Headwaters to Valley: Water quality in rivers transitioning from forest to agricultural bottomland

Doug Graber Neufeld, Isaac Alderfer, Zachary Bauman, Micah Buckwalter

Department of Biology, Eastern Mennonite University, Harrisonburg, Virginia, United States of America

Abstract

Many waterways flow out of forestlands, which tend to maintain higher water quality, into agricultural lands, which tend to degrade water quality. The roles of these two types of land cover in impacting key water quality parameters (phosphorus as orthophosphate, nitrogen as nitrate, total suspended sediment (TSS), and indicator bacteria) were investigated for the watershed of the North and South Fork of the Shenandoah River, Virginia. This area has a particularly sharp boundary between heavily forested and heavily agricultural regions. Two datasets were analyzed: 1) a large number of datapoints spanning a 20-year range in the Water Quality Portal (WQP) database, and 2) transects along three representative rivers systems over the span of a 4-year period. Overall nutrient levels were significantly better in forested regions than agricultural regions; while TSS and indicator bacteria showed some trends, but less consistently, of higher water quality in forested regions. All parameters had significant variation in both forested and agricultural areas. Transects showed that waterways flowing out of forestland maintained higher water quality for approximately the first 8 km in agricultural land. Both transect and WQP data indicated higher water quality when the percent of forested land cover in a drainage basin was about 70-80%. Thus, forestland does mitigate the impacts of agriculture on water quality to some degree, but this effect rapidly diminishes as forest cover of the watershed lessens. Furthermore, forests themselves have degraded water quality at certain times and places; for instance, nutrients level were in the medium to high stress level for aquatic life in approximately 15% of samples. This study illustrates general trends of land cover effects on water quality, while also highlighting both site-specific variability, and the dynamics of water quality as water flows out of forested areas into agricultural areas.

Introduction

Aquatic ecosystems from headwaters to larger waterways downstream have been a major focus of efforts at maintaining and improving environmental quality, due to their combined importance in providing ecosystem services, and their integral role in the lives of people [1]. Indeed, maintenance of water quality is directly related to important planetary boundaries such as the geochemical cycling of nitrogen and phosphorus [2], which have clearly exceeded safe operating zones. Numerous studies have explored key water quality parameters elucidating both patterns that are generalizable across waterways, and patterns that are specific to local spatial and temporal conditions.

Land cover, and especially continued pressure from human activities that alter land cover, are fundamental in driving water quality of watersheds. Most notable is how natural vegetation such as forests can improve water quality, while agricultural activities (both pastureland and cropland) can degrade water quality. Protection of forested lands is seen as crucial to maintaining clean water [3,4], notably illustrated by a recent study in the southeast United States found that forests had the highest water quality of all landscape types [5]. In parallel to land cover effects, headwater regions (which often are more forested) have unique characteristics compared to downstream waters [6-8]. There are clearly multiple factors beyond just land cover type that control water quality [5], and more information is needed on how water quality changes as waterways traverse different landscapes. In particular, it is not clear whether there is a sharp transition in water quality as it traverses forested and agricultural land, and how the impacts of the two landscapes combine to influence downstream water quality.

Watersheds draining into the Chesapeake Bay are the focus of a long running effort to understand how stressors such as nutrients and sediments contribute to estuarine health, and how these inputs can be reduced [9-11]. Efforts in the Chesapeake watershed have been mixed, illustrating both the ongoing challenge of reducing agricultural inputs, and the need for more information on what exactly drives nutrient trends [9,11,12]. The Shenandoah River watershed of Virginia and West Virginia is a major tributary system of the Chesapeake Bay, and stands out as having both some of the region's largest protected forestlands, and also the highest density of livestock and poultry operations in Virginia, along with significant cropland. Furthermore, there is a generally distinct boundary between the relatively flat valley bottomland which is dominated by agriculture and predominantly of limestone origin, compared to the surrounding mountains which are heavily forested, federally protected as national forest and national park land, and predominantly of sandstone origin. This watershed therefore represents a unique opportunity to study a sharp transition of waterways between heavy forestland of headwaters, and intensive agriculture downstream.

The present study combines data from a large public database (Water Quality Portal; WQP) with targeted sampling on representative river systems to compare water quality in adjacent clear heavily forested and intensively agricultural lands, and to characterize the pattern of change as rivers flow between these two types of land cover. We focus especially on nutrients (nitrate and orthophosphate), sediments, and bacteria as parameters determining both local aquatic health, and downstream impacts [13,14].

Methods

Ethics Statement

Site access outside of national forest was either at publicly accessible right-of-way along state or county highways (usually bridges), or by landowner permission. Within the national forest, permission for site access for the purpose of water sampling was granted by the US Forest Service.

North and South Fork Shenandoah River Watershed

Water quality data for the combined watersheds of the North and South Forks of the Shenandoah River watershed were accessed from the Water Quality Portal (WQP), an open access compilation of water quality results from multiple government, academic and nonprofit organizations. Since land downstream of the confluence of the North and South Forks (north of Front Royal, Virginia) does not have protected National Forest or National Park land (as dominates forestland upstream from this point), we did not analyze the watershed of the Shenandoah River downstream of the confluence. Samples from within the delineated watershed were selected for the 20 year period from Jan 1, 2004 to Dec 31, 2023. The number of sample values varied with parameter and ranged between 9,742 and 29,897. Since most sites had multiple measurements of a parameter for that period, we used the mean parameter value for each site. Outliers were identified and removed for samples having Cook's distance of 4/n (0.005 for this dataset), and $p<0.05$. The number of outliers varied with parameter, ranging from 1.4% to 3.4% of samples, and the resultant number of sites for each parameter ranged from 639 to 738.

Spatial analysis was performed using ArcGIS Pro 3.3 and QGIS 3.38.0. Since in other aquatic systems, water quality is shown to be driven by overall land use across the entire drainage basin for a collection site [15], we delineated watersheds from USGS 1/3 arc-second Digital Elevation Maps (3DEP product in The National Map), and land use/land cover (LULC) from the USGS National Land Cover Database (2021 data). Geology was mapped as Generalized Lithography in the State Geologic Map Compilation (SGMC) Geodatabase of the Conterminous United States (ver. 1.1, August 2017). To calculate a general forest boundary, we calculated the average percent forest within a 200m radius of each pixel (pixel resolution 25m) of the LULC raster using the focal statistics tool of ArcGIS

Pro. Pixels with at least 75% forest in this radius were counted as forest. This produced a smoothed general forest boundary that depicts the visual forest transition as seen on satellite images. River kilometers were then calculated upstream and downstream from the point at which the river crosses this boundary.

River Transect Sites

Three river systems were chosen for water sampling as transects – the Dry River, Briery Branch, and the North River. All three rivers originate in the mountains on the western edge of the Shenandoah watershed, converge as the lower North River, and then flow together into the South Fork of the Shenandoah River which joins the Potomac River before entering the Chesapeake Bay. The headwaters are at approximately 3000 feet, and flow through the oak-hickory forest of George Washington National Forest before abruptly entering the Shenandoah valley agricultural region.

