Supplemental Information

Temporal and Spatial Variations in Microplastic Concentrations in Small, Headwater Basins in the Southern Blue Ridge Mountains, North Carolina, USA

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Text 1: Microplastic Separation and Analysis

As noted in a detailed review of 183 papers by Lu et al. (2021), no widely accepted set of methods are available for the analysis of microplastics in freshwaters. Between 2020 and 2022 we conducted a set of experiments aimed at developing the methods that were most effective at analyzing microplastics in water and sediments in small headwater streams in the southern Appalachians that are characterized by relatively low concentrations of suspended sediments (often <25 mg/L, Miller et al., 2015) and high amounts of particulate organic matter in 10 to 500 µm size range (associate with extensive forest cover). Methods development involved, in part, experiments of recovering microplastics of varying size from sediments spiked with 6 polymer types (PP, PS, PE, PC, PVC, and PET) using different separation solutions including oils (olive and canola), and, to a lesser degree, NaCl, ZnCl, and ultimate potassium formate ($CHKO_2$) for which more data already exists. For sediments, we elected to use potassium formate on the basis of effectiveness, toxicity, costs, and degree of interference with post-separation analyses. For example, while oils were effective at separating microplastics of sediments, we were unable to consistently remove the oils using alcohol or solvents from the particles such that they would not interfere with fluorescent microscopy of filters stained with Nile Red, or Raman Spectroscopy.

While gravity separation is used in our analysis of sediment samples, we do not use a floatation method for water samples for three primary reasons: (1) during gravity separation, we found that a small percentage of particles settle with the sediments in all of the solutions tested. Settling was more pronounced for high density polymers (PVC, PET). In oil, NaCL, and ZnCl settling often result in a 20% loss in the recovery of spiked particles; (2) the use of density separation increases the potential for sample contamination, and (3) the low concentrations of mineral matter in our water samples made it possible to visually examine particles of all compositions less than <106 µm without the extra time, costs, etc. associated with gravity separation. Importantly, Lu et al. (2021) found that about half (48 %) of the 183 publications in their database did not use density separation for the analysis of water samples. The effectiveness of particle identification was tested by analyzing about 5 % of all particles within the Richland Creek basin by Raman spectrometry. Approximately, 98.5 % of particles were plastic polymers; 1.5 % were false positives.

Numerous studies have analyzed filter membranes stained with Nile Red to identify microplastics. We also examined the use of this process to help identify false negatives. It was highly effective (> 99 % recovery) at identifying microplastic standards in filtered deionized water. However, we found that the abundance of natural organic matter in our water samples led to a significant (multi-fold) overestimate in MP abundance. In the case of sediment samples, treatment with H_2O_2 and Fenton's reagent, was unable to remove all of the natural organic particles. This is consistent with the detailed studies of organic removal in soil samples (e.g., Hurley et al., 2018) and the detailed laboratory studies by Pfeiffer and Fischer (2020) that showed that the removal of organic particles was incomplete using a H_2O_2, as well as other reagents.

Thus, the use fluorescent microscopy of Nile Red stained slides appears to lead to an overestimation of microplastics in waters containing abundant particulate organics of similar size to microplastics.

Microplastics were extracted from collected sediments through density separation using a filtered, nearly saturated potassium formate solution (>1.7 g/cm3). Positive controls were performed a regular intervals to ensure that flotation of dense plastics. Following density separation, samples were filtered, and the particles analyzed, using methods similar to that applied to microplastics in water. Measures were used, however, to recover the potassium formate solution of reuse.
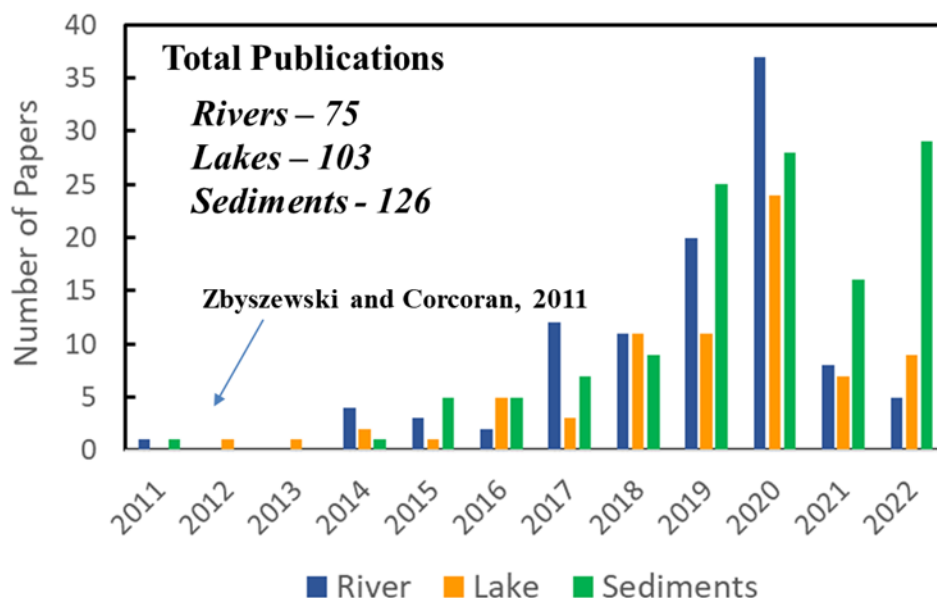
Figure S1. Number of referred papers published on MPs in rivers and lakes.

