body width may influence microplastic accumulation, with initial research not finding statistically significant relationships between these variables (de Carvalho et al. 2021). The above findings indicate the potential for small-scale physical features of watersheds to exert an influence on microplastic accumulation and abundance. However, the limited number of studies addressing such factors indicates that more research is needed.

These results also highlight the variations in microplastic distributions between sediment and water samples. Generally speaking, polymers with densities less than that of water (e.g., polypropylene, polyethylene) are more buoyant and are often found in the upper levels of the water column in calm waters (Di and Wang 2018, Wang et al. 2020). Polymers whose densities exceed that of water (e.g., polyethylene terephthalate, polyvinyl chloride) are more apt to sink and settle on the channel bottom (Wang et al. 2020). However, more than half of the studies in running water did not examine microplastics in sediment, while nearly two-thirds of studies in still water investigated sediment samples (Figure 3).

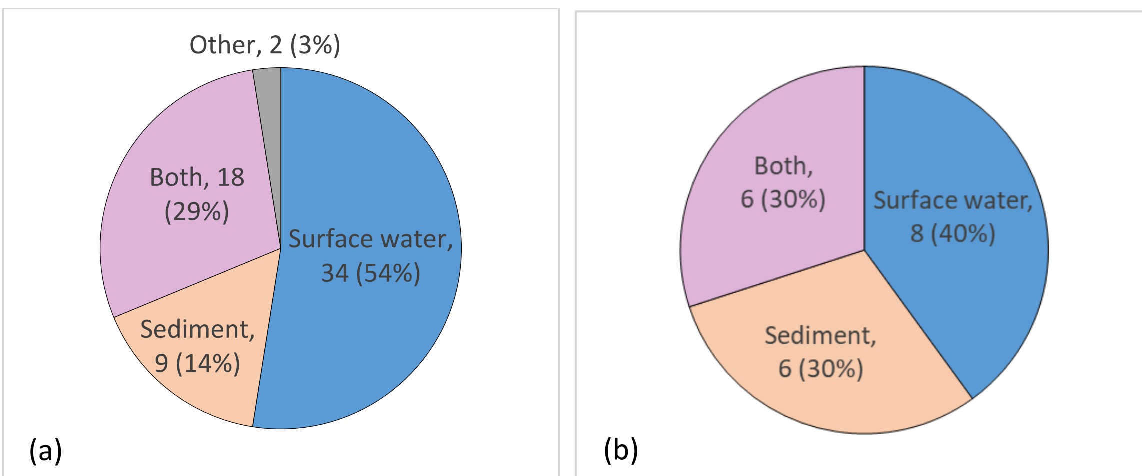


Figure 3. Number of publications addressing microplastic concentrations in surface water, in sediment, or in both. (a) represents studies addressing microplastics in running water (e.g., rivers, streams), and (b) represents those addressing microplastics in still water (e.g., lakes, ponds). Several studies sampled both running water and still water, and are thus represented in both (a) and (b). Note: The study falling into the "Other" category involved the collection of visible plastic debris on shores, which contained microplastics (Battulga et al. 2019), or the collection of pellets on shores (Corcoran et al. 2020b).

Additionally, there may exist a relationship between sediment grain size and microplastic abundance. More specifically, small-grained sediments and sand may be linked with greater numbers of microplastics, due to the ability of both to settle out of the water column in lower velocity flows (Corcoran et al. 2020a, He et al. 2020b, Dikareva and Simon 2019, Sarkar et al. 2019, Tibbetts et al. 2018). Conversely, fewer microplastics have been found at sites characterized by coarser sediments and higher flows (Tibbetts et al. 2018).

3. Factors Affecting the Temporal Distribution of Microplastics

Microplastic abundances vary on a temporal basis, which can be attributed to both hydroclimatic and hydrodynamic factors, as well as the frequency of sampling. Previous studies have focused on the impacts of precipitation, runoff, and flow rate on microplastic distributions and abundances. Table 2 shows the positive and negative relationships between microplastics and these factors, and includes 26 studies that found significant correlations. These studies indicated temporal dependence of microplastic concentrations (i.e., these studies reported significant findings with regard to temporal factors such as seasonality, precipitation, stormflow, or flow rate/discharge). Six studies indicated both spatial and temporal dependence (Chen et al. 2020, de Carvalho et al. 2021, Fan et al. 2019, Grbić et al. 2020, Sang et al. 2021, Schmidt et al. 2018) (Table 1).

3.1 Effects of precipitation seasonality on microplastic concentrations

Microplastic concentrations are influenced by factors intrinsic to the changing seasons, particularly with regard to precipitation (Xia et al. 2020). Precipitation may serve to transport land-based microplastics into aquatic environments, and high abundances of microplastics in surface waters have been observed following such rain events (Schmidt et al. 2018, Wong et al. 2020, Xia et al. 2020). In particular, precipitation may lead to a first flush event, in which microplastics that have accumulated on land during dry periods

Table 2. Temporal factors affecting MP concentrations in freshwater. Percentages in parentheses refer to the relative number of articles (as a function of either still water or running water) that assessed correlations with temporal factors.

Explanatory factors	Lakes and reservoirs			Running water		
Hydro-climatic factors	(+)	(-)	No relation	(+)	(-)	No relation
Wet season	-	Liu et al. 2019c, Mbedzi et al. 2020, Wang et al. 2021 (60%)	Hengstmann et al. 2021^, Su et al. 2016 (40%)	Campanale et al. 2020, Chen et al. 2020, Eo et al. 2019, He et al. 2020a (23.5%)	Barrows et al. 2018, de Carvalho et al. 2021, Fan et al. 2019, Wang et al. 2021, Weideman et al. 2020, Wu et al. 2020 (35.3%)	Chanpiwat and Damrongsiri 2021, Constant et al. 2020, Mani and Burkhardt-Holm 2020, Mintenig et al. 2020, Peller et al. 2019, Stanton et al. 2020, Zhao et al. 2020 (41.2%)
Precip	Xia et al. 2020 (50%)	-	Belen Alfonso et al. 2020 (50%)	Piñon-Colin et al. 2020, Schmidt et al. 2018, Wong et al. 2020 (50%)	-	Constant et al. 2020, de Carvalho et al. 2021, Mani and Burkhardt-Holm 2020 (50%)
Storm runoff	-	-	-	Cheung et al. 2019, Grbić et al. 2020, Piñon-Colin et al. 2020, Sang et al. 2021 (80%)	Hurley et al. 2018 (20%)	-
Flow velocity/ discharge	-	-	-	Campanale et al. 2020, Mani and Burkhardt-Holm 2020, Wagner et al. 2019 (23.1%)	Barrows et al. 2018, de Carvalho et al. 2021, Kapp and Yeatman 2018, Sarkar et al. 2019, Tien et al. 2020, Xiong et al. 2019 (46.1%)	Bujaczek et al. 2021, Constant et al. 2020, Dris et al. 2018, Lechthaler et al. 2021 (30.8%)

^ Indicates microplastic concentrations in lakebed sediments.

are flushed into freshwater environments in the early wet season (Schmidt et al. 2018). In this vein, antecedent precipitation may strongly influence observed concentrations of microplastics. For instance, rain events preceded by dry periods lasting several weeks can result in significantly higher microplastic levels than samples collected during the dry period, with similar yet muted results regarding microplastic samples collected after a rain event preceded by a week-long dry period (Schmidt et al. 2018). These findings suggest that dry periods may facilitate the accumulation of microplastics on land-based surfaces, with subsequent rain events flushing them into nearby rivers and streams (Schmidt et al. 2018).

The vast majority of a river's annual surface water microplastic load may be directly linked with the wet season (Eo et al. 2019), likely a product of increased runoff introducing microplastics to receiving waters as well as the resuspension of microplastics from benthic sediments (Hurley et al. 2018, Xia et al. 2020) (Table 2). It is thus not uncommon to observe significant differences in microplastic abundances between the wet and dry seasons, with indications that higher abundances in surface waters are present in the wet season (Campanale et al. 2020, Eo et al. 2019). However, these trends may not necessarily pertain to microplastics in sediment. For instance, lower concentrations of microplastics in river sediments following major flooding events indicate that floods may flush and resuspend microplastics from aquatic sedimentary environments (Hurley et al. 2018, Liu et al. 2019c). In addition, higher microplastic abundances in sediment than surface water may be present during the dry season, due to low flow facilitating the settling out of microplastics into sediment (Eo et al. 2019, Liu et al. 2019c, Mbedzi et al. 2020).

It is also suggested that such disparities exist between sediment and surface water microplastics due to more intense microplastic fluctuations in surface water. Microplastics may remain trapped in sediments for longer periods of time and thus represent more long-term concentrations (Ding et al. 2019). An examination of stormwater retention ponds in Denmark identified significant relationships between microplastic concentrations in water samples and land use categories (Liu et al. 2019a), yet when evaluating sediment samples from these same retention ponds, Liu et al (2019b) found no evidence of such relationships. Because of such disparities, it is not uncommon for analyses to find no correlations between surface water and sediment samples regarding observed microplastic abundances (Constant et al. 2020, Deng et al. 2020, Li et al. 2020b), or to find that abundances between the two are not proportional (Di and Wang 2018, Ding et al. 2019).

Microplastic abundances may also vary as a function of the type of sediment sampled. For instance, Hengstmann et al. (2021) reported substantial differences in microplastic abundances found in lakeshore sediments between seasons, with no such seasonal trend observed for lakebed sediments. Such a finding may result from the tendency for benthic sediments in particular to serve as a sink for microplastics (He et al. 2020a, Hengstmann et al. 2021).

Some studies do not report significant links between microplastics and seasonality (Chanpiwat and Damrongsiri 2021, Constant et al. 2020, Mani and Burkhardt-Holm 2020, Mintenig et al. 2020, Stanton et al. 2020, Su et al. 2016) . Additionally, negative or no relationships have been reported between microplastics and precipitation, indicating the potential for storm events and flooding to dilute microplastic concentrations in surface waters (Barrows et al. 2018, de Carvalho et al. 2021, Fan et al. 2019, Stanton et al. 2020). Increased abundances of microplastics in surface waters have also been reported during the dry season (de Carvalho et al. 2021, Fan et al. 2019, Wang et al. 2021, Weideman et al. 2020, Wu et al. 2020). These findings may be a result of microplastics being more heavily influenced by anthropogenic as opposed to environmental variables (Mani and Burkhardt-Holm 2020), or the potential for microplastics to vary more strongly as a function of spatial rather than temporal factors (Mintenig et al. 2020). Physical characteristics of microplastics (e.g., size, shape) may also play a role, in that smaller microplastics may remain in the upper water column during periods of low flow (de Carvalho et al. 2021). With varying results regarding the influence of seasonality and precipitation, future research is needed to address microplastic pollution at finer temporal and spatial resolutions.

3.2 Effects of storm runoff on microplastic concentrations

As previously noted, these findings suggest that stormwater runoff plays a critical role in delivering microplastics to freshwater bodies (Cheung et al. 2019, Grbić et al. 2020, Piñon-Colin et al. 2020, Sang et al. 2021) (Table 2). Higher precipitation rates have been correlated with increased microplastic pollution in stormwater runoff, potentially due to factors such as the flushing of discarded plastics into pipelines during storm events (Sang et al. 2021), as well as combined sewer overflows (Piñon-Colin et al. 2020). Indeed, these overflows may serve as critical transport pathways to aquatic environments. While few studies incorporate a focus on combined sewer overflows, preliminary research shows elevated abundances of microplastics in overflows, even exceeding those found in WWTP effluent (Chen et al. 2020). Future research should closely address this potentially critical link with microplastics pollution. The above results suggest that runoff may serve as a major delivery pathway of microplastics, by both introducing land-based plastics to freshwater bodies (Sang et al. 2021) as well as facilitating the delivery of microplastics to estuarine or marine environments (Zhao et al. 2020).

Selecting appropriate sampling times may be critical in evaluating the effects of rainfall and runoff on microplastics, as abundances can fluctuate greatly over relatively short periods of time. For instance, Cheung et al. (2019) sampled after a storm event and reported that microplastic concentrations decreased dramatically over the course of just two hours, and continued to decrease substantially with further samplings. Microplastic pollution is thus very closely tied to runoff processes, which can lead to quick variations in microplastic concentrations (Cheung et al. 2019, Hurley et al. 2018). As few studies incorporate an in-depth examination of microplastic concentrations over the course of a single rainfall event, additional fine temporal-scale research is needed when evaluating the role of precipitation and runoff. Knowing when these concentrations tend to be higher can provide insight regarding potential delivery pathways to riverine environments, which can assist in informing management decisions concerning microplastic waste.

3.3 Effects of flow on microplastic concentrations

There is evidence that microplastics are influenced by water velocity, in that lower flow rates and weakened hydrodynamics may facilitate their accumulation (Barrows et al. 2018, de Carvalho et al. 2021, Kapp and Yeatman 2018, Sarkar et al. 2019, Tien et al. 2020, Xiong et al. 2019) (Table 2). For instance, lower microplastic concentrations have been observed in the center of river channels themselves (Corcoran et al. 2020a, Tibbetts et al. 2018), with greater numbers of microplastics found along river banks (Dris et al. 2018). Interestingly, Wagner et al. (2019) found a positive correlation between microplastic concentrations and discharge in urban subwatersheds in Germany, with no such relationship in rural subwatersheds. The positive relationship may have been due to inputs from combined sewer overflows (Wagner et al. 2019). It is less common for studies to show no relationship between flow rate/discharge and microplastic concentrations (Bujaczek et al. 2021, Dris et al. 2018, Lechthaler et al. 2021).

As a function of both spatial and temporal variables, microplastic concentrations are highly heterogeneous within a given river (Kataoka et al. 2019, Stanton et al. 2020). These factors can greatly influence the number of microplastics that are delivered to aquatic environments, as well as the degree to which in-stream processes facilitate or hinder accumulation. Variations in seasonal microplastic abundance and distribution is at least partially a function of hydrologic variables (Campanale et al. 2020, de Carvalho et al. 2021, He et al. 2020a). If such processes are intense, microplastics are less apt to settle or to remain trapped in sediment, and are more likely to become suspended in the water column (Luo et al. 2019). Slower flow rates may lead to the accumulation of microplastics in sediments and at lower depths in the water column, as these conditions facilitate the settling of microplastics (Tien et al. 2020). In this sense, streams and rivers have the potential to serve as microplastic sinks, with microplastic concentrations varying based on the time of year. Thus, instead of being continually transported along the length of a river, they can remain trapped in sediment until a rain event occurs and spurs their resuspension (Hurley et al. 2018).

4. The Role of Scale

Scale may play an important role when evaluating the distribution of freshwater microplastics, and the studies selected for this review focused on a variety of spatial and temporal scales. As shown in Figure 4a, some specific hydrological and anthropogenic processes may dominate microplastic concentrations at specific spatial and temporal scales. From a spatial perspective, these analyses range from a single point source or river reach to the study of watersheds at a national level. From a temporal perspective, they range from a single sampling session to annual sampling sessions. As shown in Figure 4b, a majority of studies examined microplastic concentrations using a snapshot approach rather than a range of scales. In particular, only a few studies investigated a longer term with a larger spatial extent.

Some studies examined microplastic pollution as a function of watershed-scale attributes such as land use and population density (Grbić et al. 2020, Su et al. 2020, Yonkos et al. 2014). However, Dikareva and Simon (2019) argued that such attributes fail to fully explain variations in microplastic distributions, and that a focus on local-scale attributes is just as crucial. In particular, an emphasis on specific point sources (e.g., plastic production facilities and dumping sites) of microplastic pollution may provide valuable insight regarding variations in microplastic concentrations (Dikareva and Simon 2019). Similarly, Barrows et al. (2018) noted that analyses at the larger watershed scale may not provide a comprehensive picture of microplastic pollution and corresponding sources, and that future study designs may benefit from incorporating a focus on individual or specific sources of pollution. However, a sole focus on such point sources excludes the influence of important nonpoint sources such as runoff (Cheung et al. 2019).