Water sample sites were selected at approximately 5km intervals, although specific sites were dependent on availability of river access. In addition, river sections in the middle interior of the valley dried completely during periods of low rainfall, especially in midsummer. In other cases of high rainfall or winter, sites in the national forest were inaccessible for sampling. The final number of samples per site therefore varied. Samples were collected between March 2020 and July 2024 and were taken at all times of the year. As a general indication of river level in the region, flow discharge was recorded from a USGS station (#01622000) near Burketown on the North River, and varied from 114 to 1430 ft^3 per second, representing a range of discharge from 18th to 97th percentiles for the period samples were taken. Samples thus represent a wide range of seasons and river flow conditions.

At each site, parameters were either measured onsite (temperature and conductivity), or water samples were collected in dedicated water sample bottles (nitrate, orthophosphate, bacteria, sediments). All measurements or

123 bottle samples were collected in the main column below the water surface, upstream from any disturbances
124 (wading or previous sampling). Field blanks were collected at one site per transect for nitrate, orthophosphate,
125 and bacteria. A field spike bottle was also collected at one site per transect for nitrate and orthophosphate. Field
126 duplicates were collected for all nitrate, orthophosphate and bacteria samples, and for one sediment sample per
127 transect. All samples were kept on ice (for transport) or at 4°C storage until analysis.
128

129 River Transect Parameters

130

131 Water samples were analyzed for sediment, nitrate, orthophosphate, temperature, conductivity, and general and
132 fecal coliforms. A Quality Assurance Project Plan, previously approved for earlier NFWF and Virginia DEQ grant
133 sampling, guided analysis, and was based on USGS, Virginia DEQ, or EPA approved procedures (e.g. U.S. EPA 2007.
134 SW-846).
135

136 Sediments were measured gravimetrically as per EPA method 160.2. In brief, 1 L samples were filtered through
137 pre-rinsed, dried and weighted 47 mm diameter GF/F filters (0.7 μM pore-size), and then re-dried and re-weighed.
138 Conductivity was measured using a Tracer Pocketester (LaMotte) calibrated to a 84 μS/cm standard. Probe
139 calibration was verified after each transect to ensure calibration did not drift during the measurement period.
140 Temperature was measured using a Vernier temperature probe, or the internal thermometer on the conductivity
141 probe. General and fecal coliforms were measured using Coliform Easygel (Micrology, Inc; Goshen, Indiana, USA).
142 1 ml samples were plated on agar petri dishes, incubated at 37°C until colony color development (generally 36-
143 48h), and then colony identity determined by color.
144

145 Nitrogen and phosphorus were assessed as nitrate and orthophosphate, respectively. While other forms would be
146 present, these are the most common and relevant forms in these waters. For instance, nitrate is the majority of
147 total nitrogen in the region [14]. Likewise, the study of Farthing et al [16] on the effect of forestland on

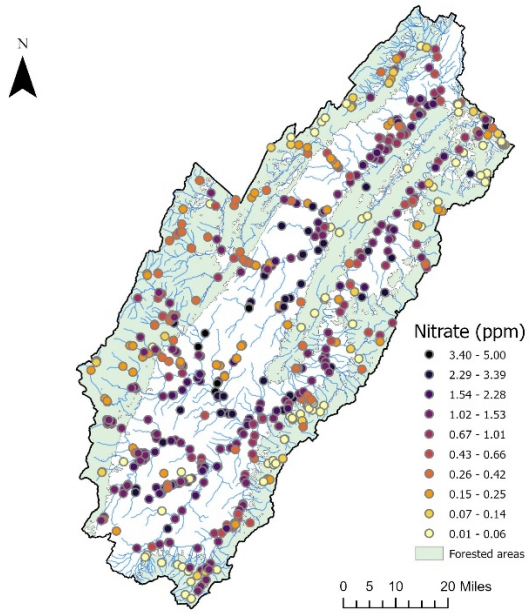
agriculturally impacted rivers found most nitrogen as nitrate, and most phosphorus as phosphate. Both nitrate and orthophosphate were measured with a colorimetric assay (Hach; for NitraVer5 and PhosVer3, respectively) according to manufacturer instructions. Duplicate sample bottles for each site were measured and the mean used as the final value. Absorbance values at appropriate wavelengths were compared to a calibration series, with blank and standard tested every 10th sample.

Results

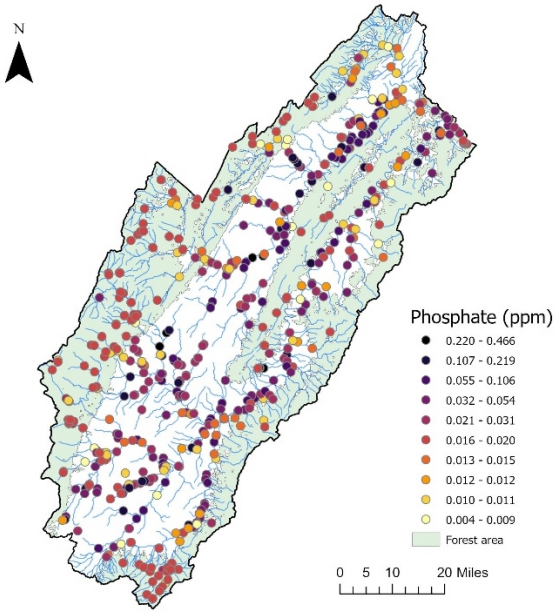
Water Quality Patterns in the North and South Forks of Shenandoah River Watershed

Water quality data from the WQP for the entire watershed of the North and South Forks of the Shenandoah River were compared with documented land cover. Parameters of concern generally correlated with the degree of forest cover in the watersheds represented by the sample sites. Nitrate, orthophosphate, and TSS all had higher concentrations outside of areas that were primarily forested (Figs 1a,b,c). This pattern was less obvious for *E.coli*, with many sites in forestland showing higher indicator bacteria concentrations (Fig 1d). Conductivity was higher in the valley (Fig 1e), due to the underlying karst geology of the valley, whereas the mountain areas are mostly silicoclastic (or basaltic, in a few regions of the blue ridge) (Fig 2). The forest boundary corresponded closely to the geological boundary, most likely because forest was historically cleared in the more agriculturally productive limestone/dolostone areas. (Fig 2). Because conductivity seemed so clearly correlated with the dramatic difference in geology that likely overwhelmed any anthropogenic increases in conductivity, we did not focus on conductivity in the analyses. The percent forestland in the drainage basin represented by sample sites reflects both site location (forested vs nonforested areas), and the area upstream draining into the point (Fig 1f).

