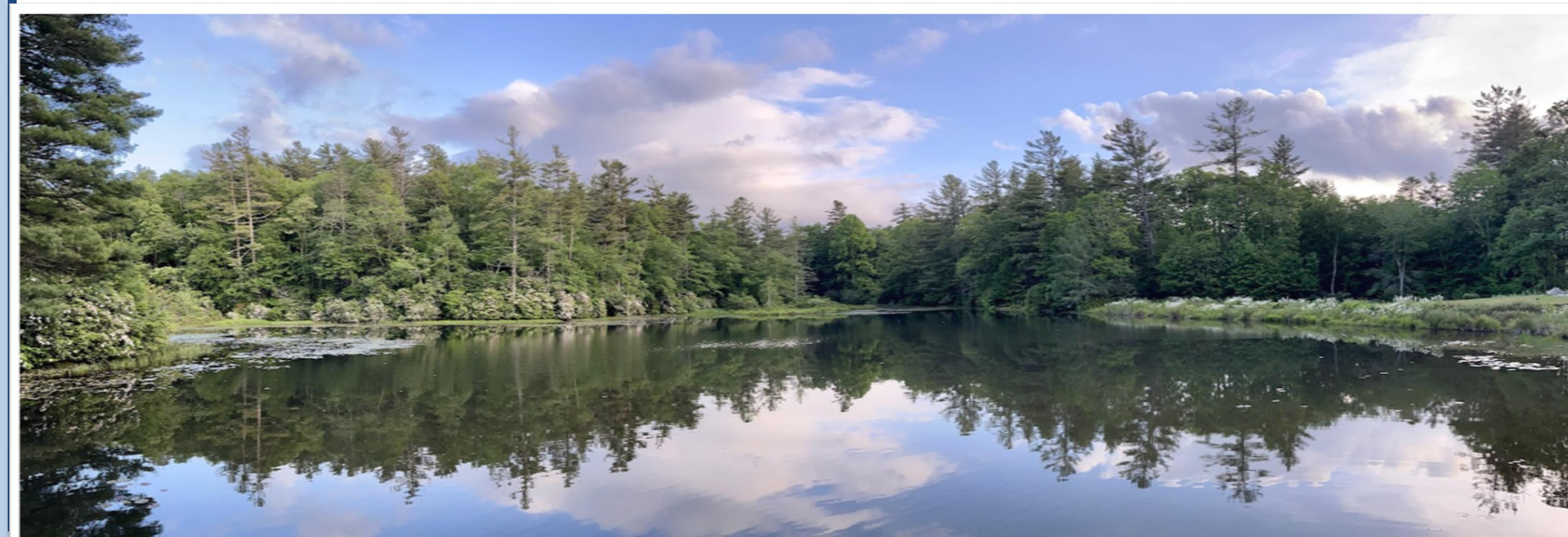


## Introduction

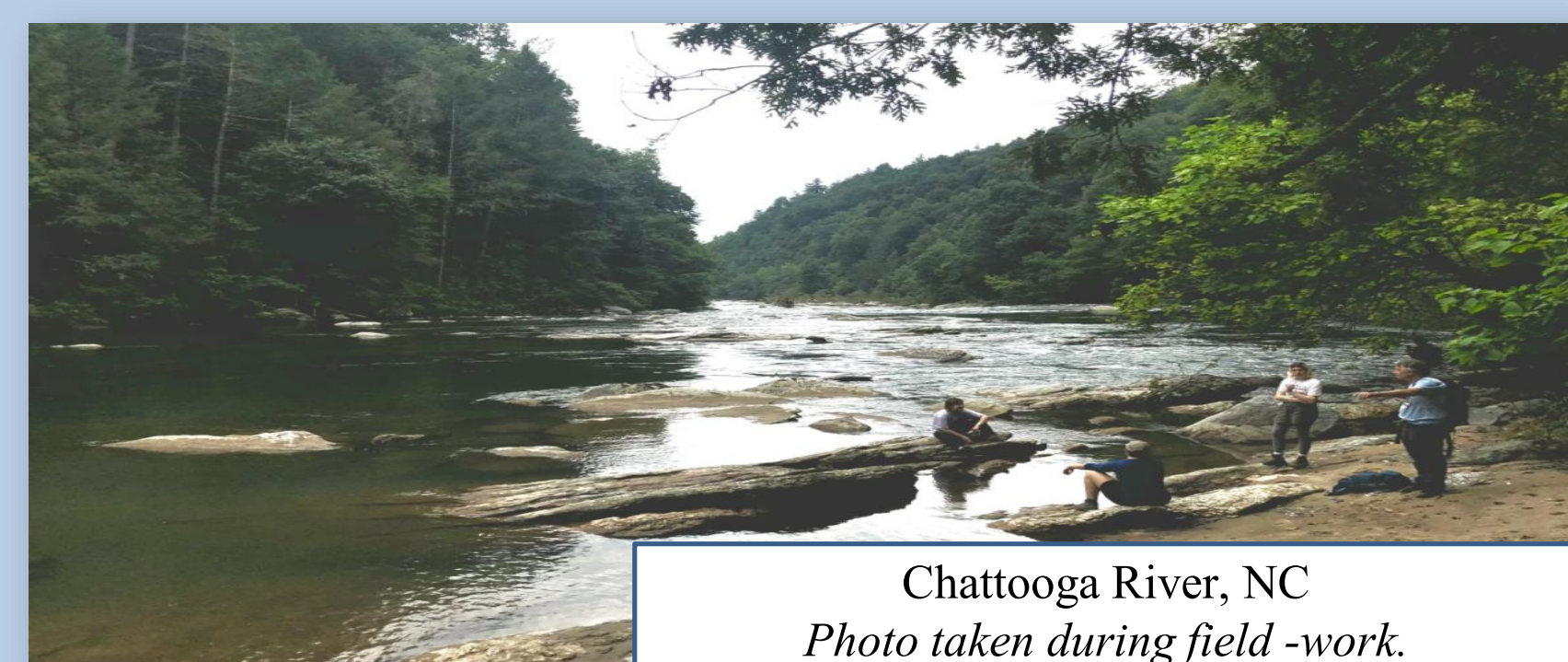
The durable, easy to produce, and cheap nature of plastics has rendered them a widespread pollutant in the environment (Jambeck et al. 2015). Only 9% of plastics ever produced have been recycled, with 79% ending up in landfills and 12% being incinerated (Geyer et al. 2017). As these plastics degrade, they form **microplastics** (MPs), which are defined as **particles between 1 μm and 5 mm in length** (Mani et al. 2015, Valsesia et al. 2021). The ubiquity of MPs in the environment presents numerous health concerns for both ecosystems and humans. When exposed to MPs, aquatic biota experience a range of toxic molecular and physiological impacts, including alterations to immune, metabolic, growth, reproductive functions (Anbumani and Kakkar 2018, Franzellitti et al. 2019).

This study aims to contribute to the growing body of evidence of MP accumulation and contamination in North American freshwater systems, considering that less than 4% of MP research is focused on freshwaters (Lambert and Wagner 2018). Research of this nature is novel considering that no freshwater MP analysis in the southeastern US has been published and there is only one published study that has examined MP concentrations during storm events (Hitchcock 2020). Microplastic research regarding aquatic systems is not only vital information for the management of freshwater systems but is also vital information for those working to preserve the southern Appalachians, a well-known biodiversity hotspot (Van Sickle 1999, Simon et al. 2005).



## Study Objectives

- The present study aims to determine the levels of microplastics in headwater streams in the southern Appalachians by **investigating how storm events influence microplastic concentrations** in streams, atmospheric deposition, and runoff, thus allowing conclusions to be made on the potential sources of contamination.
- Additional goals of this study are to **quantify and characterize** any detected microplastics to help increase the current knowledge database for the novel field of microplastic research in freshwater systems.
- Establish novel MP sampling, quality control, and characterization techniques using Raman spectroscopy.



## Acknowledgements

First and foremost, a heartfelt thank you to Jason Love (HBS) and Jerry Miller (WCU) for bringing this project to life. Many thanks to the staff of the Highlands Biological Station and the Highlands IE '21 and '22 cohorts for continuing this research. An additional thank you to Dr. Austin Gray from Virginia Tech University for allowing the use of his lab's Raman Spectrometer. A special thank you to my field-partners Noa and Grace. Lastly, I thank the UNC Office of Undergraduate Research for awarding me the SURF grant and my SURF advisor Dr. Radmila Petric for providing me with this amazing opportunity.