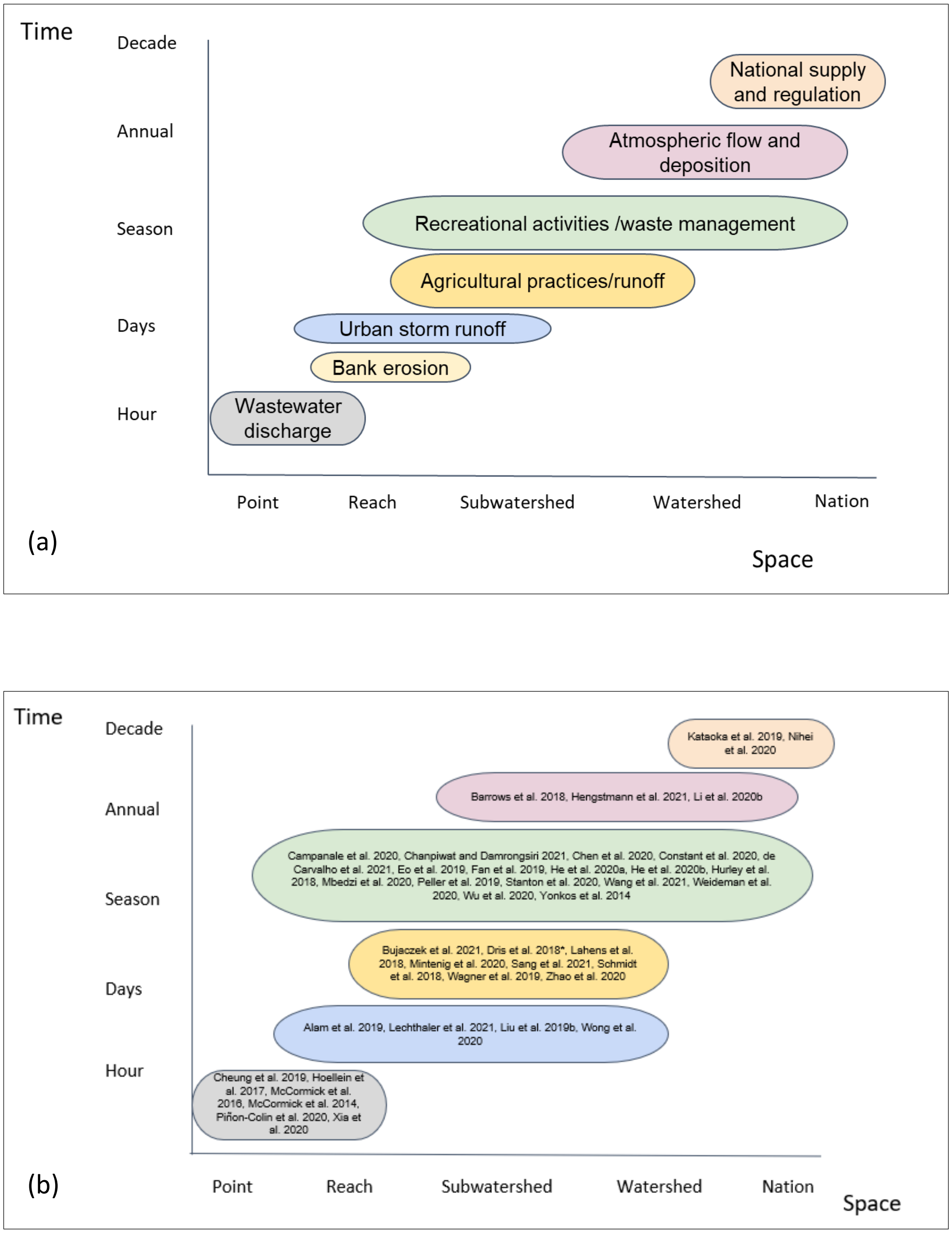


Figure 4. Hydrological and anthropogenic processes affecting microplastic concentrations in freshwater environments (a) across a range of space and time scales and (b) exemplary case studies. Asterisks denote studies that included more than one spatial or temporal scale.

Finer temporal resolutions are also becoming increasingly imperative in more fully understanding the microplastic cycle (Grbić et al. 2020, Stanton et al. 2020). As previously mentioned, microplastic concentrations can vary quite drastically over smaller temporal intervals, whether these differences are observed over several weeks (Stanton et al. 2020), from one day to the next, (Xia et al. 2020), or even over the course of a few hours (Cheung et al. 2019). With microplastic fluctuations occurring with such a high frequency, it becomes increasingly difficult for studies focusing on larger temporal intervals to not only pinpoint sources, but also to estimate accurate microplastic fluxes (Stanton et al. 2020, Zhao et al. 2019).

Additionally, few studies appear to explicitly define the spatial extent of contributing areas to microplastic pollution in freshwater bodies. The use of such well-defined scales and extents could greatly facilitate the comparison of results across studies, and allow for a greater understanding of the factors that influence the distribution and abundance of microplastic particles. For instance, this could include more specific spatial extents, such as the delineation of subwatersheds, the incorporation of riparian buffers, or the use of specific distances from study sites (Grbić et al. 2020, Wagner et al. 2019).

These variations in contributing areas may lead to differences in reported correlations, and different approaches may increase difficulty in evaluating the true impact of land cover on microplastic pollution. Some studies have used a specified radius around urban centers in the classification of urban sites, with sites exceeding this distance designated as rural (Corcoran et al. 2020a). In a similar vein, various radii around study sites have been incorporated to assess the impact of other watershed attributes such as population density (Tibbetts et al. 2018). Other studies have calculated the proportion of various land use categories within watersheds (Barrows et al. 2018, Kataoka et al. 2019), with the delineation of subwatersheds upstream of study sites used in the evaluation of watershed attributes (Nihei et al. 2020, Su et al. 2020, Wagner et al. 2019). The use of such differing techniques highlights the need for standardized spatial analysis methodologies.

Interestingly, only one study noted the use of a riparian buffer, and this was used in conjunction with analyses conducted at the full watershed level (Grbić et al. 2020). While the latter analyses produced negative correlations between microplastics and agricultural land covers, and analyses at the riparian scale showed a positive relationship between microplastics and slope in the buffer zone, there were few differences present between the two methods (Grbić et al. 2020). More research is needed at a broader range of scales to better understand the impacts on microplastic pollution.

Additionally, distance-weighted algorithms recently developed in spatial hydrology can offer new insights on sources and delivery pathways of microplastics in freshwater environments (Mainali et al. 2019). Different scales of analyses can capture different factors that are linked with freshwater contamination, with Mainali et al. (2019) noting that a major upstream source of contamination may not be identified in an analysis that focuses solely on a stream's riparian zone. Conversely, explanatory variables more closely correlated with proximity to a water body (e.g., topographic factors such as slope) may be overlooked in an analysis that incorporates the full watershed scale.

The scale-dependent processes could also vary along urbanization or flow gradient (Figure 5). This figure outlines conditions for which either microscale or large-scale processes may dominate in driving microplastic concentrations in freshwater environments, with microplastic pollution shown as a function of both flow rate and anthropogenic activities. For example, microplastic concentrations may be more subject to microscale processes resulting from spatial heterogeneity in the urban environment during low flow season. There may be lower input from terrestrial sources, and increased microplastic concentrations may be particularly apparent in riverine sediment (Hoellein et al. 2017).

Figure 5. Dominant scale processes as a function of urbanization and flow gradients. The picture in each quadrant represents the combination of flow rate and anthropogenic activities present for each condition. For instance, the lower right quadrant represents low flow conditions in a region characterized by anthropogenic activities, and is represented by stagnant water in an urban area with high levels of visible plastic pollution.

Conversely, upstream processes may become more important for determining microplastic concentrations during the high flow season, in which microplastics may either increase due to increased transport (i.e., runoff) to freshwater environments (Campanale et al. 2020) or decrease due to dilution effects (Fan et al. 2019). These large-scale processes may also be more important in regions characterized by fewer anthropogenic activities, as atmospheric sources may play a more critical role (Jiang et al.

2019). Thus, the most appropriate scale for a given study may vary depending upon the study goals. For instance, Hoellein et al. (2017) discussed the need for larger scales when investigating issues pertaining to deposition, and smaller scales for research centered around factors pertaining to microplastics distribution in riverbed sediments.

Tailoring the analytical approach to the study region may also be a worthwhile pursuit, in that multiscale analyses or distance-weighted algorithms may shed further light on microplastic sources and pathways in different environments. For instance, urban environments are comprised of a broad range of potential plastic sources, in terms of both specific point sources as well as nonpoint sources such as runoff (Deng et al. 2020, Piñon-Colin et al. 2020). In such environments, it may be critical to more fully address spatial heterogeneity (Mani and Burkhardt-Holm 2020, Mintenig et al. 2020) than in remote regions characterized by fewer anthropogenic activities. The use of an inverse distance-weighted technique, a common method employed in water quality studies (Mainali et al. 2019), was not observed in any of the reviewed studies.

5. Summary and Future Research Directions

As research in the field of freshwater microplastics is still in the developing stages, much is still unknown regarding their spatiotemporal distributions and links to potential sources. It is much more common for studies to examine microplastic concentrations as a function of either spatial or temporal factors, with very few addressing both and across scales. It is also imperative that standard sampling procedures are developed, to ensure consistency of microplastics research as well as to facilitate cross-study comparisons. For instance, a range of net mesh sizes are currently employed when collecting microplastics in surface water, and a standard size would be ideal.

Preferably these nets would include a very small mesh to capture tinier microplastics, which tend to greatly outnumber larger size categories (Chen et al. 2020, Fan et al. 2019, Schmidt et al. 2018). Additionally, replicates should be collected to capture within-site microplastic variability.

More research is needed concerning microplastic concentrations as a function of seasonality, particularly regarding variations within the wet season. Differences likely exist between microplastic concentrations in the early versus the late wet season due to factors such as the flush effect and flow dependency, and our understanding of the drivers of microplastic abundance would greatly benefit from more fine-scale temporal research. Future study designs should incorporate evaluations of microplastic variations across very short time periods (e.g., minutes/hours) as well as evaluations spanning multiple years and seasons, to more thoroughly investigate the range of factors influencing microplastic fluctuations over time. As previously noted, sample collection in surface water or sediment can greatly affect observed microplastic concentrations as well as morphologies and polymer types (Di and Wang 2018, Hoellein et al. 2017). Thus, future studies can include the collection of microplastic samples in both sediment and surface waters to obtain a more comprehensive picture of microplastic pollution within a freshwater environment.

Additionally, few studies incorporate a focus along an urban-rural gradient (Chen et al. 2021), or address the effects of landscape fragmentation on microplastic distributions. Such analyses could reveal potential sources and delivery pathways of microplastic pollution, and GIS analyses could be incorporated into future study designs to facilitate our understanding of direct relationships between microplastic pollution and various watershed characteristics. Future research could also more thoroughly address the drivers of microplastic abundance, including the role of resuspension of sediments as well as flush effects from storm events. Very little is also known regarding microplastic pollution in groundwater, and future research could address how these abundances compare to surface water and sediment microplastic concentrations. Lastly, very few studies have addressed potential relationships between microplastics and physical characteristics such as slope, elevation, and river morphologies, and this is thus an area ripe for future research.

Generally speaking, it is not uncommon for speculations to be made with regard to potential microplastic sources and links with watershed attributes, as specific sources can be quite difficult to identify and can encompass a broad range (Huang et al. 2020). While many studies may speculate regarding potential ties with variables such as urban land cover or population density, more definitive trends may not be known or examined, and this appears to be the case for both spatial and temporal analyses. Plastic pollution is becoming an increasingly serious global issue, particularly during the COVID-19 era, in which the widespread use of disposable face masks and other personal protective equipment, increase in take-away plastic containers and utensils from restaurants, and uptick in the ordering of online products has resulted in greater plastic waste (Ammendolia et al. 2021, Ma et al. 2021a). It is thus imperative for future research to incorporate more testing and statistical analyses regarding potential explanatory variables derived from a range of scales.

While some studies note atmospheric deposition as a possible explanation for elevated microplastic levels (Jiang et al. 2019, Liu et al. 2019b, Stanton et al. 2020), very few studies have incorporated the collection of such samples into their analyses. It is a growing area of research, and initial results suggest that microplastics deposited via this pathway may be much greater than observed concentrations in rivers (Brahney et al. 2020, Constant et al. 2020, Rochman and Hoellein 2020). Standardization of practices and methodologies across space may facilitate the ability to more definitively address these concerns and understand the microplastic cycle.

Evaluating microplastic concentrations is a pressing global environmental issue, and collaborations will be crucial in alleviating it (Borrelle et al. 2020, Gong and Xie 2020). Due to the wide array of sampling techniques, procedures and reporting units, it will additionally be imperative to create standardized methodologies to facilitate comparisons across studies (Campanale et al. 2020, Li et al. 2020a). With clear evidence that microplastics are ingested by a range of aquatic species, they can enter the food chain and thus potentially be ingested by humans (Li et al. 2020a). Their hydrophobic surfaces facilitate the sorption of a variety of metals and contaminants, thus exacerbating the risk to aquatic organisms (Wang et al. 2020, Zhang et al. 2020). A thorough and timely examination of microplastic sources and abundances at a range of spatial and temporal scales is therefore critical in developing policies and management procedures to reduce their release to the environment and minimize such negative consequences.

Chapter 2

Spatial and Seasonal Variations of Microplastic Concentrations in Portland's Freshwater Ecosystems

1. Introduction

1.1 Microplastic Characterization

Microplastics are an increasing concern in aquatic environments, capable of entering the food web and potentially endangering human health (Baldwin et al. 2020, Li et al. 2020a). Microplastic research first gained traction in the 1970s (Carpenter and Smith 1972), with studies largely addressing marine environments over the next several decades. Freshwater microplastic pollution is a relatively new field of research, with articles published only within the last ten to fifteen years (Talbot and Chang 2022). This research expansion has provided valuable insights into the microplastic cycle and the factors that influence their accumulation and distribution in freshwater bodies. River systems, in particular, are critical transportation pathways, carrying microplastics from inland regions to estuarine and marine environments (Jiang et al. 2019, Zhao et al. 2019). Thus, understanding their presence in freshwater environments can shed light on their abundance in marine waters, which may be greater than previously estimated due to recent evaluations of riverine microplastic flux (Hurley et al. 2018).

Generally accepted as any plastic particle between the lengths of one micron and five millimeters, microplastics form through the process of either primary or secondary production (Eerkes-Medrano et al. 2015, Horton et al. 2017). In the former, microplastics are manufactured at very small sizes, and are thus already less than five millimeters when they enter the environment. Secondary production involves the formation of microplastics through the degradation of larger pieces of plastic. Microplastic size is typically negatively correlated with abundance, with very small microplastics found in much higher concentrations than larger particles (Barrows et al. 2018, Hitchcock 2020, Huang et al. 2020, Mintenig et al. 2020). Additionally, specific polymer types may provide insight into potential origins. For instance, the Pearl River in China has elevated levels of polyamide, a common polymer found in food packaging, indicating that these microplastics likely originated from litter (Yan et al. 2019). Findings from these studies indicate that the majority of microplastics are produced via the degradation of larger pieces of plastic, with fewer resulting from the process of primary production.