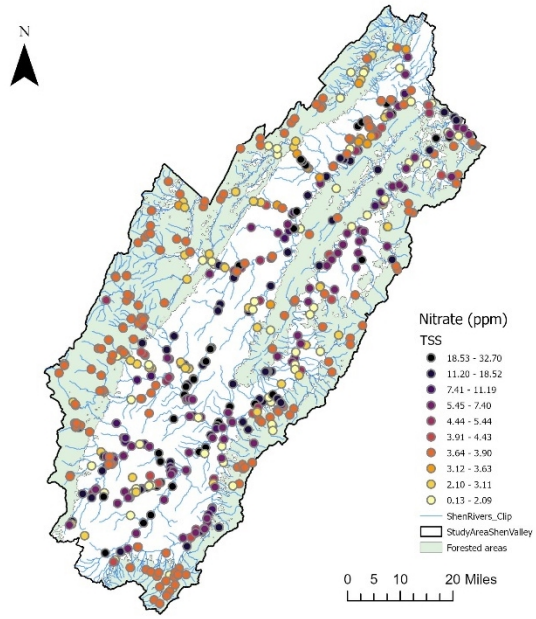
A. Nitrate



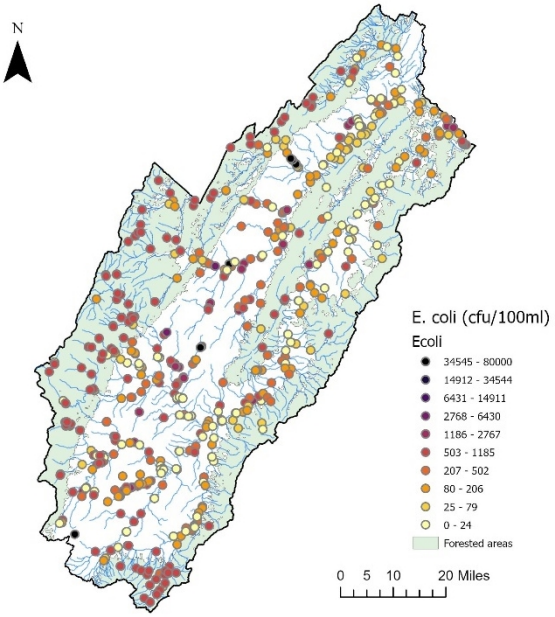
B. Orthophosphate



C. Total Suspended Solids



D. *E. coli*



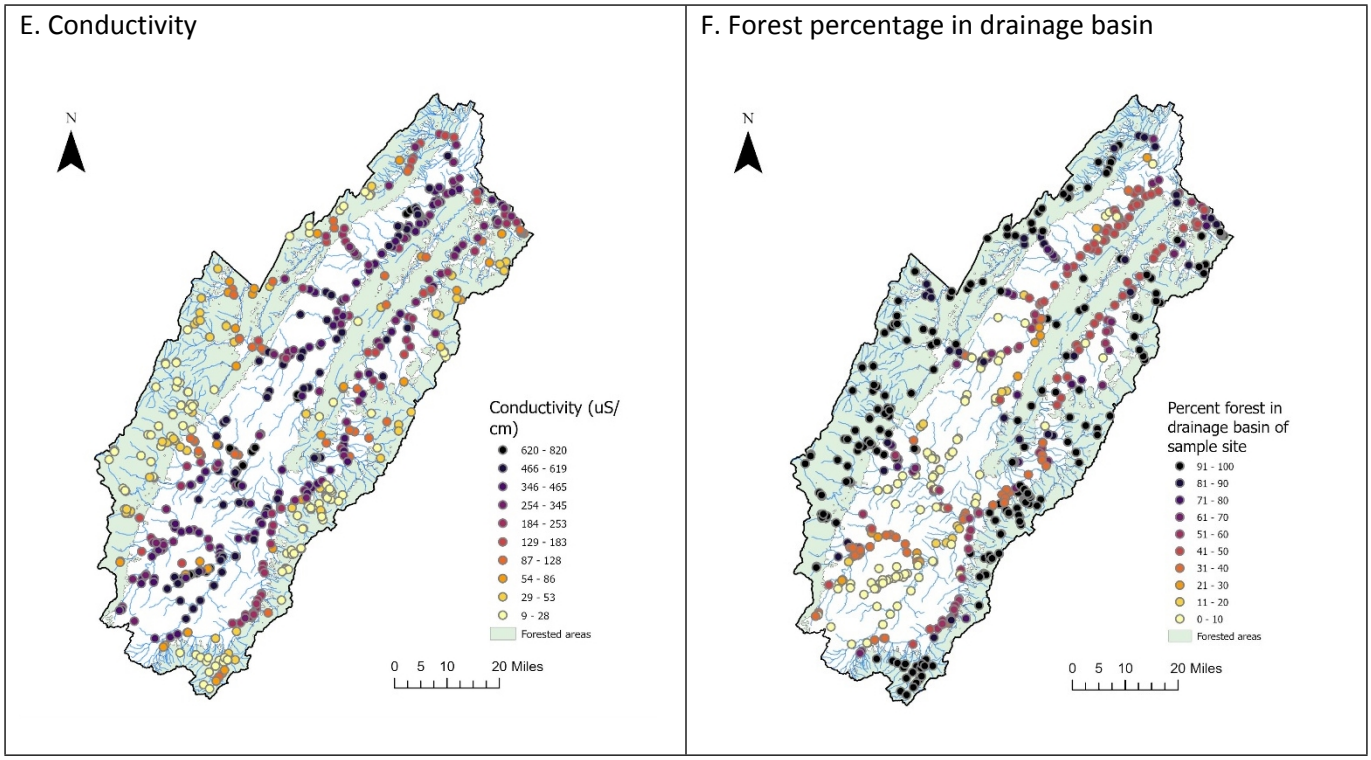


Fig. 1. Water quality parameters in watershed North and South Forks of Shenandoah River of Virginia. Data from Water Quality Portal for 2004-2023.

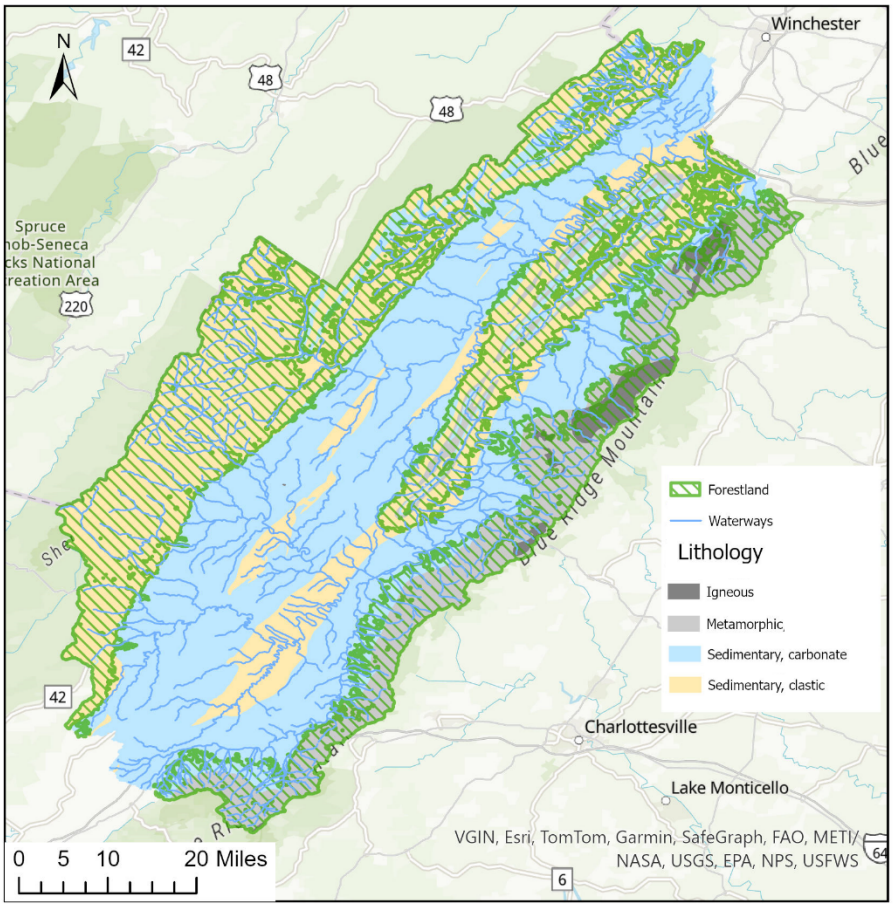


Fig. 2. Underlying lithology of the Shenandoah River watershed compared with areas with substantial forestland (areas with 75% forest cover within 200m radius; see methods).