## Methods

- Sampling was conducted in the Chattooga River watershed in Highlands, NC at two headwater streams above and below the local wastewater treatment plant and at designated atmospheric deposition sites (Fig. 1).
- Topographic and hydrologic surveying at the two stream sites was conducted regularly to calculate discharge (cms) and create representative rating curves.
- When storm events with precipitation >2.5 cm of precipitation were identified in the study region, we deployed the pre-installed Teledyne ISCO full-size portable sampler (ISCO) to collect water samples at programmed intervals during base and storm flow. Weekly quality control samples using DI water were collected from the ISCOS as well as control grab samples from the stream sites.
- Atmospheric deposition collection buckets were deployed in the terrain surrounding the two stream sites and were opened up for collection during storm events.
- All samples (stream, atmospheric, and control) were filtered using a vacuum system and then analyzed under a microscope for MP presence.
- Identified MPs were recorded, quantified, and used for later data analysis relating discharge to MP concentration.
- A select number of MPs were sent for analysis using a Raman spectrometer to identify the specific chemical composition of the plastic.

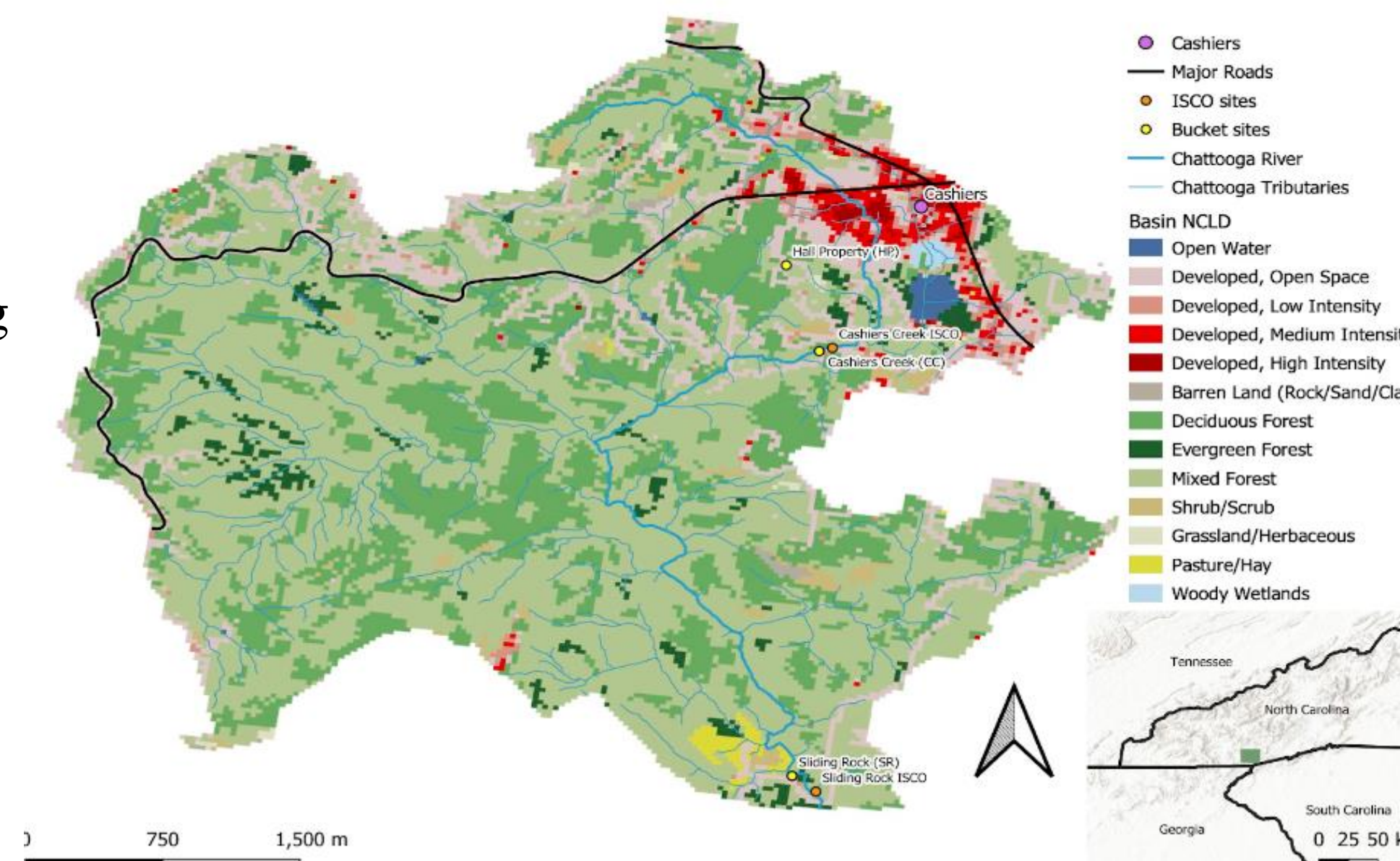
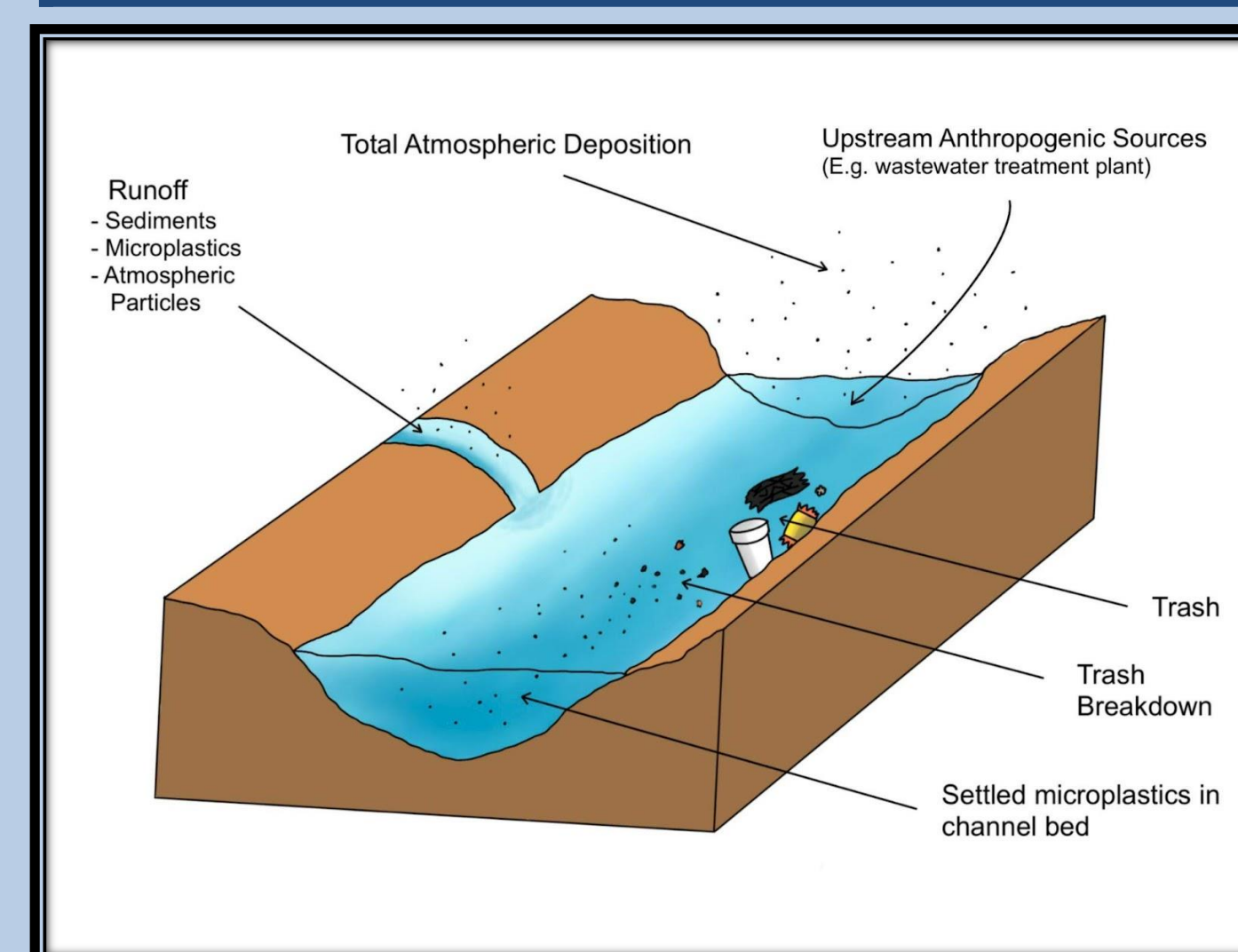