1.2 Microplastics and Influential Spatial Factors

Urban and industrial regions have been closely linked with microplastic pollution, in part due to high rates of plastic production and increased littering (Huang et al. 2020, Ma et al. 2021b, Mani et al. 2015, Townsend et al. 2019). Positive correlations have also been found between microplastic pollution and percent of impervious cover in watersheds, which greatly serves to enhance plastic transport to aquatic environments (Baldwin et al. 2016). A recent examination of microplastic pollution in four Oregon rivers found high concentrations of microfibers at an urban site by downtown Portland, with lower concentrations present at more remote sites (Valine et al. 2020). In addition, wastewater treatment plants (WWTPs) are often situated in urban areas, and have been linked with increased microplastic concentrations downstream of effluent outfalls (Estahbanati and Fahrenfeld 2016, Hoellein et al. 2017). Most treatment processes are not designed to remove tiny plastic particles, and may result in WWTPs serving as important delivery pathways of microplastics to freshwater environments (Mani et al. 2015, McCormick et al. 2016).

Microplastic pollution may also be linked with agricultural regions (Kapp and Yeatman 2018). WWTP sludge frequently contains high concentrations of microplastics, and this sludge is often treated and applied to agricultural lands as biosolids (Leslie et al. 2017, Mahon et al. 2017). Biosolids tend to be nutrient-rich and thus act as a fertilizer for crops, yet their application on agricultural lands can result in the introduction of microplastics to these environments. While agricultural soils may serve as a sink for many of these plastic particles (Feng et al. 2020), these soils and the plastics they contain may also be vulnerable to reentering surface water bodies during storms and subsequent runoff events (Kapp and Yeatman 2018, Peller et al. 2018). Additionally, agricultural regions tend to include the heavy use of plastics, such as baling twine and tarps. These plastics can break down over time and potentially enter freshwater bodies, and may result from the degradation of tarps, irrigation pipes, and plastic film mulching (Campanale et al. 2020b, Feng et al. 2020, Grbić et al. 2020, Guerranti et al. 2017).

While microplastic concentrations are heavily influenced by proximity to anthropogenic activities, they have been found in remote regions as well. A study of six rivers located in a remote region of Tibet showed the existence of microplastic pollution, despite no nearby industrial or developed regions (Jiang et al. 2019). These elevated levels of microplastics may be due to activities of nearby residents or tourists, or through the transport of these plastics to remote regions via air currents (Jiang et al. 2019). Atmospheric deposition may thus play a critical role in the transport of microplastics to more remote regions, with recent research in the Rocky Mountains of Colorado finding elevated levels of microplastic fibers in atmospheric deposition samples (Wetherbee et al. 2019).

Population density has also been positively correlated with microplastic concentrations (Battulga et al. 2019, Huang et al. 2020, Kataoka et al. 2019, Ma et al. 2021b, Mani et al. 2015, Valine et al. 2020, Yonkos et al. 2014). This may be due to factors such as increased levels of microfibers present in washing machine effluent, as well as household sewage containing pellets from personal care products (McCormick et al. 2016). Indeed, Baldwin et al. (2016) found a positive correlation between population density and the presence of pellets and microbeads. Rodrigues et al. (2018) found a prevalence of foams, often a by-product of food packaging, adjacent to highly populated regions.

1.3 Microplastics and Influential Temporal Factors

In addition to variations in spatial distribution, microplastic concentrations vary on a temporal basis as well. Seasonality and fluctuations in precipitation have been shown to influence the distribution and abundance of microplastics in freshwater environments (Baldwin et al. 2016, Campanale et al. 2020b, Wu et al. 2020). Many studies report increased microplastic concentrations during the wet season, as land-based microplastics may be introduced to waterways via storm runoff (Eo et al. 2019, Hurley et al. 2018). As such, precipitation may serve to flush microplastics into aquatic environments with subsequent increased microplastic concentrations reported (Hitchcock et al. 2020, Schmidt et al. 2018, Wong et al. 2020). However, negative relationships have also been observed between precipitation/discharge and microplastic abundance, with the former potentially causing decreased concentrations of the latter due to dilution effects

(Barrows et al. 2018, Stanton et al. 2020). These findings indicate the need for additional research conducted on finer temporal scales.

Flow rate has also been linked with microplastic concentrations, with gentler hydrodynamics potentially facilitating their accumulation (Kapp and Yeatman 2018, Mani et al. 2015, Xiong et al. 2019, Watkins et al. 2019). This phenomenon may be particularly apparent in slower moving bodies of water such as lakes and reservoirs (Free et al. 2014, Tibbetts et al. 2018, Watkins et al. 2019a), and in waters located immediately upstream of dams (Huang et al. 2020, Shruti et al. 2019, Zhang et al. 2015). Because of weakened hydrodynamics in relatively closed freshwater bodies such as lakes, microplastics are more easily able to accumulate (Wang et al. 2017). Conversely, higher flow rates in the center of rivers have resulted in observations of decreased microplastic concentrations, with river banks serving as microplastic sinks (Tibbetts et al. 2018).

1.4 Research Focus

As research in the field of freshwater microplastics is still in the early stages, much is still unknown regarding their spatial and temporal distributions and links to potential sources. Few studies have examined variations in microplastic concentrations as a function of seasonality, with even fewer addressing variations observed within the wet season. Differences likely exist between microplastic concentrations in the early versus the late wet season due to factors such as the flush effect and flow dependency (Watkins et al. 2019). Thus, our understanding of the drivers of microplastic abundance would greatly benefit from such a seasonal comparison. Additionally, there are few studies that address microplastic concentrations along an urban-rural gradient. Analyses of this type are particularly critical, as their examination could reveal potential sources and delivery pathways of microplastic pollution. Furthermore, while the presence of other pollutants and contaminants has been well-documented in rivers in the Portland area (Chang et al. 2019, Chen and Chang 2019, Chen and Chang 2014, Pratt and Chang 2012), much remains unclear regarding the degree to which microplastics impact Portland's freshwater bodies (Valine et al. 2020).

This study aims to address these data and knowledge gaps, by investigating microplastics in Portland watersheds with varying degrees of urban development, and by evaluating seasonal variability in microplastic concentrations with different flow regimes. In particular, the objectives of this research are to (i) evaluate how watershed attributes such as land use, arterial road length, elevation, and slope influence microplastic concentrations, (ii) evaluate the influence of seasonality on microplastic concentrations, (iii) evaluate the influence of water velocity and precipitation on microplastic concentrations, and (iv) determine the most common forms of microplastics to evaluate links with potential sources. It is hypothesized that higher concentrations of microplastics will be found adjacent to developed and agricultural areas as well as in wet season samples, particularly early in the season due to flush effects.

2. Methods

2.1 Study Area

Two Portland area watersheds served as the focal points for this study, including the Clackamas River watershed and the Johnson Creek watershed (Figure 1). These watersheds were selected to assess potential microplastic distributions along an urban-rural gradient in the Portland metropolitan area. Both are comprised of a range of land cover characteristics, thus exposing their waterways to a multitude of anthropogenic activities. The upper reaches of Johnson Creek flow through a continuum of rural and agricultural lands, and the lower reaches are exposed to a much greater degree of urban development. The Clackamas River also spans an urban-rural gradient, with upper reaches adjacent to forested and mountainous regions and lower reaches located near agricultural lands and varying degrees of urban development. The Clackamas is a major tributary to the Willamette River and serves as a source of drinking water to 350,000 residents in the Portland metro area (Chen and Chang 2019). The main soil type present in the study region is silt loam (NRCS 2021).

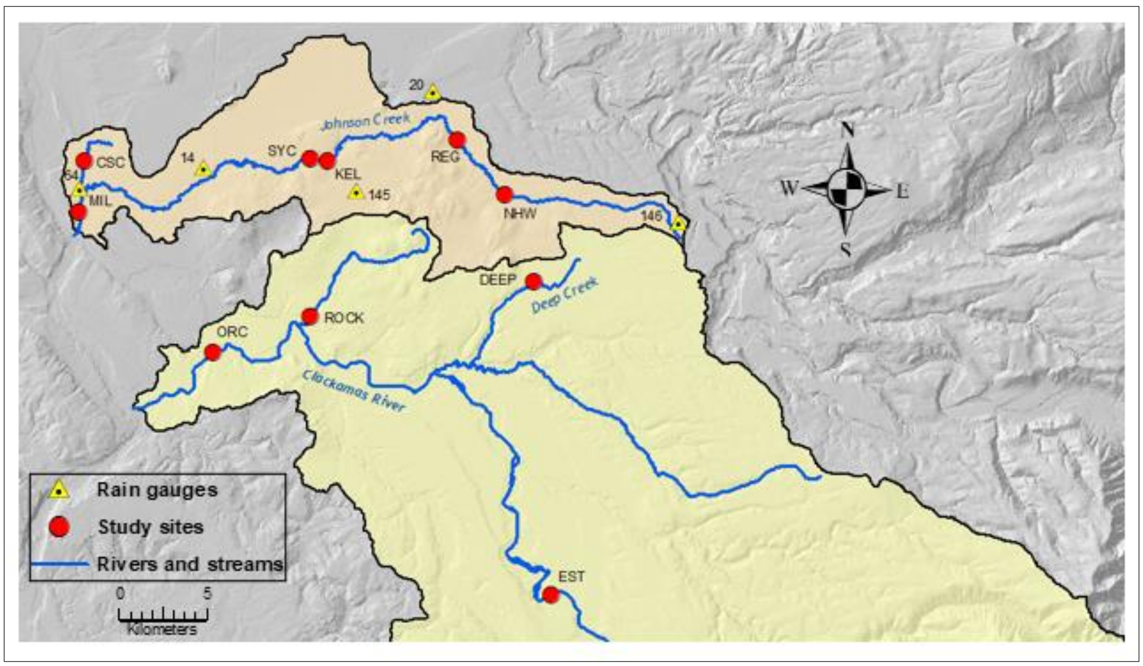


Figure 1. Study sites and rain gauges.

Samples were collected from 10 study sites, with four located in the Clackamas River watershed and six in the Johnson Creek watershed (Table 1). The majority of sites were selected to coincide with USGS gaging stations, with the intent of using USGS flow data when riverine conditions prevented the collection of such data in situ. There are three exceptions to this, including one site located near the confluence of Rock Creek and the Clackamas River that was selected to further represent potential impacts of urban/residential development on microplastic pollution. Additionally, one site is located on the North Fork of Deep Creek, a tributary to the Clackamas that is heavily influenced by agricultural activities. Lastly, one site is located near the headwaters of Johnson Creek to further shed light on microplastic sources to the river.

Table 1. Stream monitoring sites used for analysis.

Monitoring Sites	USGS gaging number	Drainage Area (km^2)	Elevation (m)	Dominant land cover (%)
Johnson Creek				
Near headwaters* (NHW)	-		119	Ag/Forest
Regner Rd (REG)	14211400	39.78	91.5	Ag/Urban
Kelley Creek (KEL)	14211499	12.15	74.7	Ag/Urban
Sycamore (SYC)	14211500	69.41	68.5	Urban
Milwaukie (MIL)	14211550	137.71	6	Urban
Crystal Springs Creek (CSC)	14211542	7.90	13.3	Urban
Clackamas River				
Estacada (EST)	14210000	1737.88	87.4	Ag/Forest
Deep Creek* (DEEP)	-		141	Ag/Urban
Rock Creek* (ROCK)	-		39	Ag/Urban
Near Oregon City (ORC)	14211010	2434.59	9	Urban

*****Not a USGS monitoring site

2.2 Data and Derivation of Explanatory Variables

Average flow velocity measurements over 60-second intervals were collected during each sampling session with a Marsh McBirney Flo-Mate flow meter. When river conditions prevented the safe collection of these data (namely the February sampling sessions for the Sycamore and Milwaukie sites), USGS data were downloaded from the National Water Information System (NWIS) database (USGS Water Data for the Nation, 2021) for stream gauges corresponding with the microplastic sampling locations. In these instances, similar historical discharge/gauge measurements were identified and the corresponding velocity readings were included in the current study. For the Estacada site, 15-minute incremental precipitation data were obtained from the USGS gaging station located on site. Hourly precipitation data were obtained from the City of Portland HYDRA Rainfall Network for the remaining nine sites, and data from the gauges closest in proximity to the study sites were used (particularly if they were located upstream), and are thus estimates of precipitation at the sites (Bureau of Environmental Services, City of Portland, 2021). Elevation data were obtained through Google Earth by inputting site latitude/longitude coordinates.

Subwatersheds were delineated for each study site using ArcHydro Tools in ArcGIS 10.8.1 (ESRI 2020), resulting in the creation of distinct, non-overlapping polygons (Mainali and Chang 2018, Pratt and Chang 2012). A land cover raster layer of Oregon was downloaded from the National Land Cover Dataset (NLCD) 2019 (Multi-Resolution Land Characteristics Consortium 2020), and clipped to the subwatershed boundaries. Because the NLCD dataset includes a broad range of land cover categories, they were combined into a single category as appropriate (e.g., deciduous forest, evergreen forest, and mixed forest were combined into a single ‘forest’ category). Total percentage of each land cover category (agricultural, developed, forest, shrub, and barren) were then derived for each subwatershed by utilizing the zonal histogram tool in ArcGIS. Additionally, subwatershed area was derived for each site.

Nearstream buffer zones were also created in ArcGIS, in which a 500m upstream buffer was derived for each study site. Mainali and Chang (2018) found that nearstream buffer zones (i.e., a 100m circular upstream buffer and a 1km riparian upstream buffer) more fully accounted for processes involved in water quality, with watershed-scale processes being less influential. As the current study included a single nearstream analysis, and as a 1km upstream buffer would have resulted in overlapping buffer zones across several sites, 500m was deemed an appropriate upstream buffer (Figure 2). Slope was derived in ArcGIS at both scales, in which the zonal statistics by table tool was used to calculate the average slope within each subwatershed and within each nearstream buffer zone (Table 2). In addition, total arterial road length in the nearstream buffer zones for each site was computed using the statistics function in ArcGIS.

Table 2. Variables used in analysis of microplastics in two Portland metro watersheds.

Variable type	Agency source	Data	Derived variable	Original data
Independent	MRLC	National land cover dataset 2019 (30m)	Agriculture (%)	Pasture, cultivated crops, hay
			Urban (%)	Low, medium, high intensity developed
			Forest (%)	Deciduous, evergreen, mixed
Independent	Google Earth	Elevation	Site elevation (m)	Elevation above sea level (m)
Independent	PSU GISData	Oregon 30m DEM	Subwatershed and nearstream slope averages (deg)	Slope (deg)
Independent	USGS	Streamflow (15-60 min intervals)	M/s at time of sampling	Discharge (cms)
Independent	HYDRA USGS	Precipitation (60-min intervals) Precipitation (15-min intervals)	24- and 72-hour antecedent precip (mm)	Precipitation amount (mm)
Dependent	This study	Microplastic concentration	Count per volume (particle/m^3)	Total microplastic count and water volume sampled

2.3 Data Collection

2.3.1 Preparatory Work

Before collecting samples at the study sites, materials were prepared in the Applied Coastal Ecology (ACE) lab at Portland State University. Quart-sized glass mason jars were rinsed three times with filtered deionizied (DI) water, with a layer of aluminum foil present underneath the cap to prevent contamination from the plastic ring present in the cap. Jars were then filled partway with filtered DI water, to be used for rinsing the contents of the cod end into the sample mason jar. Mason jars were also labeled with appropriate sampling information, including the month, site, and subsample number.