We quantified how water quality correlates with forest cover first by comparing each water quality parameter to the percent forest cover of the calculated watershed draining into the sample sites. Linear regression of site watershed forest cover compared with parameters of concern indicated a significant relationship for nitrate and TSS, but not for orthophosphate or *E. coli* (Table 1). Given that parameters may relate nonlinearly to the proportion of forest cover, we further analyzed the relationship by grouping watersheds into 5 bins representing 20% increments of forest cover. Statistical analysis (ANOVA, with Tukey's HSD post-hoc) indicated significant differences with forest cover for all parameters (Fig. 3). For nitrate and orthophosphate, nutrient levels were significantly lower in the highest category of forest cover (80-100%), whereas the other categories of forest cover (60-80%, and lower) had higher and similar levels. Nitrate and orthophosphate data therefore suggested that the

187 greatest decrease in water quality occurred when rivers moved from heavily forested land to moderate or low

188 forest cover. This pattern was different for TSS, which showed a more gradual increase in concentration with

189 decreased forest cover, and for *E. coli*, which showed a less consistent pattern with forest cover.

190

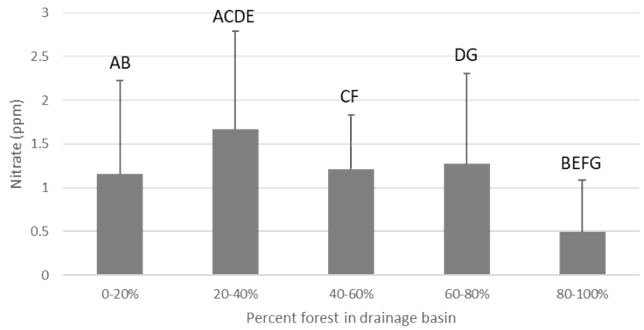
191 Table 1

Parameter	Relationship of parameter (y) to forest cover (x)	r^2	significance (p value)	sample size (N)
Nitrate	y=1.26x + 1.80	0.17	**p<0.001**	759
Orthophosphate	y=-0.036 + 0.064	0.03	p=0.373	708
TSS	y=-7.87 + 11.94	0.13	**p<0.001**	674
E. coli	y=-0.005 + 2.077	<0.01	p>0.500	648

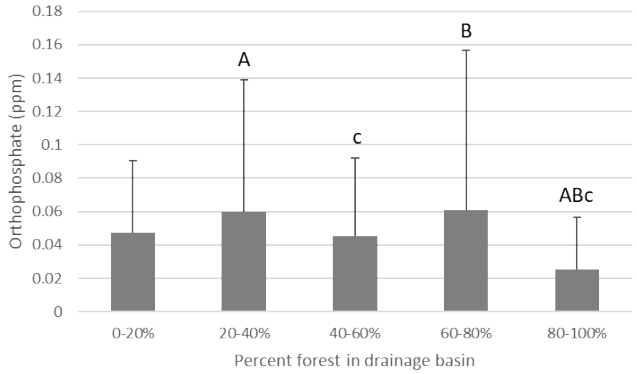
192

193

A. Nitrate



B. Orthophosphate



C. Total Suspended Solids

D. *E. coli*

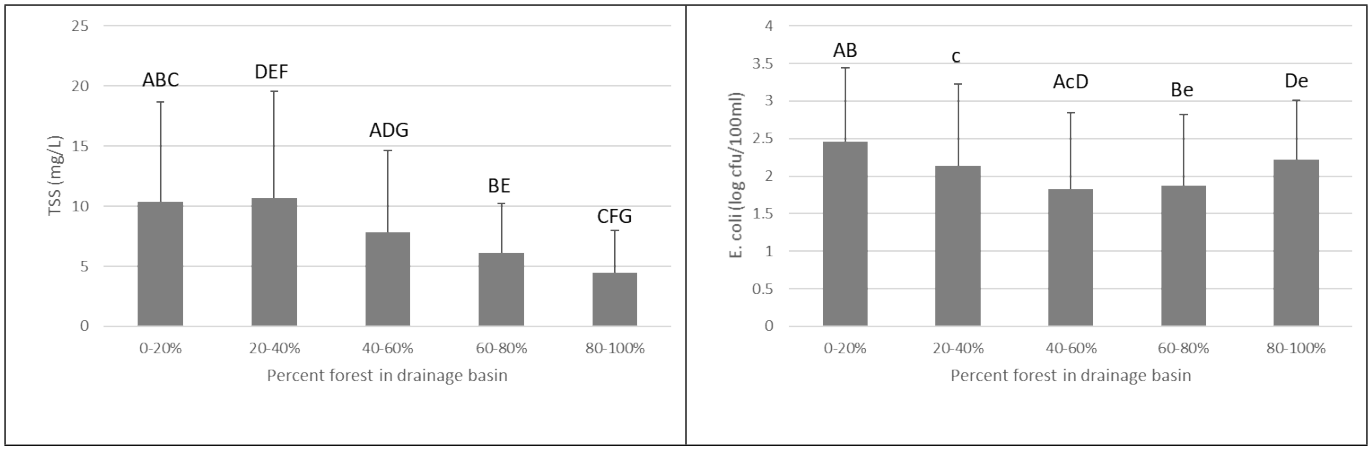


Fig. 3. Water quality in forest percent categories. Significant differences between categories indicated by letters on error bars (upper case $p<0.05$; lower case $0.05<p<0.1$).

Cluster and outlier analysis (Anselin Local Moran's I) confirmed that clusters of low nitrate, orthophosphate and TSS tended to be located in heavily forested land, but in some cases were just outside the forestland, or along major waterways that would take significant volumes of water into the valley (Fig. 4; light blue points). Clusters of high values were located in the valley, usually at some distance from the forested areas (Fig. 4; light red points). For all parameters, there were outliers (high values in low cluster areas, dark red points; or low values in high cluster areas, dark blue points), pointing to significant localized variation.

Low clusters for *E. coli* tended to be on the edge of forested land, either from waterways flowing out of the forest, or main the branches of the rivers that flow along the edge of forest land in the northern section of the watershed. *E. coli* in forest land was almost all classified as not clustered and not outliers – in other words, an even distribution of moderate values.