Table S1. Summary of land-use/land-cover conditions upstream of sampling sites within Richland Creek.

	Upstream Basin Area (km)							
	Site 8	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Open Water	0.00	0.00	0.18	0.18	0.00	0.18	0.18	0.18
Low intensity development & Open	0.06	5.45	1.43	22.09	3.66	26.33	26.89	31.53
Medium to High Intensity Development	0.00	0.53	0.19	2.71	0.42	3.47	3.56	3.99
Forest	7.23	20.81	41.26	86.24	1.31	87.67	88.00	100.92
Barren	0.00	0.00	0.22	0.25	0.00	0.25	0.25	0.26
Other Vegetation	0.01	1.16	0.84	4.53	0.05	4.69	4.95	10.54
Wetlands	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Totals	7.30	27.95	44.12	115.99	5.43	122.58	123.82	147.42

|	Percent of Upstream Basin (%)							
	Site 8	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Open Water	0.00	0.00	0.40	0.15	0.00	0.14	0.14	0.12
Low intensity development & Open	0.81	19.49	3.24	19.04	67.34	21.48	21.71	21.39
Medium to High Intensity Development	0.00	1.90	0.44	2.34	7.68	2.83	2.88	2.71
Forest	99.09	74.45	93.53	74.35	24.06	71.52	71.07	68.46
Barren Land	0.00	0.00	0.49	0.25	0.00	0.25	0.25	0.26
Other Vegetation	0.12	4.15	1.90	3.90	0.88	3.83	4.00	7.15
Wetlands	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Totals	100	100	100	100	100	100	100	100
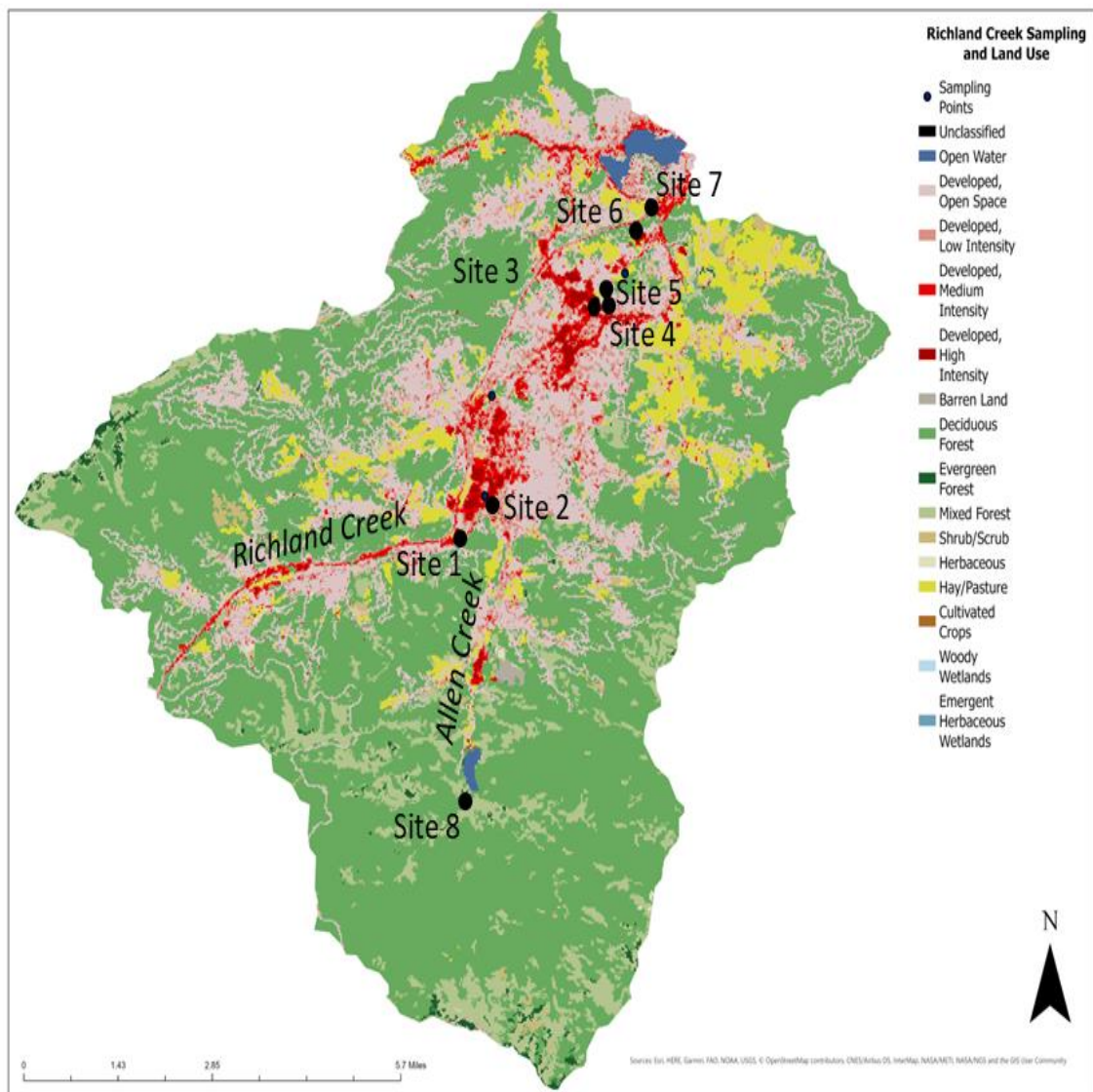
Figure S2. Land-use map of the Richland Creek study area (data obtained from the National Land-Cover database).
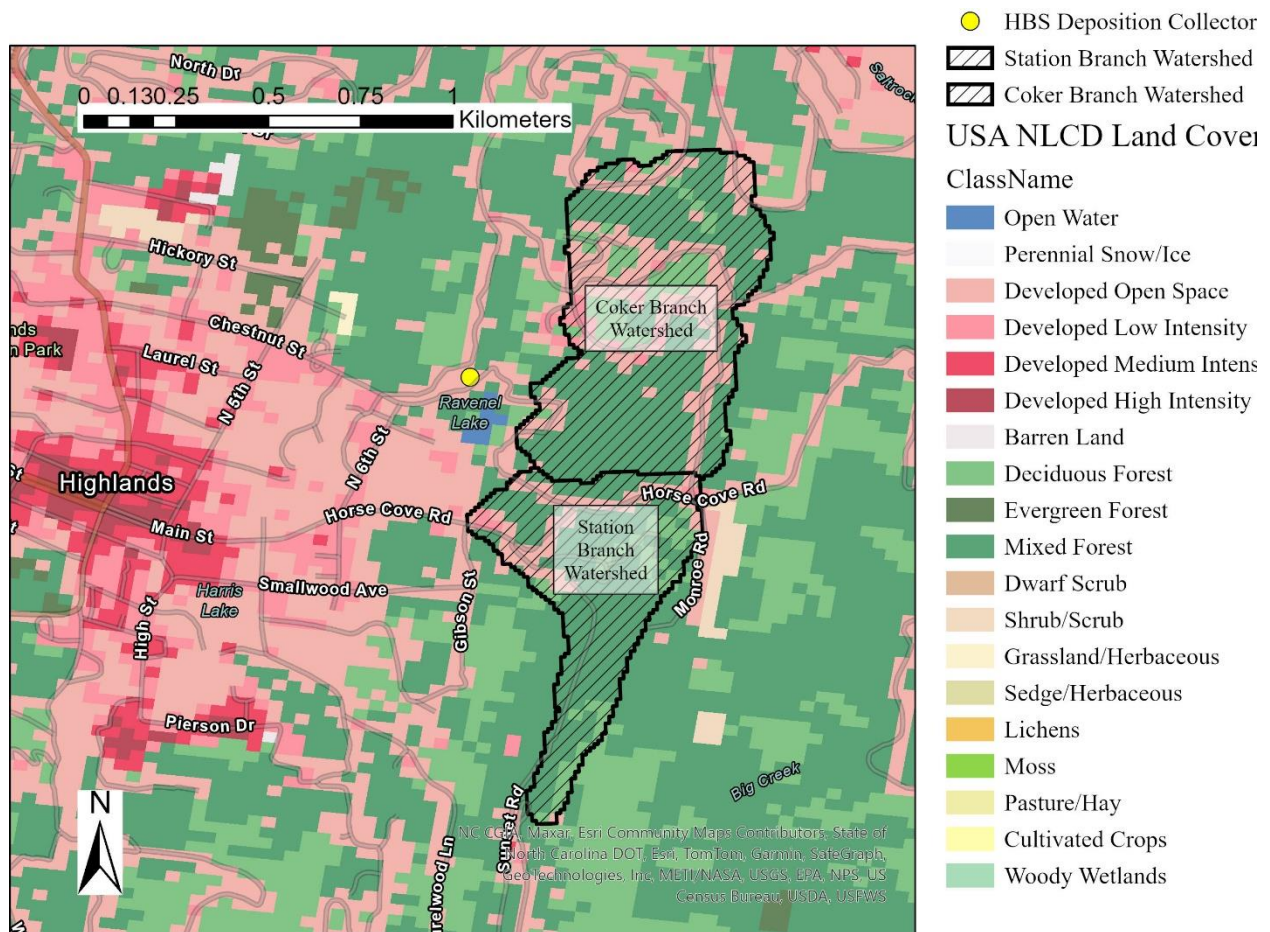
Figure S3. Land-use map of the Cullasaja River study area (data obtained from the National Land-Cover database).