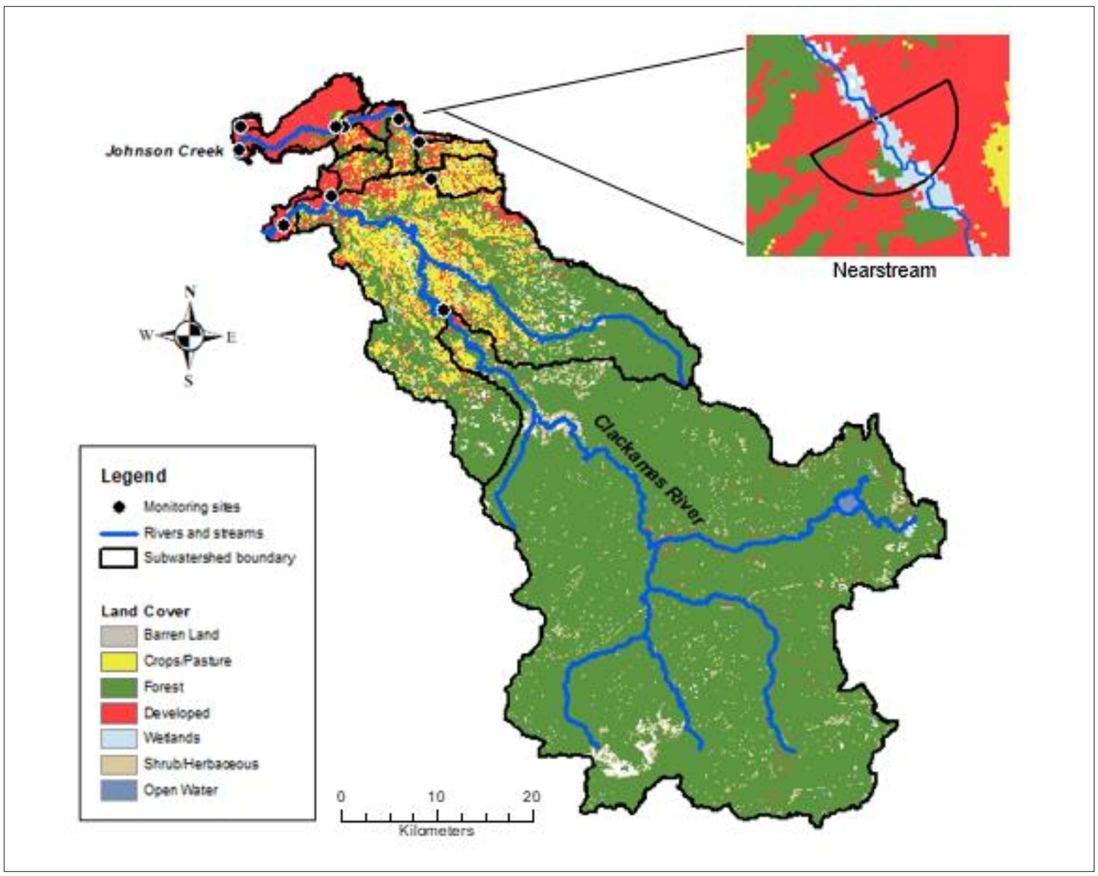


Figure 2. Map of study area land cover and monitoring sites, at the subwatershed and nearstream scales of analysis.

2.3.2 Sample Collection

Samples were collected via wading from the center of the stream, where possible. Sites for which this was not possible (namely sites directly along the Clackamas) required a different approach, which involved wading into the river and collecting samples at a standard depth of one meter. Otherwise, water depth at each sampling location was recorded using a meter stick. Where possible, stream width was also measured and recorded using a transect tape. Before beginning sample collection, the plankton net and cod end were rinsed three times in the river water to prevent cross-contamination from previous sites.

Samples were captured by submerging an 80μm mesh plankton tow net equipped with flow meter for 15-minute intervals (Valine et al. 2020) and holding it stationary. While excess water flowed directly through the net, microplastic particles and bits of organic debris were captured in the cod end that was attached to the tapered end of the plankton net. Three replicates were collected per site to assess within-site microplastic variability (Jiang et al. 2019, Wang et al. 2020). A control jar filled with DI water was placed next to the sampling site, and the lid was removed when each sampling session commenced and closed at the completion of sampling to capture airborne microplastics.

At the end of each sampling interval, the net was positioned upright and rinsed down thoroughly with river water to move microplastics down into the cod end. The cod end was tapped periodically as necessary to allow for excess water to escape, and the sample was poured into the appropriately labeled mason jar. The cod end was then rinsed thoroughly with filtered DI water to collect any microplastics that may have been stuck to the sides, and poured into the mason jar. Once the lid was placed over the sample, the lid for the control jar was also replaced. The net and cod end were then thoroughly rinsed in the river before departing for the next site. All samples were stored in refrigerators until the commencement of laboratory procedures.

Samples were collected during three sampling sessions to investigate the impacts of seasonality on microplastic concentrations (Barrows et al. 2018). The first session took place on August 28-30, 2020 and represented microplastic abundances during the dry season and thus without the influence of antecedent precipitation. Only one session was conducted in the dry season, as microplastic concentrations are unlikely to vary significantly throughout summer baseflow conditions. The second sampling session took place on September 24-25, 2020, representing microplastic concentrations in the early wet season when land-based microplastics have been flushed into aquatic environments (Hitchcock et al. 2020, Kataoka et al. 2019). The last sampling session occurred in the middle of the wet season on February 2-4, 2021, when microplastic concentrations in rivers are potentially more flow-dependent and less impacted by flush effects (Kataoka et al. 2019, Yonkos et al. 2014).

2.3.3 Sample Processing

In preparation for microscope analyses, a series of laboratory procedures were conducted to isolate microplastics on filter papers (Whatman 1820-047 Glass Microfiber Binder Free Filter, 1.6 Micron, 4.3 s/100mL Flow Rate, Grade GF/A, 4.7cm Diameter) (Valine et al. 2020). Samples were first put through an organic matter digestion step using potassium hydroxide (methods adapted from Baechler et al. 2020), followed by density separation using a hypersaline solution. Lastly, they were vacuum filtered onto filter paper, each of which was stored in a petri dish in a covered cardboard box until microscope analysis with a Leica dissecting microscope. Further details regarding laboratory and microscope procedures are given in Appendix A.

2.3.4 Quality Control

To minimize the risk of contamination, orange cotton jumpsuits were worn during sample collection, lab procedures, and microscope analyses. In addition, nitrile gloves were worn during all lab procedures and analyses. Any orange particles noted in samples were excluded from the final microplastic counts.

2.3.5 µFTIR Analysis

A subset of particles was sent to the Ecotox and Environmental Stress Lab at Oregon State University for micro-Fourier transform infrared (µFTIR) spectroscopy analysis to identify specific polymers and validate total counts (Baechler et al. 2020, Wang et al. 2020). As part of the selection process, samples were first randomized, as were the 12 sections of each petri dish. Once under the microscope, the third observed microplastic within the specified section was removed for µFTIR analysis. One hundred and one particles from field samples were selected by this randomized process, and an additional five were specifically selected to examine particles of interest. In addition, ten particles from controls were included for analysis. The selected particles represented approximately 10% of the total recorded microplastics. µFTIR methodologies were similar to those detailed by Harris et al. (2021).

2.3.6 Statistical Analysis

Statistical analyses were conducted in R, version 1.4.1717 (R Core Team, 2021). Because assumptions of normality and equal variance were not met, nonparametric statistics were used to assess potential relationships between explanatory variables and microplastic concentrations. Water volume for each subsampling was computed using the following equation:

Volume = A x T x V (m/s)

Where: *A*=area of the net opening (m^2); *T*=sampling duration (s); *V*=average velocity of the water (m/s) (Campanale et al. 2020a).

Microplastic concentrations were computed by dividing the total count of each subsample by the water volume sampled, thus standardizing the data (de Carvalho et al. 2021). The microplastic concentrations of each subsample were then averaged at each site during each sampling session, resulting in a single microplastic concentration per site per season.

To determine potential influences of seasonality, a Kruskal-Wallis rank sum test was run to compare average microplastic concentrations across the three sampling sessions. To assess whether differences may exist between sites, the ten study sites were first divided into two groups based on land use. At the subwatershed scale, the Urban group was comprised of sites with subwatersheds characterized by greater than 40% developed land (CSC, MIL, SYC, ROCK, REG, and KEL), with the Mixed/Rural group comprised of the remainder (EST, ORC, DEEP, and NHW). At the nearstream scale, the Urban group was comprised of sites with nearstream regions characterized by greater than 60% developed land (DEEP, CSC, NHW, REG, SYC, and MIL), with the Mixed/Rural group comprised of the remainder (EST, ROCK, ORC, and KEL). These groups were then further subdivided based on sampling session, for a total of six groups. Kruskal-Wallis rank sum tests were then run to compare average microplastic concentrations based on these site categories as a function of sampling session, and to specifically compare tire wear particles across the sampling sessions.

Spearman's rank correlation was used to compare average microplastic concentrations with spatial and temporal predictor variables. Spatial variables included subwatershed area, total arterial road length, land use, elevation, and slope (the latter three included both subwatershed and nearstream scales). Temporal variables included average water velocity during each sampling session, 24-hour antecedent rainfall, and 72-hour antecedent rainfall.

Additionally, two sets of principal component analyses (PCA) with varimax rotations were conducted. The first focused on the temporal and nearstream spatial explanatory variables, with one PCA associated with each sampling session and all subsamples included to evaluate within-site variability. The second addressed microplastic morphologies, again with one PCA associated with each sampling session and all subsamples included. These analyses were conducted in order to represent multivariate data as a reduced number of related variables in order to more easily identify trends, and the Kaiser rule was used to determine the number of principal components to retain. As land cover data were represented in terms of proportion, remaining spatial variables were also normalized on a 0 to 1 scale using the following equation:

$$V_i = (X_i - X_{imin})/(X_{imax} - X_{imin})$$

"Where V_i = normalized value of indicator X_i, X_{imin}, and X_{imax} represent the minimum and maximum values of a specific indicator *i*, respectively" (Chang et al. 2021).

3. Results

3.1 Characteristics of Microplastics

Microplastics were found at all sites, with a total of 1009 particles observed across the 90 field samples. While the highest microplastic abundances for each sampling session were observed at the MIL site (August: n=30; September: n=207; February: n=135), the KEL site had the highest concentration for August (37.73 p/m^3), the MIL site had the highest concentration for September (1.76 p/m^3), and the NHW site had the

Table 3. Microplastics found in laboratory and field controls.

	Aug		**Sept**		**Feb**		**LC**	**Types of Contamination**				
	L	F	L	F	L	F		Fiber	Frag	TWP	Film	Foam
EST	12	9	8	7	6	5	-	44	-	2	1*	-
DEEP	5	8	7	6	7	2	-	27	1	6*	-	1*
ROCK	4	8	9	4	3	3	-	30	1*	-	-	-
ORC	15	10	13	6	5	3	-	50	1	1*	-	-
CSC	10	6	7	9	3	6	-	38	3*	-	-	-
NHW	4	4	13	9	4	5	-	35	2	2	-	-
REG	12	7	13	10	3	3	-	45	3	-	-	-
KEL	6	7	8	3	4	5	-	31	1	1*	-	-
SYC	2	6	7	9	7	6	-	33	-	3*	1	-
MIL	8	6	5	7	2	7	-	31	1	3*	-	-
Grp4	-	-	-	-	-	-	8	7	-	-	-	1
Grp8	-	-	-	-	-	-	5	5	-	-	-	-
Grp11	-	-	-	-	-	-	10	10	-	-	-	-
Grp16	-	-	-	-	-	-	5	5	-	-	-	-
Grp19	-	-	-	-	-	-	2	1	-	-	1	-
Grp22	-	-	-	-	-	-	10	9	1	-	-	-
Grp25	-	-	-	-	-	-	3	3	-	-	-	-
Grp28	-	-	-	-	-	-	7	6	1	-	-	-
Grp31	-	-	-	-	-	-	1	-	-	1	-	-
Grp34	-	-	-	-	-	-	4	4	-	-	-	-
Grp37	-	-	-	-	-	-	1	1	-	-	-	-
Grp40	-	-	-	-	-	-	3	2	-	1	-	-
Grp43	-	-	-	-	-	-	1	1	-	-	-	-

L = Lab control; F = Field control. "Grp" refers to a group of field controls, which were processed in batches separate from field samples and had their own lab controls (LC).
*Particles are present in field controls only, not lab controls.

Table 4. Microplastics found in density separation (DS) controls and triplicate NaCl controls.

Additional Controls	Count
DS Control #1	5

DS Control #2	2
DS Control #3	2
DS Control #4	1
DS Control #5	2
DS Control #6	6
DS Control #7	1
DS Control #8	1
DS Control #9	3
NaCl Control #1	2
NaCl Control #2	3
NaCl Control #3	3

highest concentration for February (0.89 p/m^3). An additional 490 particles were found across the 30 field and 53 lab controls (Tables 3 and 4). Scope controls revealed minimal aerial deposition of microplastics, with a fiber typically noted every few field samples (Table 5).

Four microplastic morphologies were observed in field samples, including fragments (n=505, 50.1%), fibers (n=173, 17.1%), films (n=71, 7%), and foams (n=23, 2.3%) (Figure 3). Additionally, 237 tire wear particles (23.5%) were observed in field samples. Morphologies in field samples varied by site and across the sampling sessions (Figure 4). Of particular note is the relatively low proportion of fibers present at the EST and ORC sites in the dry season, which then became the dominant morphology for both sites in the mid-wet season. Conversely, three Johnson Creek sites (REG, KEL, and SYC) demonstrated the opposite trend, in which fibers were the dominant morphology in the dry season and then dropped to much lower proportions in the mid-wet season.

Table 5. Microplastics found in microscope controls.

Sample ID	Morphology	Color
*Aug KEL#1	Fiber	Blue
	Fiber	Blue
Aug NHW#2	Fiber	Blue
Aug ROCK#3	Fiber	Purple
Aug ORC#1	Fiber	Blue
Aug ORC#3	Fiber	Gray
Aug EST#3	Fiber	Green

Aug CSC#1	Fiber	Blue
Sept SYC#3	Fiber	Black
Sept KEL#2	Fiber	Black
Sept DEEP#1	Fiber	Gray
*Sept MIL#1	Fiber	Blue
	Fiber	Blue
*Sept MIL#2	Fiber	Black
*Sept MIL#3	Fiber	Blue
Sept EST#3	Fiber	Blue
Sept ROCK#1	Fiber	Blue
Feb REG#1	Fiber	Blue
Feb KEL#3	Fiber	Blue
Feb SYC#2	Fiber	Blue
Feb SYC#3	Fiber	Blue
Feb CSC#3	Fiber	Blue
Feb ROCK#2	Fiber	Blue
Feb DEEP#2	Fiber	Blue
Feb DEEP#3	Fiber	Blue
*Feb MIL#1	Fiber	Gray
	Fiber	Black
Feb MIL#2	Fiber	Blue

*Uncovered under the microscope for three or more hours, thereby potentially exposed to additional aerial contamination.

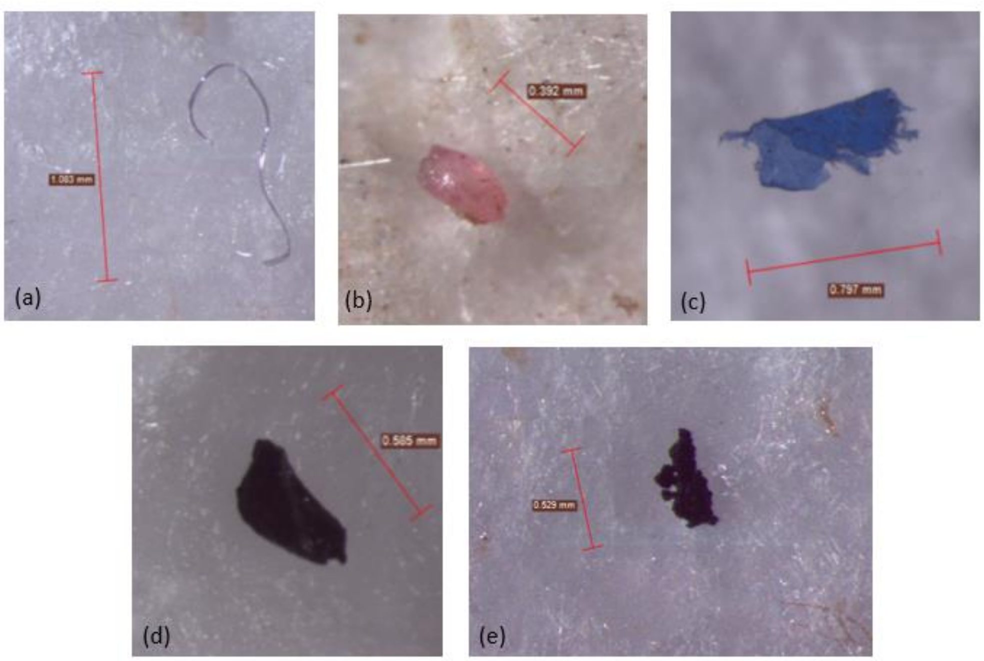


Figure 3. Examples of microplastics found in Johnson Creek and the Clackamas River, Oregon USA: (a) purple fiber; (b) pink fragment; (c) blue film; (d) black foam; and (e) black tire wear particle.