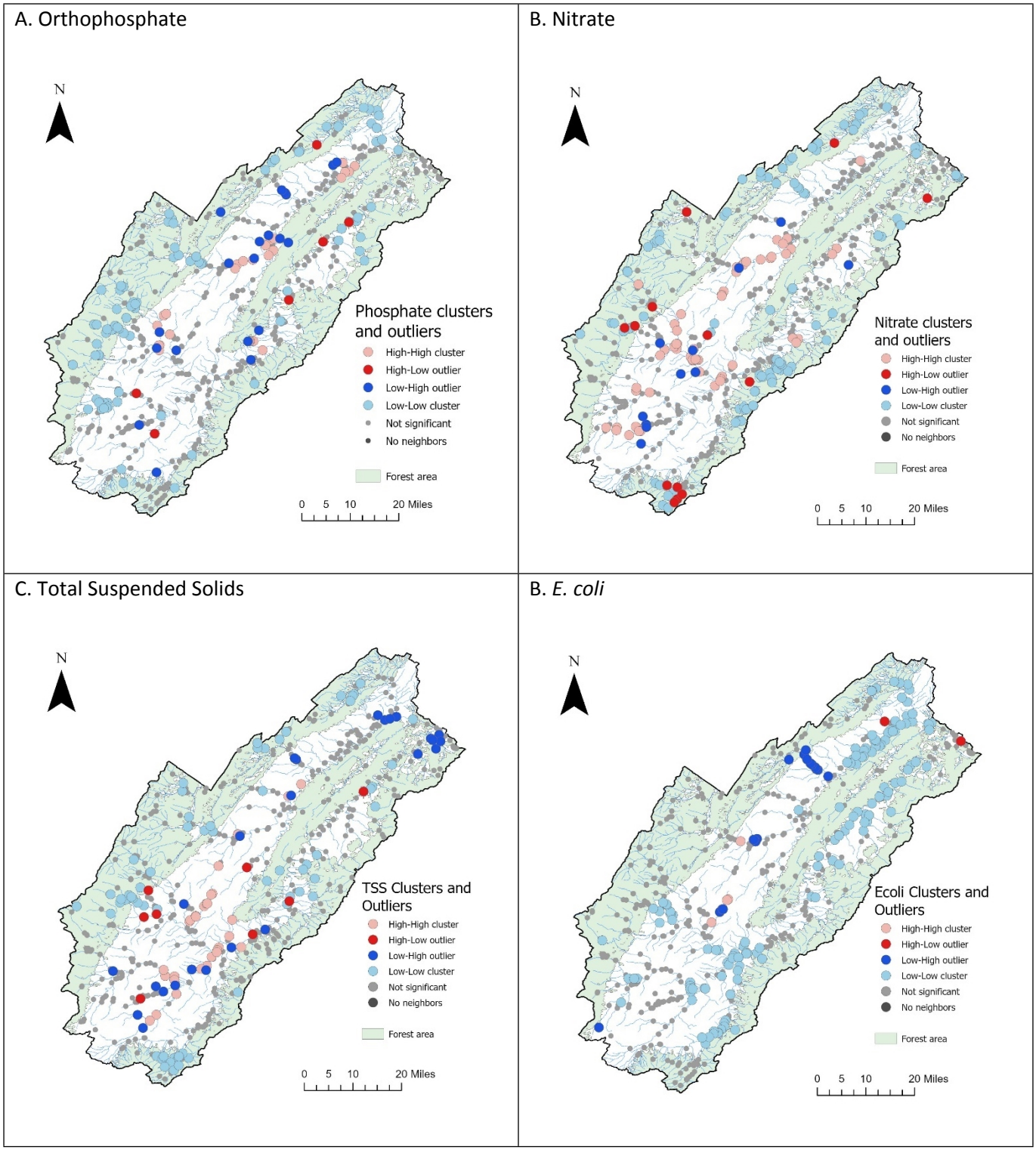


Fig. 4. Cluster and outlier analysis of water quality parameters for North and South Fork Shenandoah River, Virginia. "High-High" and "Low-Low" are points in high and low cluster areas, respectively. "High-Low" and "Low-High" are high values in low cluster areas, and low values in high cluster areas, respectively.

Overall, these results suggested that any effects of forestland on water quality extend into nonforested land, and that there is significant localized variation in all parameters.

Water Quality in Transects of Three Rivers Flowing from Forest to Agricultural Bottomland

We measured water quality along transects for three specific rivers – the Dry River, Briery Branch, and North River – located on the western side of the Shenandoah watershed. All three rivers flow from significant distances through national forest land, and then enter areas primarily used for agriculture (as both pasture and cropland), allowing us to explore more how water quality changes with the transition from forest to agriculture (Fig. 5).

There was an increase in orthophosphate, nitrate, and general and fecal coliforms (Figs. 6a,b,d,e) at 8-10 km downstream of the forest boundary. This pattern was less obvious for TSS (Fig. 6c), which varied more at sample sites, and had some higher concentrations in the forestland. When we examined temperature differentials (how much temperature differed from the average temperature across the transect for that sampling run), we found a different pattern – temperature appeared to more gradually increase rather showing the abrupt shift at 8 river kms (see supplemental data). There was significant variation for all variables, indicating (as did the previous analysis of sites across the Shenandoah watershed; Figs 1,4) that there are significant localized effects that impact water at any particular point and time.

Conductivity also showed a clear change after rivers moved from forest to agricultural lands (Fig. 6f), with the increase happening about 8-10 river kilometers downstream from the forest edge. Since the boundary between silicoclastic and karst geology corresponded closer to the forest edge, it appeared that enough water was flowing out of forestland that ions from the karst geology were not impacting water quality until the 8-10 km mark. This

increase corresponds to when sample site watersheds began to represent a more significant area of agricultural land (although the total area in watersheds was still mostly forestland) (Fig. 6f).