Table S2. Source and online links to data used to develop the geographic information systems for the study basins.

| Mapped Feature	Data Source
Watershed delineations	USGS StreamStats; https://streamstats.usgs.gov/ss/
Hydrography lines (streams)	NC OneMap https://www.nconemap.gov/datasets/6ad241c95985415d819156c767938b9d_4/explore?location=35.485311%2C-82.993036%2C13.68
Soils Data	Waynesville Open Source Hub; https://opendata-hayco.hub.arcgis.com/search?tags=environment https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx
Topographic Data (Base maps)	ESRI; https://www.arcgis.com/home/item.html?id=7378ae8b471940cb9f9d114b67cd09b8
Topographic	USGS; https://ngmdb.usgs.gov/topoview/viewer/#10/35.5976/-83.2468
Land-Use/land-cover	National Land Cover Database; https://datagateway.nrcs.usda.gov/GDGOrder.aspx
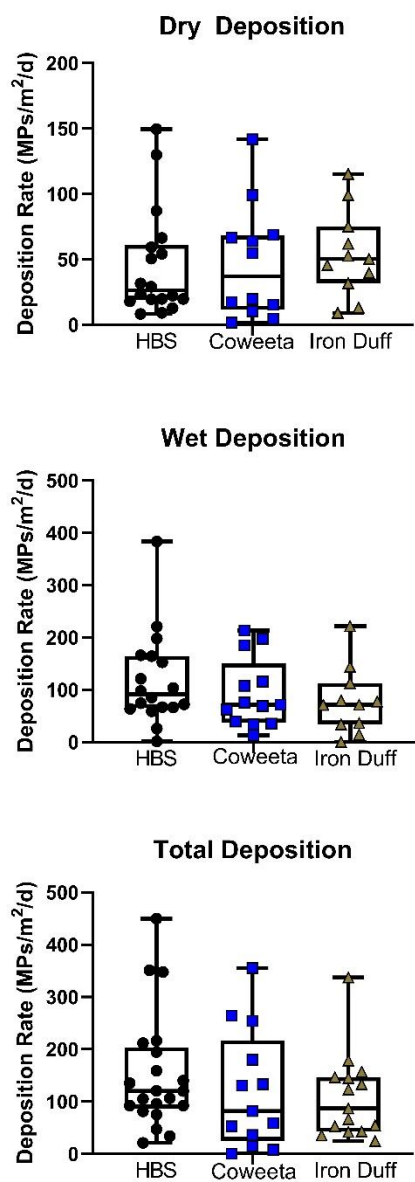
Figure S4. Comparison of atmospheric deposition rates between the three monitoring sites.