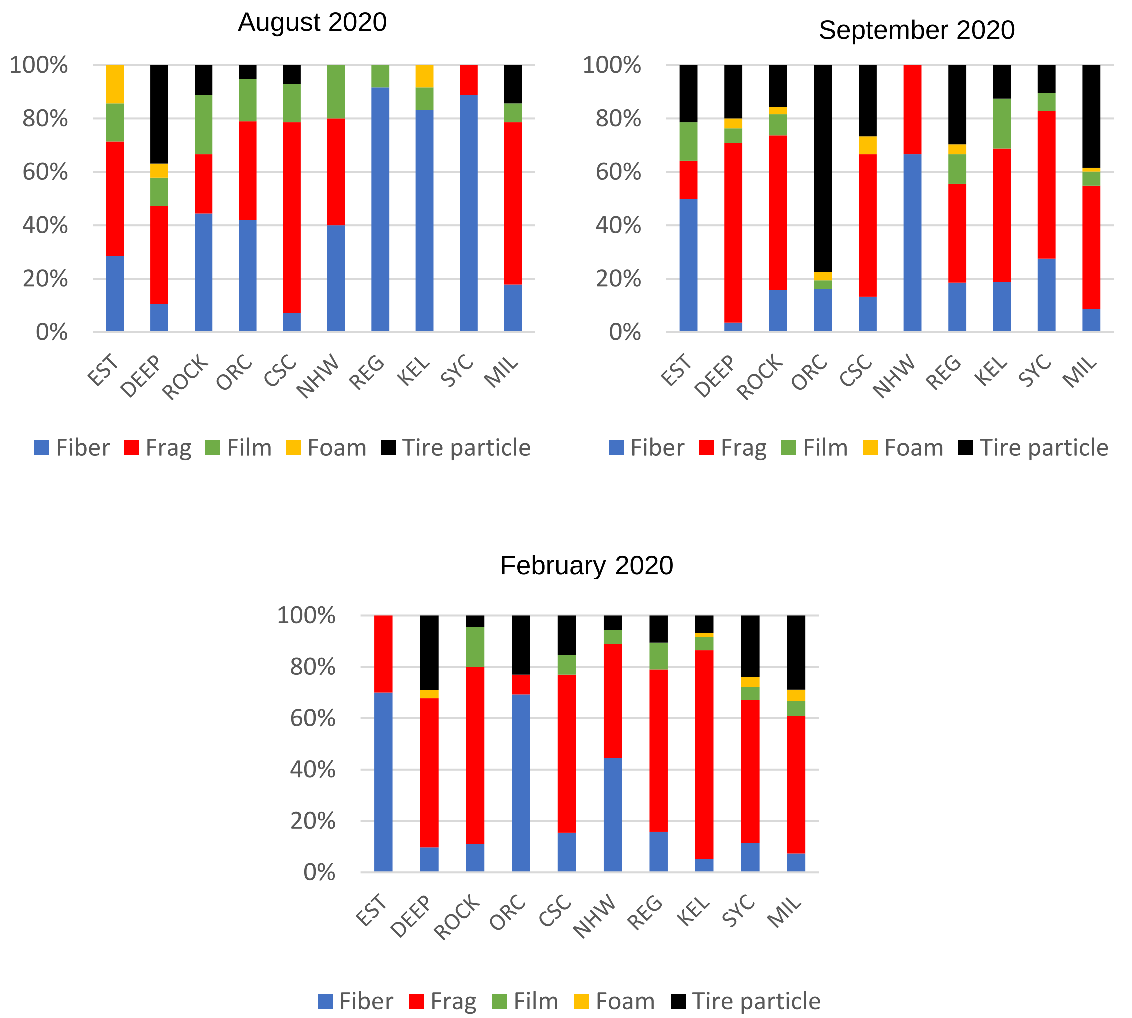


Figure 4. Proportion of microplastic morphologies observed by site and by sampling session.

Microplastics fell into one of nine color categories: gray (n=367, 36.4%), black (n=313, 31%), blue (n=174, 17.2%), white/clear (n=66, 6.5%), pink (n=39, n=3.9%), green (n=17, 1.7%), red (n=17, 1.7%, purple (n=10, 1%), and yellow (n=6, 0.6%) (Figure 5). Microplastics were also divided into five size classes, similar to Campanale et al. (2020b); 63-100µm (n=17, 1.7%), 101-500µm (n=402, 39.8%), 501-1000µm (n=318, 31.5%), 1001-2000µm (n=184, 18.2%), and 2001-5000µm (n=88, 8.7%) (Figure 6).

Thus, microplastics less than 0.5mm in length comprised over 40% of the observed plastics, with nearly three-fourths of particles measuring less than 1mm.

µFTIR analyses of the 116 submitted particles identified a total of nine polymer types: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), cellulose, cellophane, ethylene vinyl acetate, polyvinyl acrylonitrile, and styrene butadine. Dominant polymers in field samples included PE (30%), PP (27%), cellulose (17%), and PET (9%) (Figure 7). Every particle evaluated by µFTIR was either synthetic or anthropogenically impacted.

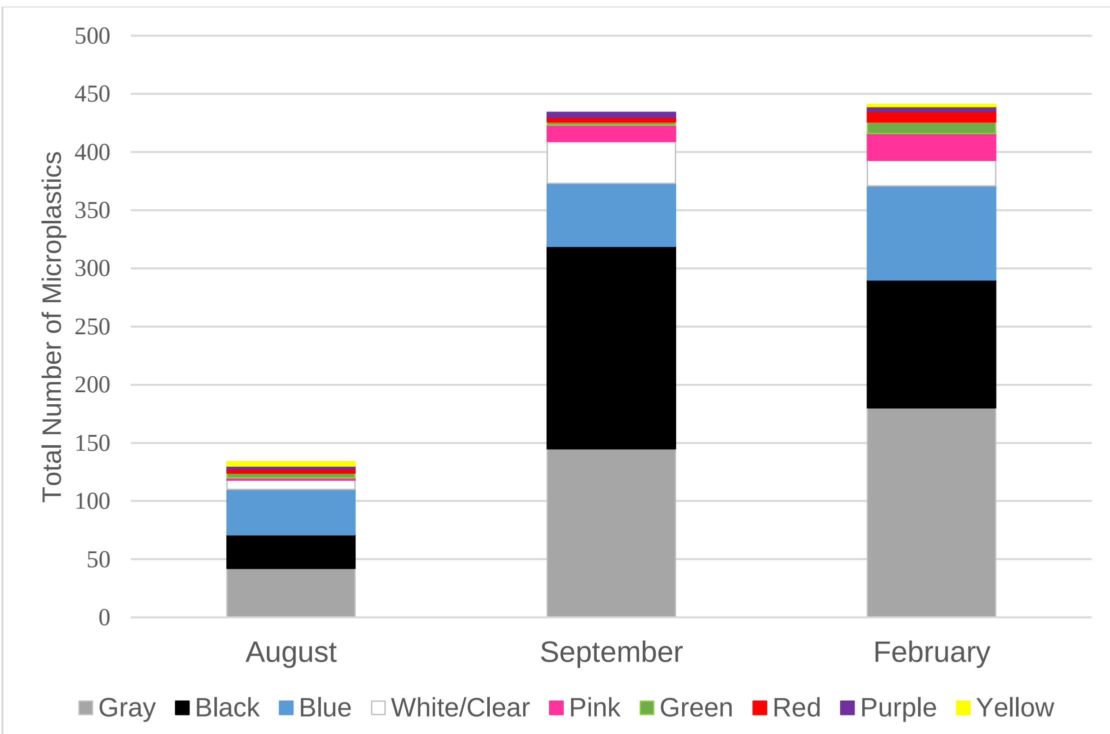


Figure 5. Color composition of microplastics across three sampling sessions.

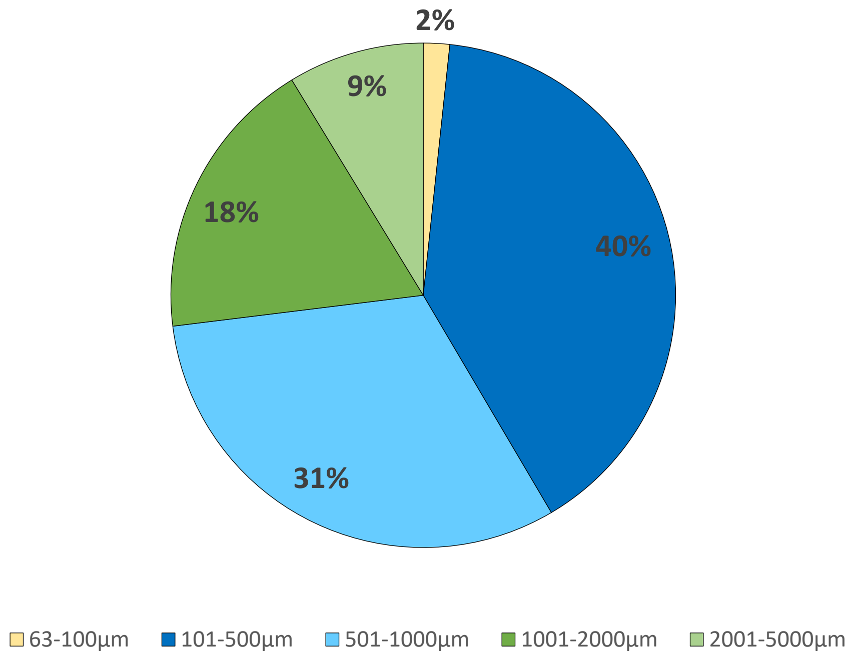


Figure 6. Size composition of microplastics.

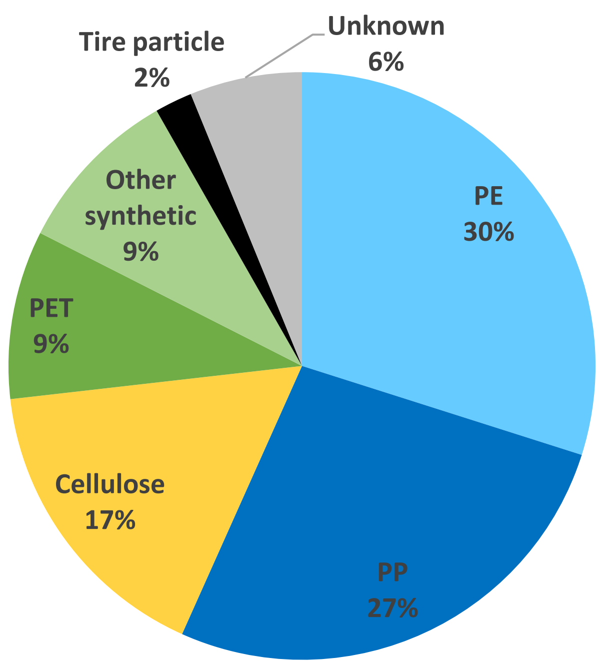


Figure 7. Polymer composition of microplastics evaluated by µFTIR spectroscopy. Note: only one PE particle was characterized as high-density PE, the rest were comprised of low-density PE.

3.2 Seasonal Analysis

Differences were found between the average microplastic concentrations observed during the three sampling sessions (Kruskal-Wallis, $H(2)=6.1342$, $p<0.05$). Results of a post-hoc Dunn test were inconclusive due to low statistical power, but an examination of boxplots showed that a significant difference existed between August and February. More specifically, average microplastic concentrations were highest in August (3.24 ± 1.84 p/m^3) and lowest in February (0.365 ± 0.076 p/m^3) (Figure 8). Microplastic concentrations ranged from 0.19 – 18.86 p/m^3 in August, 0.43 – 1.69 p/m^3 in September, and 0.09 – 0.85 p/m^3 in February (Figure 9).

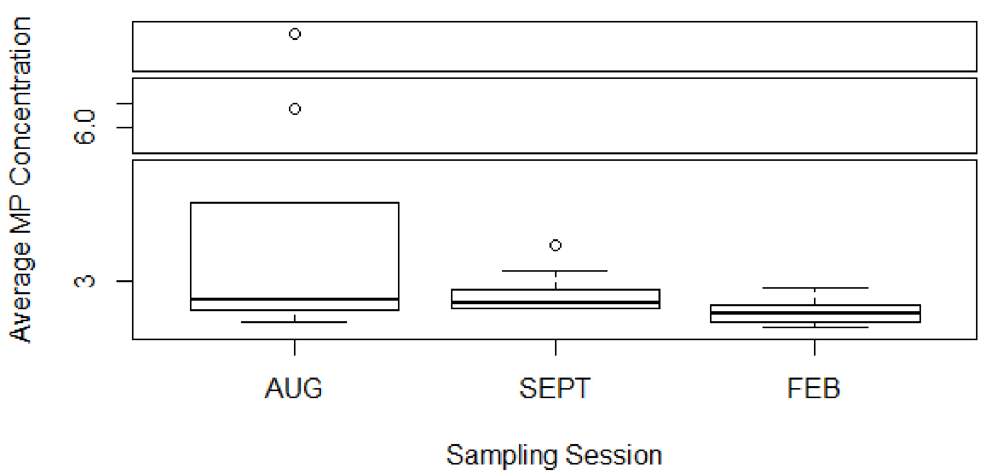


Figure 8. Average microplastic concentrations in Johnson Creek and the Clackamas River, Oregon, USA during three sampling sessions in 2020 and 2021.