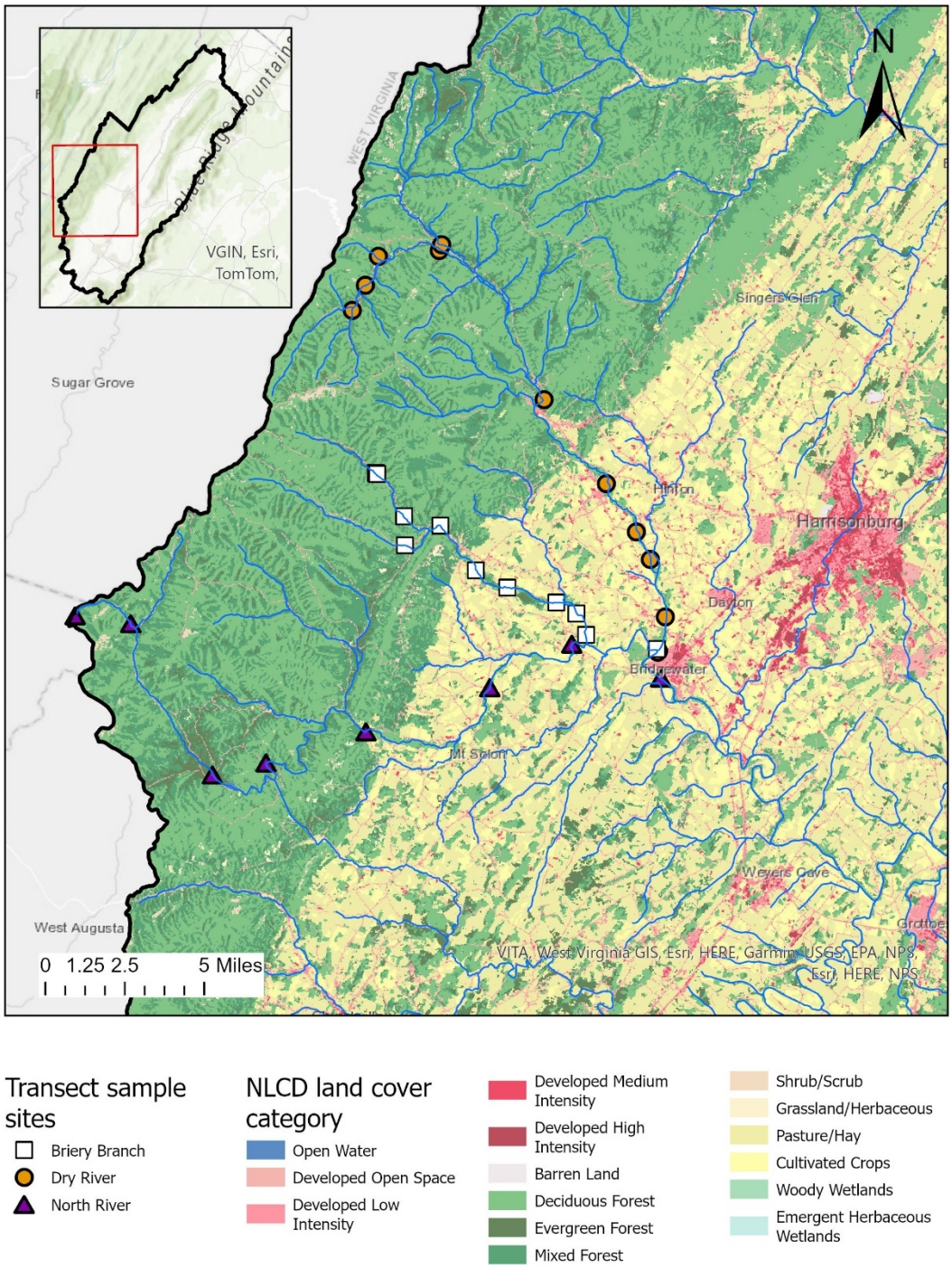
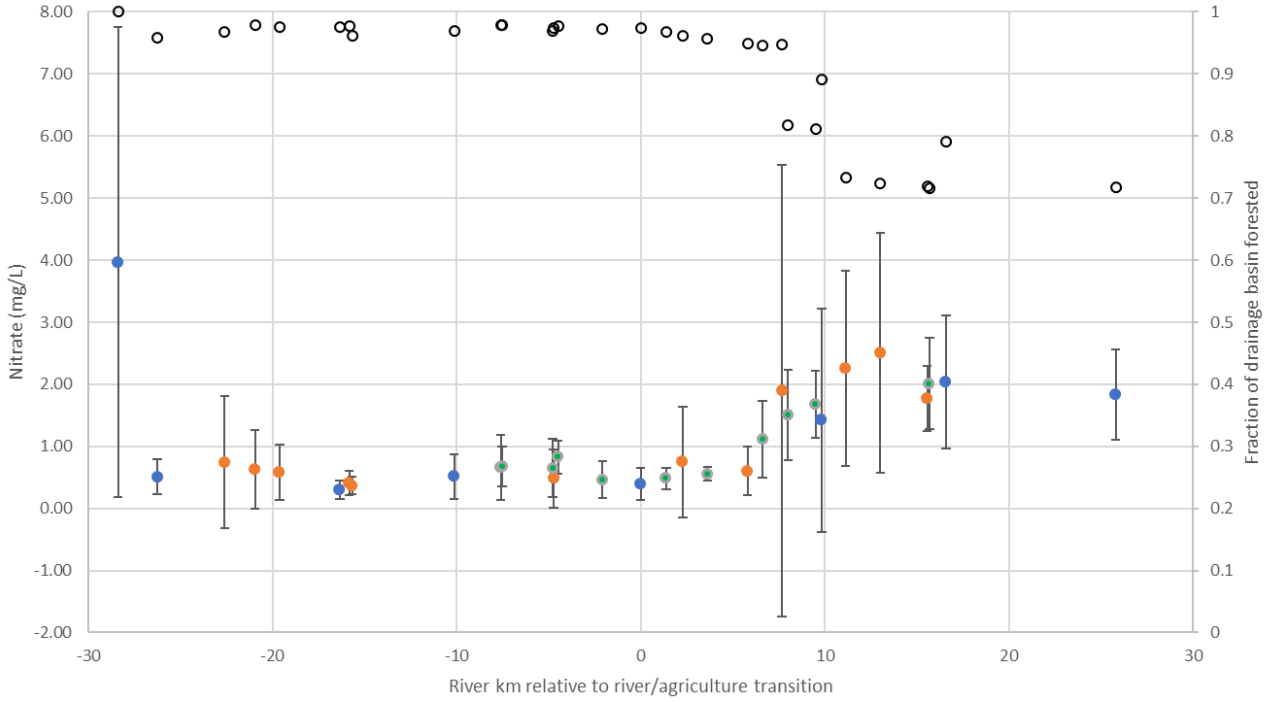
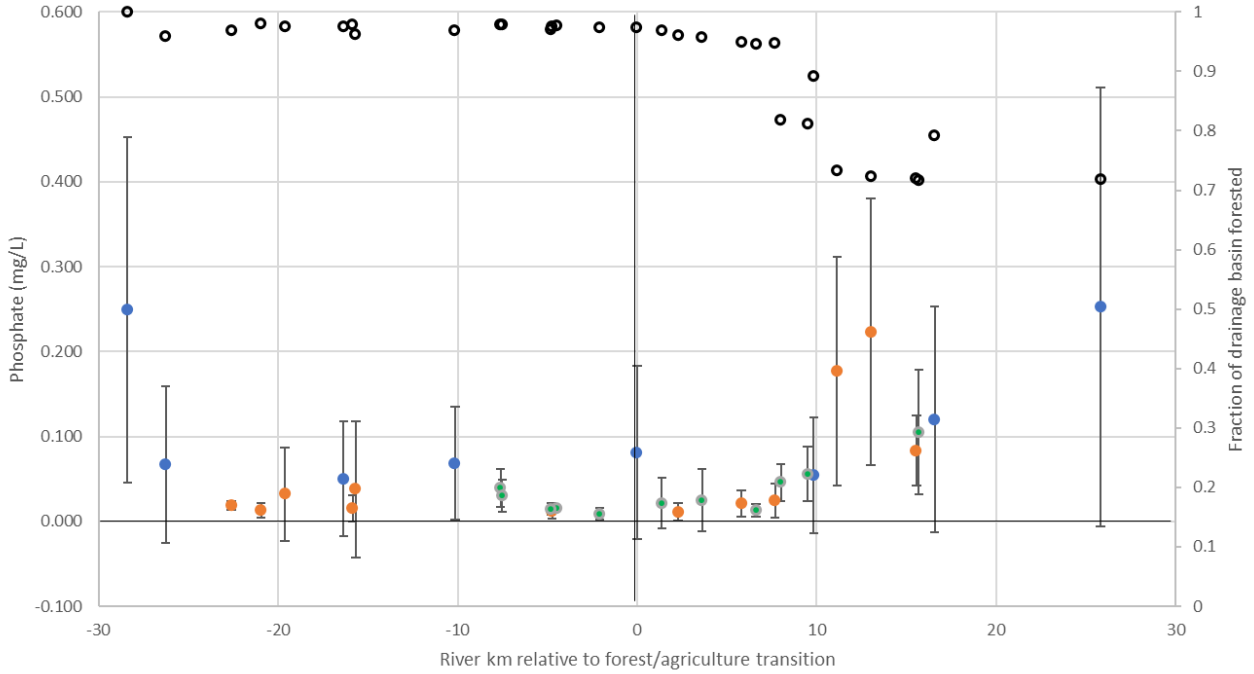


Fig. 5. River transect sites relative to land cover designations.