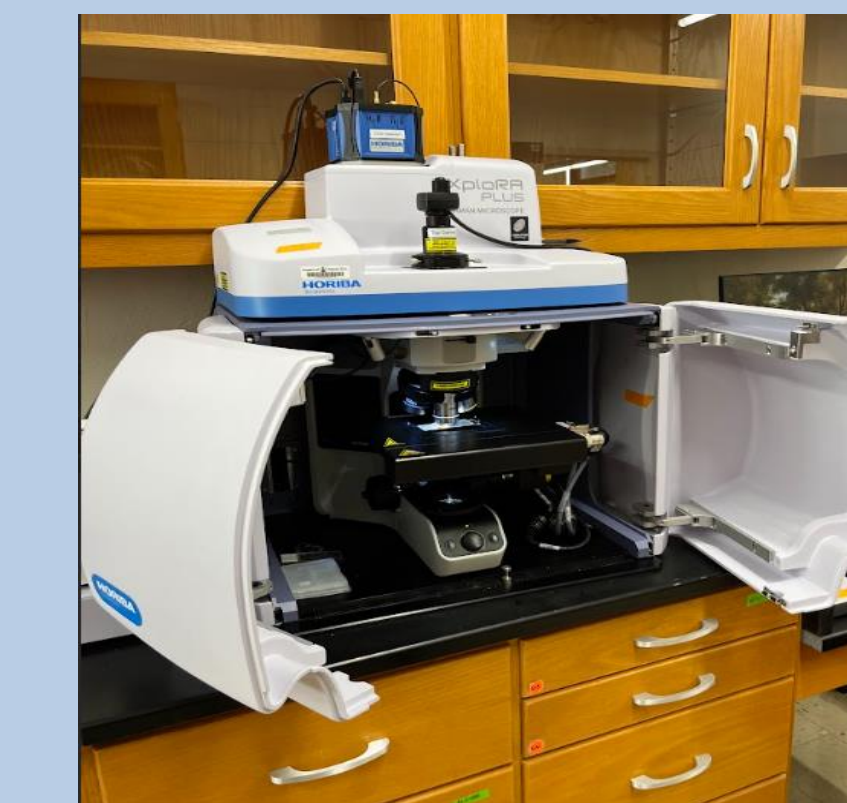
Fig. 1. Map of sample sites in the Chattooga watershed overlaid with National Land Cover Data. The watershed is primarily forested with scattered low density housing developments. The unincorporated town of Cashiers sits in the northeastern part of the watershed. QGIS.



ISCO water samples after a storm event.



Image of detected microplastic (purple fiber) after filtration.



Raman Spectrometer in Dr. Austin Gray's lab at VT.