At the subwatershed scale, no differences in average microplastic concentrations were observed between the Urban and Mixed/Rural groups (Kruskal-Wallis, $H(5)=8.2333$, $p>0.1$). A difference was observed at the nearstream scale (Kruskal-Wallis,

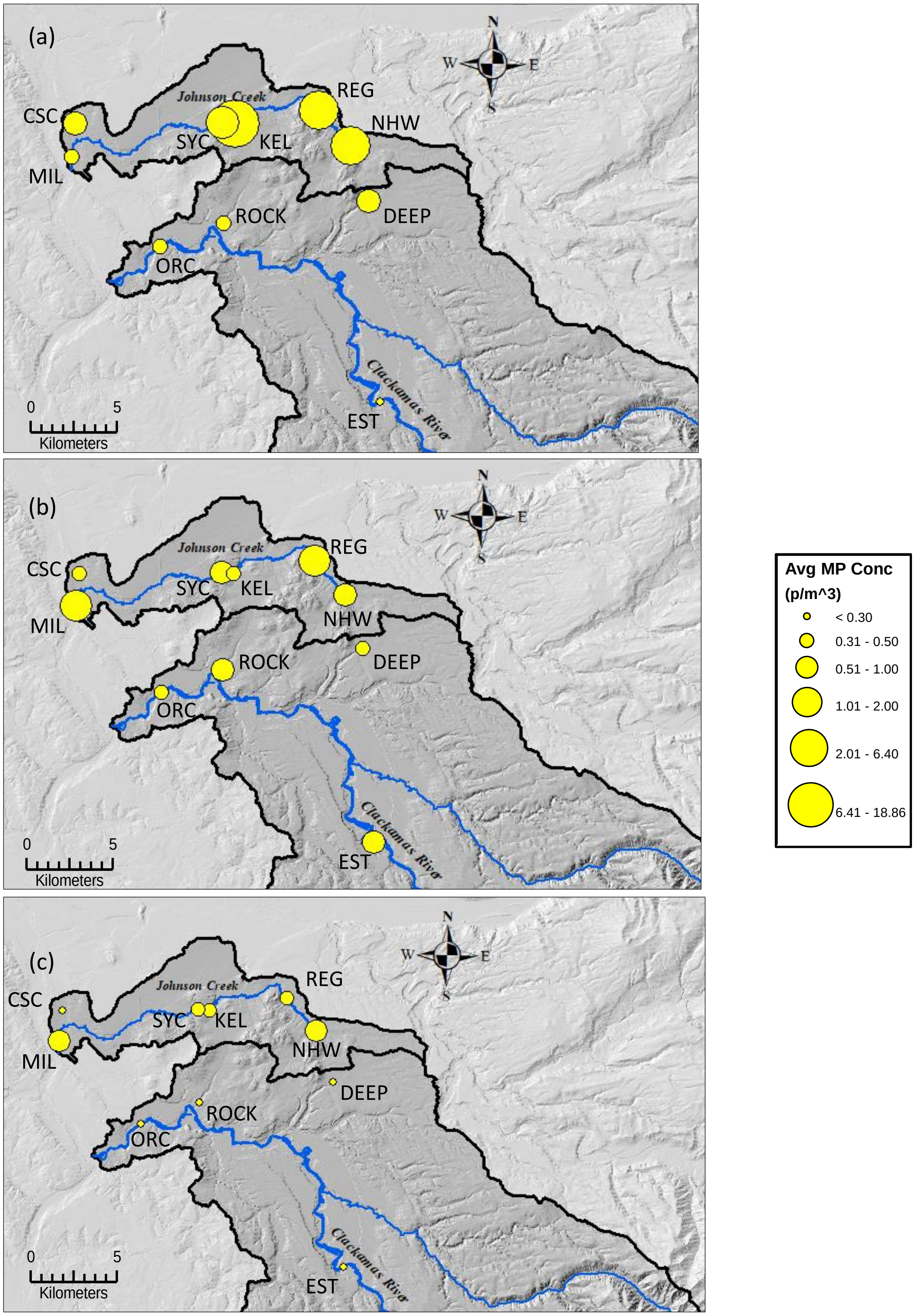


Figure 9. Microplastic concentrations at each monitoring site in Johnson Creek and the Clackamas River, Oregon, USA during the sampling sessions of (a) August, 2020 (b) September, 2020 and (c) February, 2021.

H(5)=11.852, p<0.05), with a post-hoc test revealing a significant difference between the August sampling at Urban sites (higher concentrations) and the February sampling of Mixed/Rural sites (lower concentrations) (Dunn's test, p<0.05). Thus, despite sampling across multiple land use types and sampling sessions, there only existed a difference between August and February, with no differences between the Urban and Mixed/Rural groups observed during any single sampling session.

In addition, tire wear particle (TWP) concentrations differed by sampling session (Kruskal-Wallis, H(2)=8.6157, p<0.05). A post-hoc Dunn test revealed that August TWP concentrations differed significantly from September concentrations (p<0.05), with higher concentrations observed in September.

3.3 Correlation Analysis

Average microplastic concentrations for each of the three sampling sessions were correlated with several spatial and temporal variables (Tables 6 and 7, respectively). Only two correlations were significant with regard to temporal factors, with higher microplastic concentrations in August linked with lower average water velocities (r = -0.854, p<0.05) and higher microplastic concentrations in February coinciding with increased rainfall in the 24 hours preceding sample collection (r = 0.638, p<0.05). At the spatial level, both September and February microplastic concentrations were lower in predominantly agricultural lands at the nearstream scale (r = -0.721, p<0.05 and r = -0.673, p<0.05, respectively). Sites with greater proportions of shrub land had lower microplastic concentrations in February at the subwatershed scale (r = -0.721, p<0.05), and greater subwatershed area was linked with lower microplastic concentrations in August (r = -0.673, p<0.05).

Table 6. Correlations between average microplastic concentrations and spatial factors.

		Area	Elev	Slope	Rd Dens	Barren	Crops	Dev	Forest	Shrub
Aug	SWS	-0.673*	0.261	-0.321		-0.532	0.261	0.455	-0.358	-0.479
	Near			0.042	0.278	-0.337	-0.491	0.455	-0.274	-0.560
Sept	SWS	0.127	0.042	0.115		-0.625	-0.091	0.236	-0.127	-0.527
	Near			-0.212	0.127	0.078	-0.721*	0.297	-0.085	0.143
Feb	SWS	-0.430	0.042	-0.370		-0.607	0.152	0.564	-0.515	-0.721*
	Near			-0.285	0.491	-0.017	-0.673*	0.564	-0.432	-0.198

*significant at the 0.05 level; Rd Dens = Road Density; SWS = Subwatershed scale

Table 7. Correlations between average microplastic concentrations and temporal factors.

	Velocity (m/s)	24P (mm)	72P (mm)
August	-0.854*	-	-
September	-0.382	0.309	-0.486
February	-0.212	0.638*	-0.006

*significant at the 0.05 level
24P = 24-hour antecedent precipitation
72P = 72-hour antecedent precipitation

3.4 Principal Component Analyses

3.4.1 Explanatory Variables

Two principal components explained 64.3% of the total variance in microplastic concentrations between sites as a function of spatial variables for August, 61.2% of the total variance observed for September, and 62.7% of the total variance observed for February (Figure 10). For all three sampling sessions, the same four variables constituted PC1 when using the absolute value of 0.4 as a cutoff value: subwatershed area, average subwatershed slope, nearstream developed land, and nearstream forested land. Nearstream barren and shrub land constituted PC2 in August and September, with 72-hour antecedent precipitation dominating for September and February.

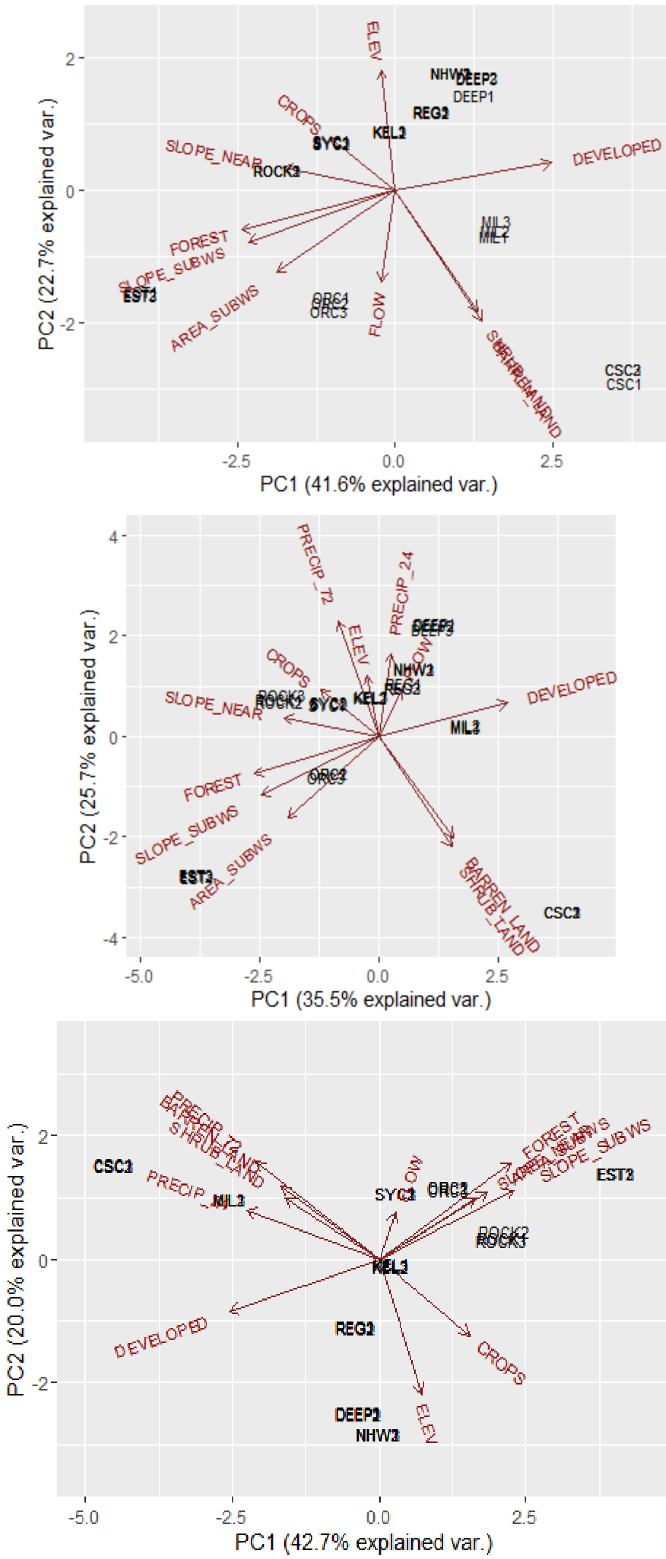
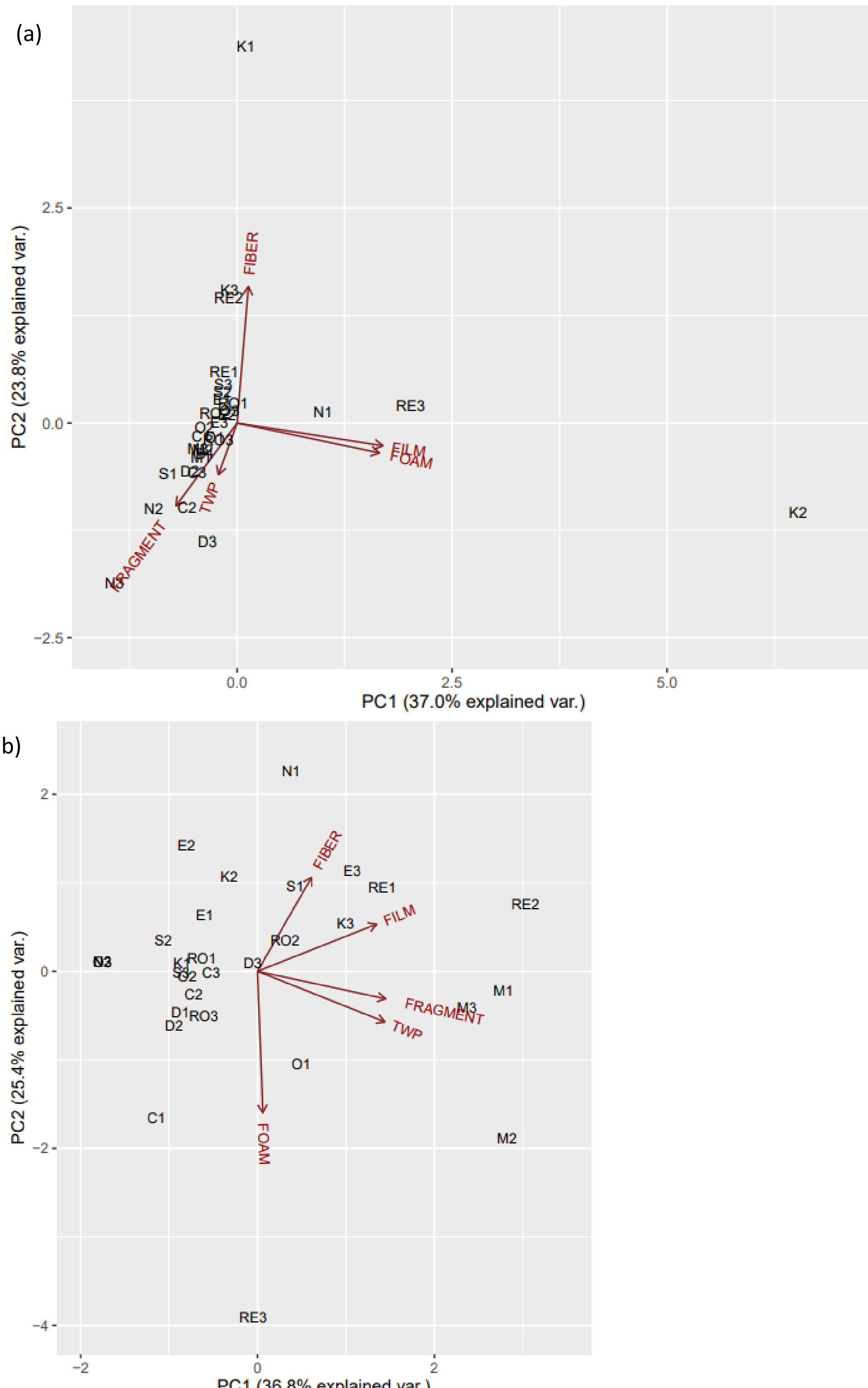


Figure 10. Principal component analysis of the temporal and nearstream spatial variables during (a) August, 2020, (b) September, 2020 and (c) February, 2021.


(a)
PC2 (23.8% explained var.)
PC1 (37.0% explained var.)
FIBER
FILM
FOAM
TWP
FRAGMENT
K1
K2
K3
RE2
RE1
RE3
N1
N2
N3
S1
C2
D3
2.5
0.0
−2.5
5.0
(b)
PC2 (25.4% explained var.)
PC1 (36.8% explained var.)
FIBER
FILM
FRAGMENT
TWP
FOAM
N1
E2
K2
S1
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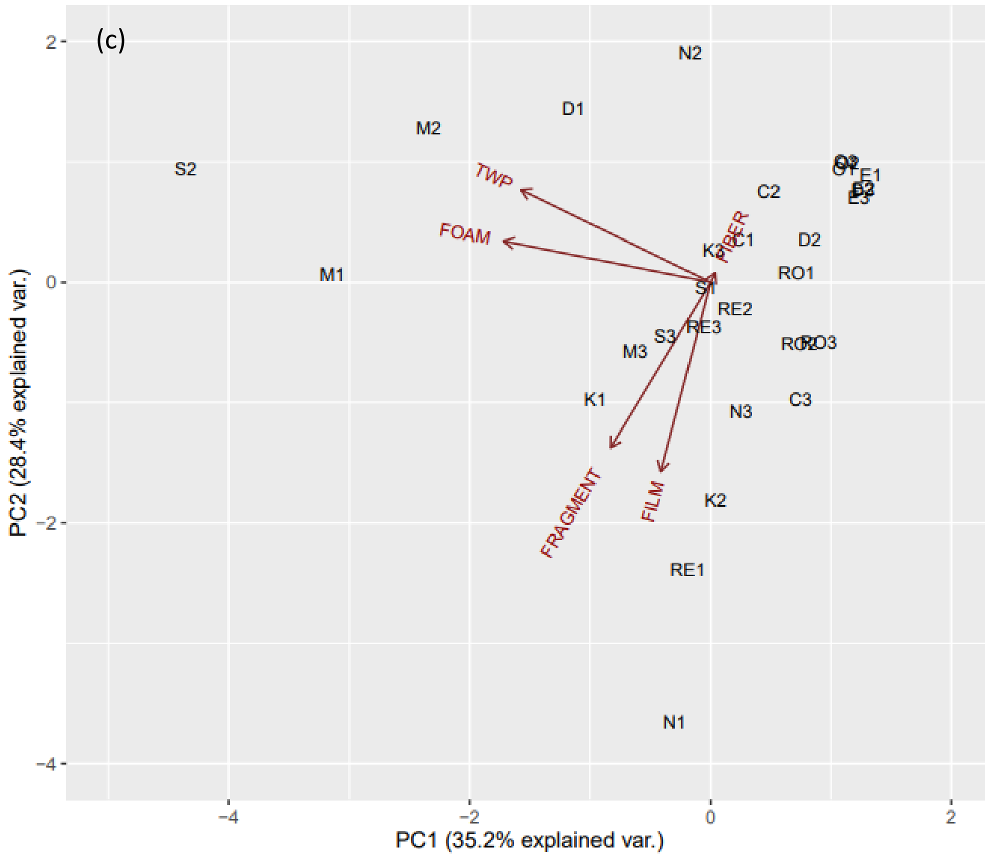

Figure 11. Principal component analysis of microplastic morphologies observed during (a) August, 2020, (b) September, 2020 and (c) February, 2021. Letters refer to sampling site (C = CSC; D = DEEP; E = EST; K = KEL; M = MIL; N = NHW; O = ORC; RE = REG; RO = ROCK; S = SYC). Numbers refer to subsample (1 = subsample #1; 2 = subsample #2; 3 = subsample#3).

3.4.2 Microplastic Morphologies

Two principal components explained 60.8% of the total variance observed in morphologies between sites for August, 62.2% of the total variance observed for September, and 63.6% of the total variance observed for February (Figure 11). When using the absolute value of 0.4 as a cutoff value, different combinations of variables constituted the principal components for August (PC1 – film, foam; PC2 – fiber, fragment; PC3 – TWP), September, (PC1 – fragment, film, TWP; PC2 – fiber, foam), and February (PC1 – foam, TWP; PC2 – fragment, film; PC3 – fiber).

4. Discussion

4.1 Microplastic Characteristics

Fragments were the dominant morphology observed in this study, similar to findings from previous studies (Bertoldi et al. 2021, Mai et al. 2021, Tibbetts et al. 2018). Fibers have also been noted as a dominant morphology (Belen Alfonso et al. 2020, Chen et al. 2020, Feng et al. 2020, Hu et al. 2020), though they were the second most common morphology at my study sites. The dominance of gray particles in the current study is unusual, as the literature shows that dominant colors typically include blue (Barrows et al. 2018, Dris et al. 2018, Miller et al. 2017, Strady et al. 2020), clear/white (Baldwin et al. 2020, Di and Wang 2018, Han et al. 2020, Huang et al. 2020), and black (Guerranti et al. 2017, Qin et al. 2020, Sang et al. 2021). Greater proportions of clear/white plastics in particular may result from processes such as photodegradation (Fan et al. 2019). Additionally, the dominance of plastic particles under 1mm in length is consistent with previous research (Bertoldi et al. 2021, Bujaczek et al. 2021, Sang et al. 2021, Wang et al. 2021).

4.2. Seasonality and Hydrodynamic Variables

Microplastic pollution varies as a function of both space and time, with concentrations fluctuating in part due to precipitation and runoff (Cheung et al. 2019, Xia et al. 2020). Heterogeneous distributions within a body of water may also be due to factors such as point sources of contamination (Crew et al. 2020, McCormick et al. 2014). Many studies include a snapshot of microplastic pollution (i.e., a single sampling session) in freshwater bodies (Di and Wang 2018, Hoellein et al. 2017, Mao et al. 2020), or incorporate a narrow land use gradient into their study designs (Campbell et al. 2017, Campanale et al. 2020b, Lahens et al. 2018). Because of the fluctuating nature of microplastic concentrations and varying sources over space, it is becoming increasingly critical to understand the spatial and temporal heterogeneity of microplastic sources and delivery pathways.

Microplastics were identified at all sites throughout all sampling sessions, and an investigation of the literature shows similar findings of microplastic ubiquity (Ballent et al. 2016, Constant et al. 2020, Shruti et al. 2019, Yin et al. 2020). Microplastic pollution varied across seasons, with significantly higher concentrations observed during August (dry season) than during February (mid-wet season). It was expected that higher concentrations would be found during the wet season, particularly during the early wet season, yet there was no evidence for this in the current study. While some studies find higher microplastic concentrations in the wet season (Eo et al. 2019, He et al. 2020a), others have found that microplastics dominate in dry season samples (de Carvalho et al. 2021, Fan et al. 2021). This study's finding has been attributed to the potential for increased precipitation and runoff associated with the wet season to result in dilution effects and lower observed microplastic concentrations (Fan et al. 2019, Stanton et al. 2020, Wu et al. 2020). As microplastic concentrations were negatively correlated with average flow rates in August, dilution effects may have played a role in lower observed concentrations in the wet season.

With the exception of a positive correlation between February microplastic concentrations and 24-hour antecedent precipitation, there was little evidence that precipitation amount influenced microplastics at the study sites. Despite several previous studies showing no relationship between the two (e.g., Constant et al. 2020, de Carvalho et al. 2021), it is important to note that the vast majority of precipitation values for each study site were estimates, with the closest HYDRA rain gauge at times located several miles away from a particular study site. As a result, data from a particular gauge were at times used for more than one site. Due to the coarse scale of this analysis, the use of HYDRA precipitation data can be considered a study limitation. To ensure a clearer picture of potential relationships between precipitation and microplastics, obtaining precipitation data on a finer spatial scale with gauges located within very close proximity to study sites is ideal.

It is also important to note that the timing of data collection may be critical in evaluating the influence of precipitation. As previously noted, microplastic concentrations can vary drastically over very short periods of time as a function of hydroclimatic variables (Xia et al. 2020), even over the course of several hours (Cheung et al. 2019). Wet season sampling for the current study was conducted over a period of two or three days, due to the limited number of researchers involved in data collection. If multiple collection teams had been available to complete each wet season sampling event in a standardized amount of time and within a single day, this could have contributed to a clearer analysis regarding the impacts of precipitation on microplastic concentrations.

4.3 Watershed Attributes

Few spatial variables were found to have significant relationships with microplastic concentrations, for either the subwatershed scale or the nearstream scale. Of important interest, however, were negative correlations between September and February microplastic concentrations and proportion of agricultural lands in the nearstream zones. This indicates that fewer microplastics were found in regions where the immediately upstream area was characterized by a greater degree of croplands, even during wet season periods when runoff is most likely to introduce plastic particles to freshwater bodies. The use of microplastic-rich biosolids and/or the degradation of a multitude of plastics used for agricultural purposes thus may not result in the flushing of substantial amounts of microplastics into nearby freshwater bodies, but these microplastics may instead remain trapped in permeable agricultural soils (Feng et al. 2020). Very few studies to date have evaluated microplastics in soils, and additional research is needed to shed further light on the microplastic cycle in both agricultural regions and other land use categories (Amrutha et al. 2020, Feng et al. 2020).

Only one significant relationship was observed at the subwatershed scale, and included a negative correlation between February microplastic concentrations and proportion of shrub land. In addition, solely nearstream land use categories were represented in the first two principal components of the PCA. It is thus possible that nearstream analyses may shed more light when determining relationships between microplastic pollution and potential explanatory factors. Microplastics likely share delivery pathways with other contaminants and nutrients that threaten water quality (Mishell Donoso and Rios-Touma 2020, Sarkar et al. 2019, Zhou et al. 2020), and as previously noted, recent water quality modeling research has highlighted the importance of nearstream as opposed to watershed-scale processes (Mainali and Chang 2018). Similar sentiments were expressed by Barrows et al. (2018), whose analyses at the subwatershed scale spurred the belief that more localized analyses (e.g., on specific point sources) may be more useful in understanding the role of potential explanatory factors. A similar emphasis on specific sources of microplastics addressed at local scales was noted by Dikareva and Simon (2019).

While total microplastic counts were highest at the Milwaukie site, which is characterized by a high proportion of urban land cover, an unexpected finding was the lack of a correlation between microplastic concentrations and developed land, at either the subwatershed or nearstream scales. As previously mentioned, the two watersheds included in this study represent a range of land covers, yet it is possible that the selected sites may not represent the full urban-rural gradient, thus clouding potential relationships. For instance, many of the sites were located in mostly developed regions. Perhaps the incorporation of a greater number of study sites spanning a broader range of the gradient may reveal more specific results (Belen Alfonso et al. 2020, Dikareva and Simon 2019), and this may also be the case with other watershed attributes such as slope and elevation. Additionally, as the net was submerged just under the surface of the water to ensure that water volume could be calculated, it is possible that some microplastics on the surface circumvented the net. Lastly, it is important to note that factors not evaluated by the current study (e.g., various microscale processes, sediment resuspension) may have exerted an influence on observed microplastic concentrations. Evaluating microplastic concentrations in sediment samples would have provided further insight regarding influential factors as well as a more comprehensive picture of the microplastic cycle at the monitoring sites.

4.4 Potential Sources

Broad links can be made with regard to observed microplastic morphologies and potential sources. As previously mentioned, fragments were the dominant observed morphology, indicating that the breakdown of larger pieces of plastic and litter may be a critical source of microplastics in Portland's freshwater bodies. Fibers were also common, indicating that factors such as washing machine effluent (e.g., in residential regions surrounding the Regner and Kelley Creek sites) or recreational activities (e.g., at sites characterized by a high degree of water activity such as the Estacada and Near Oregon City sites) may play a role in microplastic pollution as well. Additionally, given the significant increase in tire wear particle concentrations observed between the August and September sampling sessions, it is likely that these particles accumulated on land during the dry period and were flushed into nearby waterways during the first wet season storm event. The influx of tire wear particles in the early wet season is particularly alarming, as recent research has highlighted the severe threat they pose to salmon (Tian et al. 2021).

The identification of specific polymer types can also shed light on potential sources of microplastic pollution. Polyethylene (PE) was the most commonly observed polymer, which is consistent with previous findings (Fan et al. 2019, Xiong et al. 2019). In particular, PE particles were composed of two sub-polymers with very different applications. Of the 116 particles assessed by µFTIR spectroscopy, low-density polyethylene (LDPE) particles were found at all but two of the study sites. These plastics are typically found in thin plastic bags, such as those used in grocery stores (Mishra et al. 2021). In contrast, only one high-density polyethylene (HDPE) particle was reported, and it was observed at the Milwaukie site. As HDPE particles are commonly used in construction activities and PVC pipes (Mishra et al. 2021), its presence at the more industrial Milwaukie site is unsurprising. Polypropylene (PP) was very common as well, and is often found in a variety of packaging materials as well as in synthetic clothing (Mishra et al. 2021). Of the samples that underwent µFTIR analyses, PP particles were found at all but two of the sites, underscoring their ubiquity.

4.5 Conservative Estimates of Microplastics

The observed microplastic concentrations in this study are likely conservative, which may be due to several factors. For instance, the use of a hypersaline solution during density separation does not result in the flotation of 100% of plastic particles, as higher density plastics in particular often remain trapped with sediment (Mishra et al. 2021). Therefore, the vacuum filtration step may have missed microplastics that remained at the bottom of the sample jars (Di and Wang 2018, Valine et al. 2020), thus resulting in a subset being isolated on the filter paper for microscope analysis.

Additionally, the current study included the use of glass microfiber binder free filters, which are white in color. While the current study identified some white microplastic particles under the microscope, it is likely that others were missed due to the difficulty in identifying these particles against a white background. The inclusion of clear polycarbonate filters in future studies may facilitate the identification of white microplastics.

5. Conclusion

This study showed that microplastic concentrations in the Portland metropolitan area may be influenced by certain hydroclimatic variables and subwatershed characteristics. In the dry season, lower flow rates appeared to facilitate the accumulation of microplastics, with concentrations also potentially influenced by antecedent rainfall in the mid-wet season. Additionally, microplastic concentrations may be influenced more strongly by nearstream as opposed to subwatershed factors, particularly with regard to adjacent agricultural lands. Fragments were dominant in both watersheds, likely due to the breakdown of larger pieces of plastic. Gray particles were particularly common, and the 101-500μm size class of microplastics was the most highly represented. Higher concentrations of tire wear particles in the wet season suggest a flushing effect.

The findings of this study further our knowledge of riverine microplastic pollution in the Portland metro area and contribute to our understanding of potential sources of microplastics in freshwater environments. This information is beneficial to local officials and agencies in Portland, who are increasingly interested in knowing the potential sources and pathways of microplastics in their water bodies. Armed with such knowledge, they may be better equipped to enact policies that result in decreased concentrations of microplastics reaching aquatic environments. In addition, the findings of the research can identify hotspots of microplastic pollution that may benefit from remediation, and can potentially assist in projections of microplastic concentrations in other locations with similar characteristics, for which no microplastics data have yet been collected.

Chapter 3

Conclusion

Freshwater microplastic research has afforded us insights into potential sources and delivery pathways of these plastics to downstream environments. The examination of a broad range of spatial and temporal variables has resulted in a clearer understanding of the microplastic cycle and the factors that influence their distribution and abundance. Studies of microplastics in freshwater environments have increased substantially in recent years and show no signs of slowing down. With plastic production rates rapidly increasing and plastic waste continuously accumulating in the environment, knowledge gained from this body of research can assist in the development of management plans and in more effectively addressing the ubiquity of plastic pollution.

A variety of spatial factors can influence freshwater microplastic pollution, including anthropogenic variables such as land use, population density, and impervious surfaces, and topographic variables such as slope and elevation (Baldwin et al. 2016, Chen et al. 2020, Grbić et al. 2020). While it is important to stress the overarching influence of anthropogenic activities, it is also critical to note that microplastic pollution is not unique to regions located in close proximity to human influence. The transportation of microplastics to more remote environments via atmospheric deposition, a growing body of research, highlights the pervasive nature of these plastics as well as the immediate need for their management (Jiang et al. 2019, Wetherbee et al. 2019).

Assessments of microplastics at the watershed and subwatershed scale can shed light on the role of upstream variables and those not located in direct proximity to a waterway. However, a sole focus on larger scale variables may overlook the influence of local sources of microplastic pollution and nearstream riparian variables. Thus, addressing spatial factors at multiple scales of analysis can provide a more comprehensive picture of the distribution and potential sources of microplastic pollution.

In addition, microplastic concentration fluxes are influenced by a range of temporal variables, including storm events, runoff, and instream flow rates (Cheung et al. 2019, Sang et al. 2021). It is important to note the highly variable nature of microplastic concentrations due to such factors, and to recognize the subsequent complexities in estimating the microplastic flux of a given body of water. Fine-scale temporal research can provide critical insights into the microplastic cycle, potentially revealing when concentrations may be highest in such waterways.

This study aimed to examine potential relationships between observed microplastic concentrations and a range of spatial and temporal variables. Spatial variables included land use, total arterial road length, slope, and elevation, and analyses were conducted at both the subwatershed scale and the nearstream scale. In addition, temporal factors such as water velocity at the time of sampling and precipitation received in the 24- and 72-hours preceding sample collection were evaluated for relationships with microplastic concentrations. While few correlations were revealed during statistical analysis (e.g., nearstream agricultural regions in two sampling sessions, 24-hour antecedent precipitation in one sampling session, water velocity in one sampling session), these correlations were generally not consistent over the course of all three sampling sessions. For instance, while lower water velocities in August were linked with higher microplastic concentrations, no links were found between microplastics and water velocity for either the September or February sampling sessions.

Additionally, this study did not demonstrate correlations between microplastic concentrations and multiple variables (e.g., developed or forested land at either scale of analysis, total arterial road length at the nearstream scale, 72-hour antecedent precipitation). As previously indicated, modifications to the study design may reveal additional relationships, such as the inclusion of sites spanning a broader range of the urban-rural gradient and with greater topographic variability, as well as the inclusion of sampling sessions conducted at much finer temporal scales (e.g., collecting samples multiple times over the course of several hours at each study site).