## Results

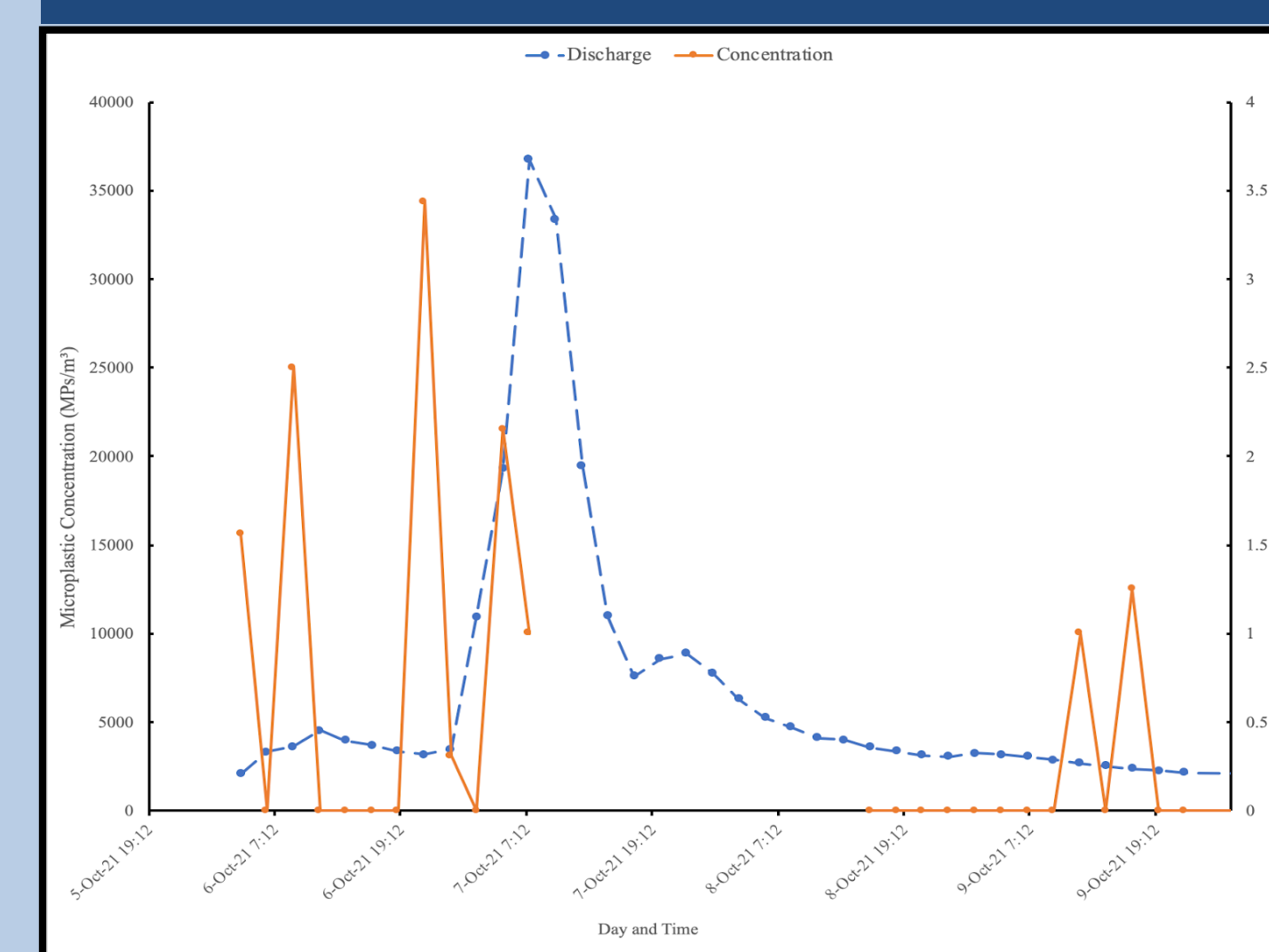


Fig. 5. Change in MP concentration at Cashiers Creek as discharge (in cubic meters per second) changed during the storm event.