This study can potentially serve as a baseline for microplastic pollution in two of Portland's freshwater ecosystems, and can be used as a reference for future microplastic research in the metro area. The expansion of freshwater microplastic research in Oregon may assist with future decision-making of agencies and stakeholders regarding management and mitigation strategies.

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Appendix A. Laboratory procedures for sample processing.

Organic Matter Digestion

Due to appreciable amounts of biotic material in the collected samples, organic matter digestion was a necessary first step in the process of isolating microplastics and facilitating their identification under a microscope (Baechler et al. 2020). Samples were first filtered through a 63um sieve, with excess water draining into a waste beaker and plastics and biological material trapped on the sieve. Any large debris present on the sieve, including but not limited to leaves, pine needles, and twigs, was rinsed thoroughly with filtered DI water over the sieve and discarded. To capture any plastics stuck to the sides of the beaker, the beaker was rinsed with filtered DI water and poured over the sieve. The contents of the sieve were rinsed into a clean beaker with 270 mL of filtered DI water to standardize the volume. A glass stir bar was added to each beaker, and samples were placed on hot plates in a fume hood. Using an aluminum foil boat, 30 grams of potassium hydroxide (KOH) were transferred to each beaker. Samples were digested at 40C with the stir function set to 350 rpm for between 24-72 hours, depending upon the amount of organic material present and the murkiness of the sample. For each set of samples in a fume hood, a lab control containing 270mL of filtered DI water and 30 grams of KOH was used to capture microplastics resulting from contamination (e.g, microplastics present in the KOH itself, airborne microplastics).

After digestion, samples were removed from the hot plates and filtered again through the 63um sieve, and each beaker was rinsed with filtered DI water through the sieve to ensure that all materials exited the beaker. The contents of each sieve were poured into clean and prelabeled petri dishes. Using as little filtered DI water as possible, any remaining sieve contents were rinsed into each dish using a 5mL pipette. Petri dishes were then covered and stored in boxes to await density separation. This procedure was adapted from digestion methods conducted by Baechler et al. (2020).

Density Separation and Vacuum Filtration

Due to the presence of sediments and lingering biotic material in many samples, a density separation step was taken to further isolate microplastic particles from the rest of each sample. As many of the samples had dried out in their petri dishes, they were rehydrated overnight using a thin layer of filtered DI water. A hypersaline solution was prepared in 2.5L glass jars and remade as necessary, in which 168.4 grams of sodium chloride ($NaCl$) was added to 2L of filtered DI water. The jar was vigorously shaken for 2 minutes, and 400mL of solution was transferred to rinsed and labeled quart-sized mason jars. Samples were rinsed out of their petri dishes into the mason jars, using as little DI water as possible. A shucking tool was used as necessary to scrape sample remnants out of the petri dishes. Jars were covered and shaken for 60 seconds, and then placed on a lab bench for 24 hours at ambient temperature to allow the contents to stratify. While the heavier sediments settled out, lighter and more buoyant plastic particles floated closer to the surface of the samples. For each session involving this procedure, a density separation lab control containing 400mL of hypersaline solution was used.

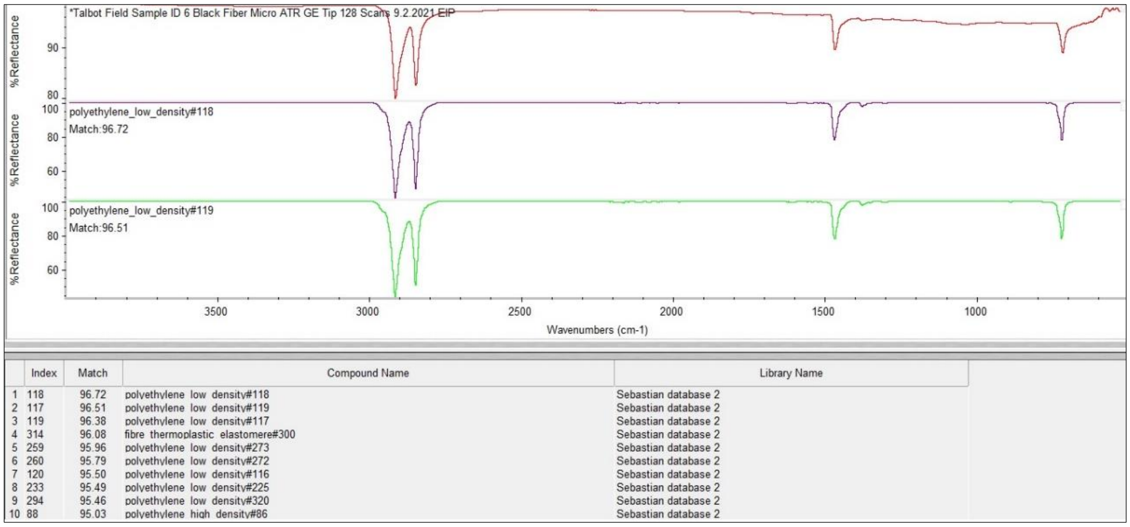
Following stratification, the hypersaline solution was vacuumed out with a filtration apparatus. In this setup, a piece of filter paper was placed on top of a glass base with sintered disc, which was mounted on a 2L Erlenmeyer flask. A glass funnel was secured to the glass base with a metal clamp, effectively pinning the filter paper between the funnel and the flask. Vacuum suction was created by connecting the apparatus to the sink with a rubber hose and turning the faucet on. Roughly two-thirds of each stratified quart sample jar was poured slowly into the funnel, with care being taken to minimize the inclusion of sediments. The suction caused the hypersaline solution to be pulled into the flask, leaving any plastic particles trapped on the filter paper (Valine et al. 2020). Each filter was then transferred to a clean petri dish and stored in a box to await microscope analysis. As before, a control containing filtered DI water was used to capture any airborne microplastics.

Microscope Analyses

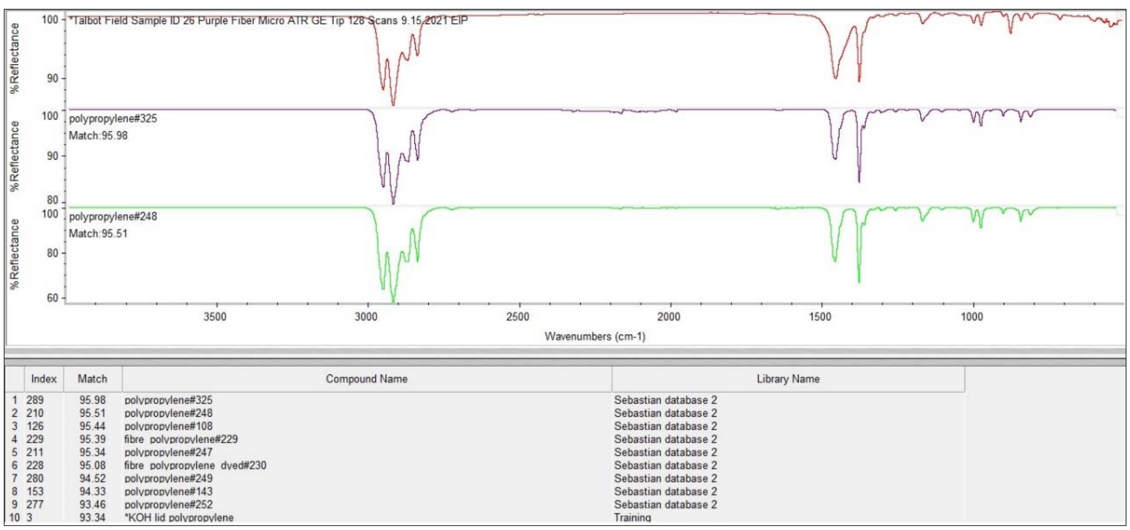
Stickers showing 12 numbered pie wedges were affixed to the bottom of each petri dish to aid in both orientation and the tracking of relative locations of plastic particles. Filters were examined using a Leica MZ6 dissecting microscope, and methodologies outlined in the Guide to Microplastic Identification (Marine & Environmental Research Institute, nd) were followed to aid in the distinction between microplastics and biotic material. For instance, particles showing cellular structure were excluded, along with fiber-like particles characterized by tapering. Additionally, particles that broke apart upon manipulation with a metal probe were also excluded. In these instances, the particle in question was assumed to be biological or non-plastic in nature.

Filter inspection began in the upper left section and continued in a straight line across the filter paper, with the aforementioned metal probe used to explore and prod particles to determine flexibility. Inspection of the row below commenced at the right side of the paper and continued to the left, and this horizontal pattern was repeated for each row of the filter paper. When a suspected microplastic was identified, information regarding type (fiber, fragment, film, foam), color, maximum width, maximum length, and magnification level were recorded on a datasheet. In addition, photographs were taken of each suspected microplastic and saved to a google drive for future reference and use. A control petri dish with a clean filter was placed next to the scope to assess contamination from airborne particles, and was evaluated for microplastics between each scoped study sample (Valine et al. 2020).

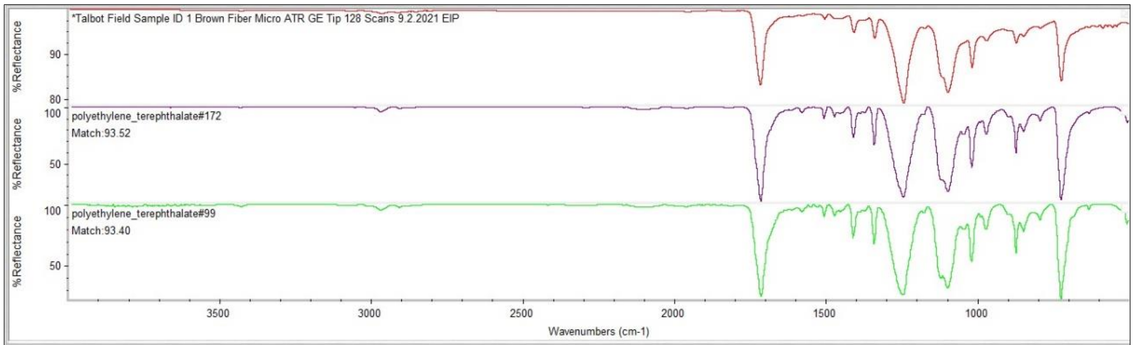
Appendix B. Examples of μFTIR spectra observed in study samples: (a) low density polyethylene; (b) polypropylene; (c) polyethylene terephthalate; (d) cellulose.



(a) Low-density polyethylene

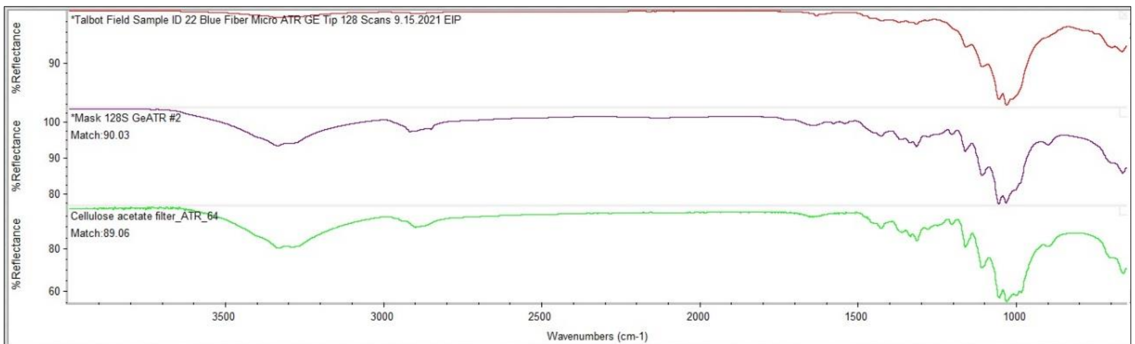


(b) Polypropylene



	Index	Match	Compound Name	Library Name
1	187	93.52	polyethylene terephthalate#172	Sebastian database 2
2	75	93.40	polyethylene terephthalate#99	Sebastian database 2
3	160	92.45	polyethylene terephthalate#136	Sebastian database 2
4	76	92.16	polyethylene terephthalate#98	Sebastian database 2
5	231	92.04	polyethylene terephtalate#227	Sebastian database 2
6	191	91.96	fibre polyester#217	Sebastian database 2
7	185	91.15	polytehylene terephthalate#174	Sebastian database 2
8	192	90.23	fibre polyester#216	Sebastian database 2
9	188	88.89	polyester#171	Sebastian database 2
10	157	88.20	polyester#139	Sebastian database 2

(c) Polyethylene terephthalate



	Index	Match	Compound Name	Library Name
1	2	90.03	*Mask 128S GeATR #2	Training2
2	1	89.06	Cellulose acetate filter ATR 64	NOAA plastics
3	68	88.73	fibre cotton combers#47	Sebastian database 2
4	12	87.67	cellulose#31	Sebastian database 2
5	69	86.91	fibre cotton US pima#48	Sebastian database 2
6	70	86.44	fibre cotton uzbekistan#49	Sebastian database 2
7	114	85.27	fibre linen#122	Sebastian database 2
8	85	84.73	fibre hemp fine#89	Sebastian database 2
9	123	84.65	cellulose wipe#113	Sebastian database 2
10	84	84.52	fibre hemp rough#90	Sebastian database 2

(d) Cellulose

Appendix C. Samples potentially experiencing contamination from improper rinsing of glassware.

Date	Order	Sample	# of MPs
3/16/2021	1	SEPT Regner Field Con (Grp 22)	10
	2	SEPT Estacada Field Con (Grp 22)	7
	3	AUG Milwaukie Field Con (Grp 22)	6
	4	Lab Control (Grp 22)	10
	5	SEPT Estacada Sample #1 (Grp 21)	2
	6	SEPT Estacada Sample #2 (Grp 21)	1
	7	SEPT Estacada Sample #3 (Grp 21)	2
	8	Lab Control (Grp 21)	8
	9	SEPT Regner Sample #1 (Grp 20)	12
	10	SEPT Regner Sample #2 (Grp 20)	10
	11	SEPT Regner Sample #3 (Grp 20)	5
	12	Lab Control (Grp 20)	13
3/23/2021	1	SEPT Rock Field Con (Grp 28	4
	2	SEPT Crystal Springs Field Con (Grp 28)	10
	3	Lab Control (Grp 28)	7
	4	SEPT Crystal Springs Sample #1 (Grp 27)	3
	5	SEPT Crystal Springs Sample #2 (Grp 27)	1
	6	SEPT Crystal Springs Sample #3 (Grp 27)	3
	7	Lab Control (Grp 27)	7
	8	SEPT Rock Sample #1 (Grp 26)	7
	9	SEPT Rock Sample #2 (Grp 26)	10
	10	SEPT Rock Sample #3 (Grp 26)	8
	11	Lab Control (Grp 26)	9
3/26/2021	1	AUG Oregon City Sample #1 (Grp 1)	5
	2	AUG Oregon City Sample #2 (Grp 1)	11
	3	AUG Oregon City Sample #3 (Grp 1)	4
	4	Lab Control (Grp 1)	15
	5	AUG Estacada Sample #1 (Grp 2)	2
	6	AUG Estacada Sample #2 (Grp 2)	2
	7	AUG Estacada Sample #3 (Grp 2)	3
	8	Lab Control (Grp 2)	12
	9	AUG Regner Sample #1 (Grp 3)	2

10	AUG Regner Sample #2 (Grp 3)	7
11	AUG Regner Sample #3 (Grp 3)	3
12	Lab Control (Grp 3)	12
13	AUG Regner Field Con (Grp 4)	7
14	AUG Oregon City Field Con (Grp 4)	10
15	AUG Estacada Field Con (GRP 4)	9
16	Lab Control (Grp 4)	8
17	AUG Crystal Springs Sample #1 (Grp 12)	3
18	AUG Crystal Springs Sample #2 (Grp 12)	4
19	AUG Crystal Springs Sample #3 (Grp 12)	3
20	Lab Control (Grp 12)	10