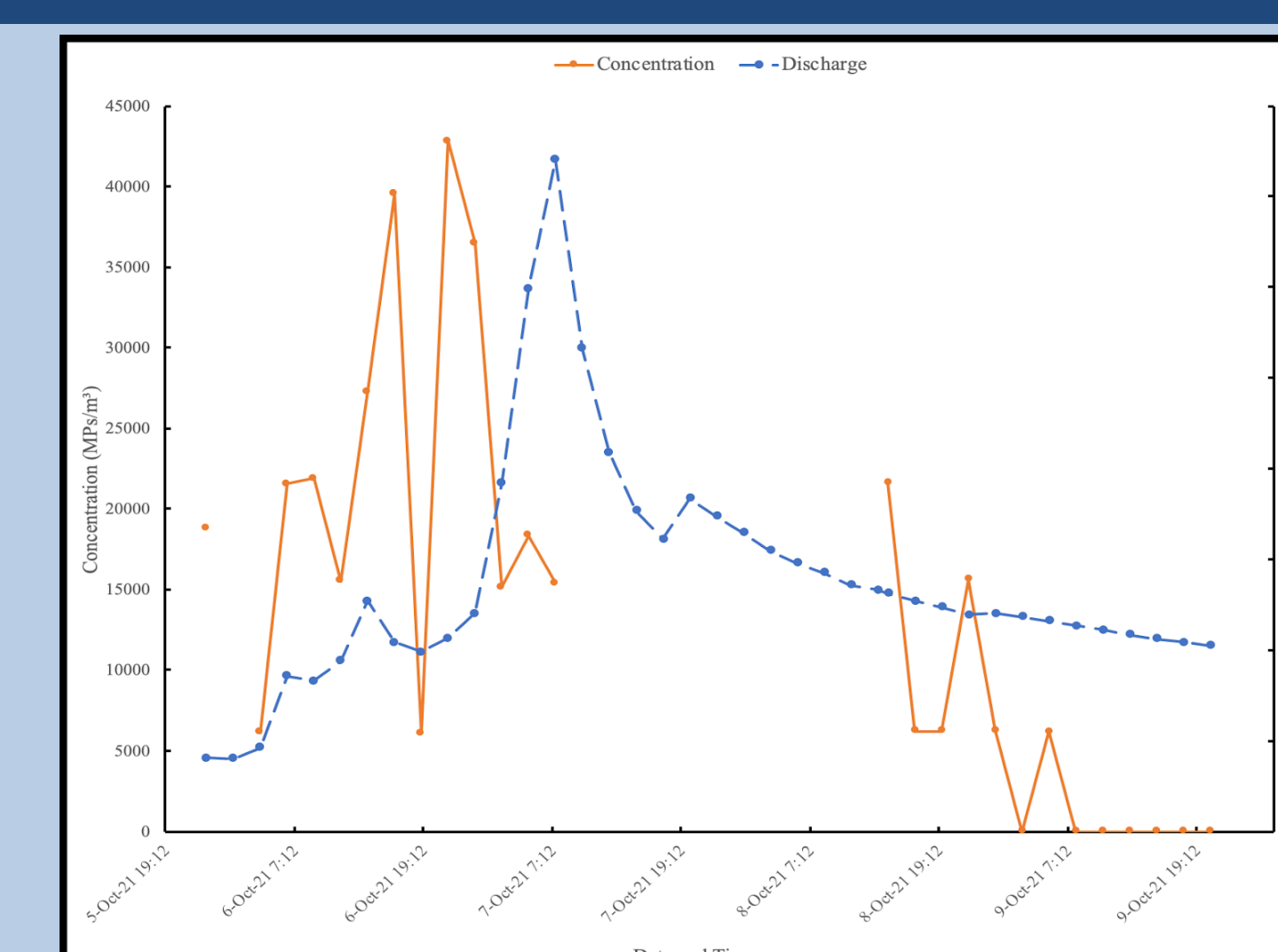


Fig. 6. Change in MP concentration at Sliding Rock as discharge (in cubic meters per second) changed during the storm event.

TABLE 1. Amount of atmospheric deposition (MPs/m<sup>2</sup>/day) at sites in the upper Chattooga River watershed, Jackson Co., NC between 1 and 21 October 2021. Sliding Rock and Hall Property sites were throughfall deposition. Deposition was highest at Cashiers Creek which was an open field site.

Location	Date Deployed	Date Collected	Total Time (days)	MPs (m <sup>2</sup> /day)
Sliding Rock	1 Oct	8 Oct	7.0	175.7
	8 Oct	15 Oct	6.9	1.1
	15 Oct	21 Oct	6.1	23.6
Cashiers Creek	1 Oct	8 Oct	6.9	190.8
	8 Oct	15 Oct	6.9	12.0
	15 Oct	21 Oct	6.1	8.7
Hall Property	1 Oct	8 Oct	7.1	162.0
	8 Oct	15 Oct	6.9	12.0
	15 Oct	21 Oct	6.2	18.38

## Discussion and Conclusions

- No significant relationship between discharge and MP concentration was determined, however similarities between atmospheric and stream MPs indicate **potential atmospheric circulation and deposition of microplastics into freshwater systems**.
- Our study's estimated maximum number of MPs found in one sample was  $5.08 \times 10^4$  MPs/m<sup>3</sup> during storm flow. This result is magnitudes higher than other studies findings and indicates **potential high contamination levels for the Chattooga river watershed**. We confirmed approximately 82% of collected particles as anthropogenic microparticles using Raman spectroscopy.
- MP contamination poses a unique threat to the biodiversity hotspot that is the southern Appalachians, the streams in this region are the "water towers" of the Southeast and should be monitored closely.
- Significant MP contamination can occur during sampling and it is vital to correct for this contamination. This study is ongoing and additional work will help to delineate the sources, and drivers of MPs.

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