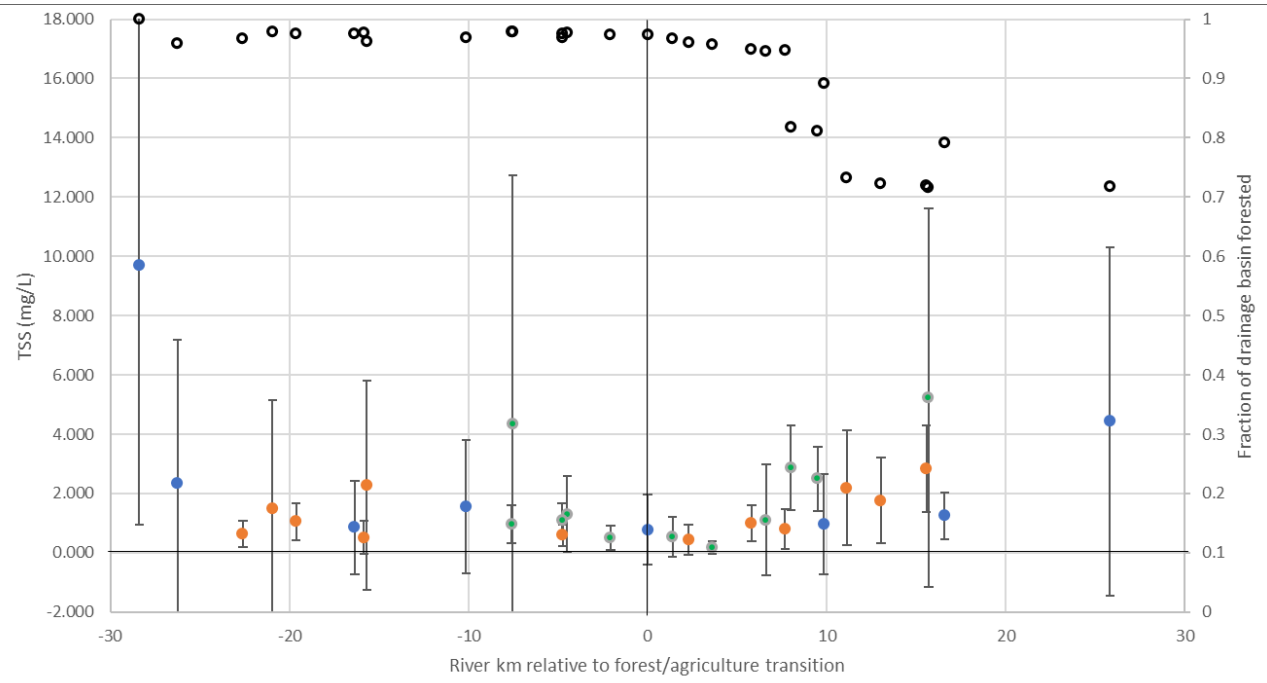
A. Nitrate



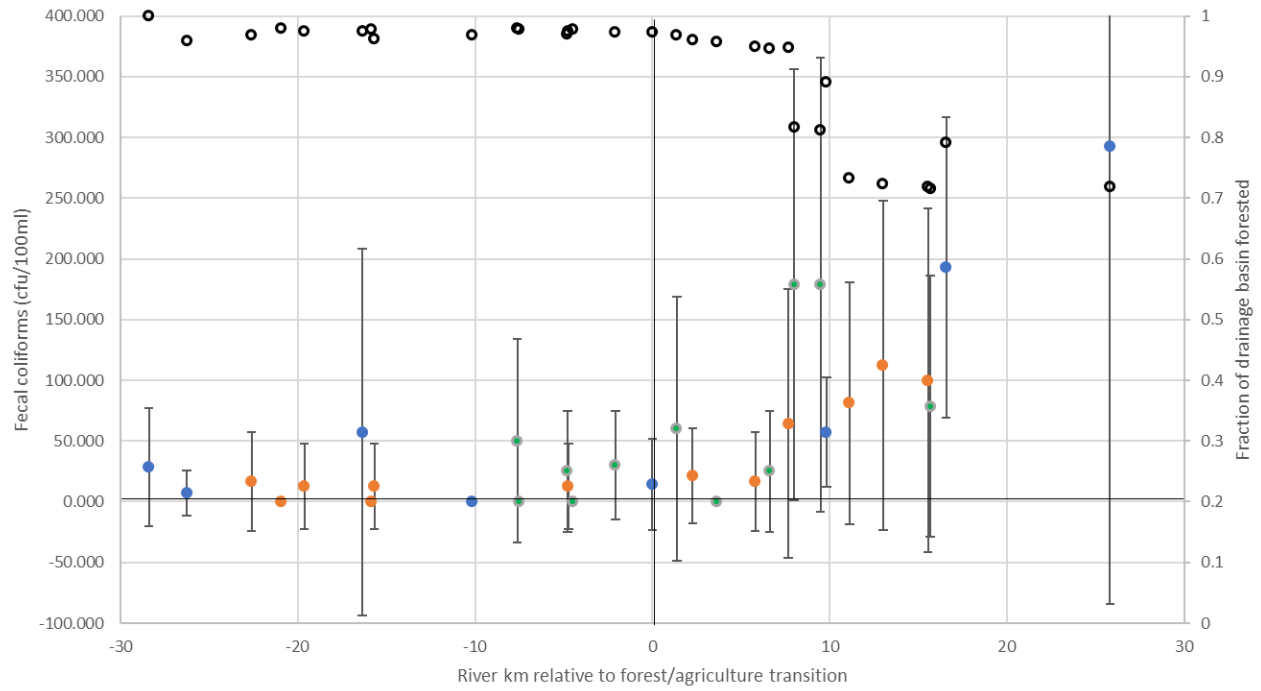
B. Orthophosphate



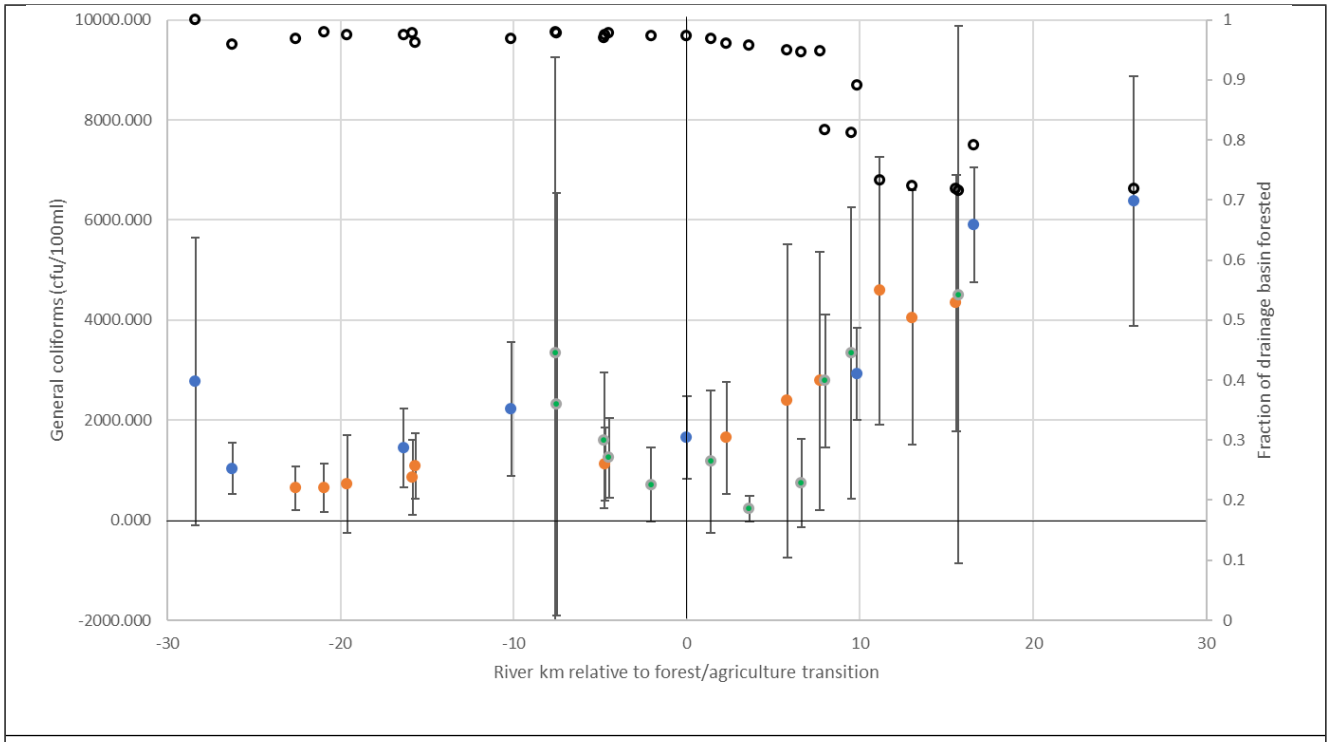
C. Total Suspended Solids



D. Fecal coliform



E. General coliforms



F. Conductivity

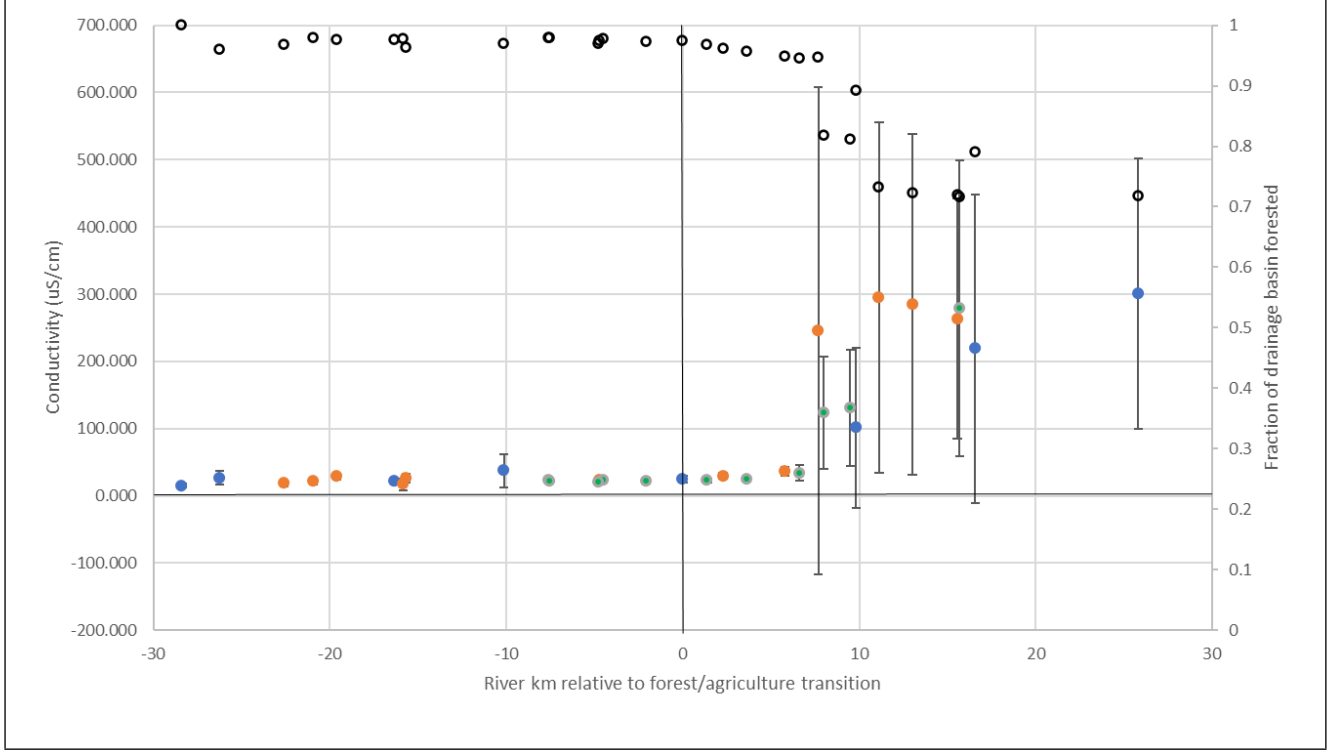


Fig. 6. Water quality parameters along river transects, from forest headwaters to agricultural bottomland. Blue, orange and green points represent parameter mean ± standard deviation values for the North River, Dry River, and Briary Branch, respectively. Points are Open points represent percent forest fraction for each corresponding parameter point.

Since there was an abrupt shift in some parameters at the 8-10 km mark, we pooled samples from the transects into three categories: forestland, edge of valley (up to 8 river kms), and interior of the valley (beyond 8 river kms). Given the known impacts of flow rate on water quality parameters, we furthered binned samples into three flow rate categories (low, medium and high). Two-way ANOVA did not show any effects of flow rate ($p>0.05$ for all parameters). We therefore dropped the flow rate bins, and used one-way ANOVA with post-hoc to investigate the effect of location. For nitrate, orthophosphate, and general and fecal coliforms, forestland and edge of valley generally did not statistically differ from each other, whereas a significant shift occurred once water was in the interior of the valley (>8 km downstream) (Fig. 7). This pattern did not hold for sediments.

We note that the 8 km mark, where percent of overall watershed generally drops to less than 80%, corresponds with the previous analysis of all Shenandoah valley sites indicating the biggest change in parameters likewise occurring with this change from heavily forested to moderately forested watersheds (Fig. 3). The water quality benefits of forested land continue for some distance into agricultural valley land, but this likely depended on there being a large area of forest in the drainage basin (thus the overall watershed was still mostly forest). If there isn't a large area forest (such as some of the watersheds in the valley represented by sampling points in the overall Shenandoah watershed analysis), there are sites of higher water quality, but they are apparently determined by other factors besides forest cover.

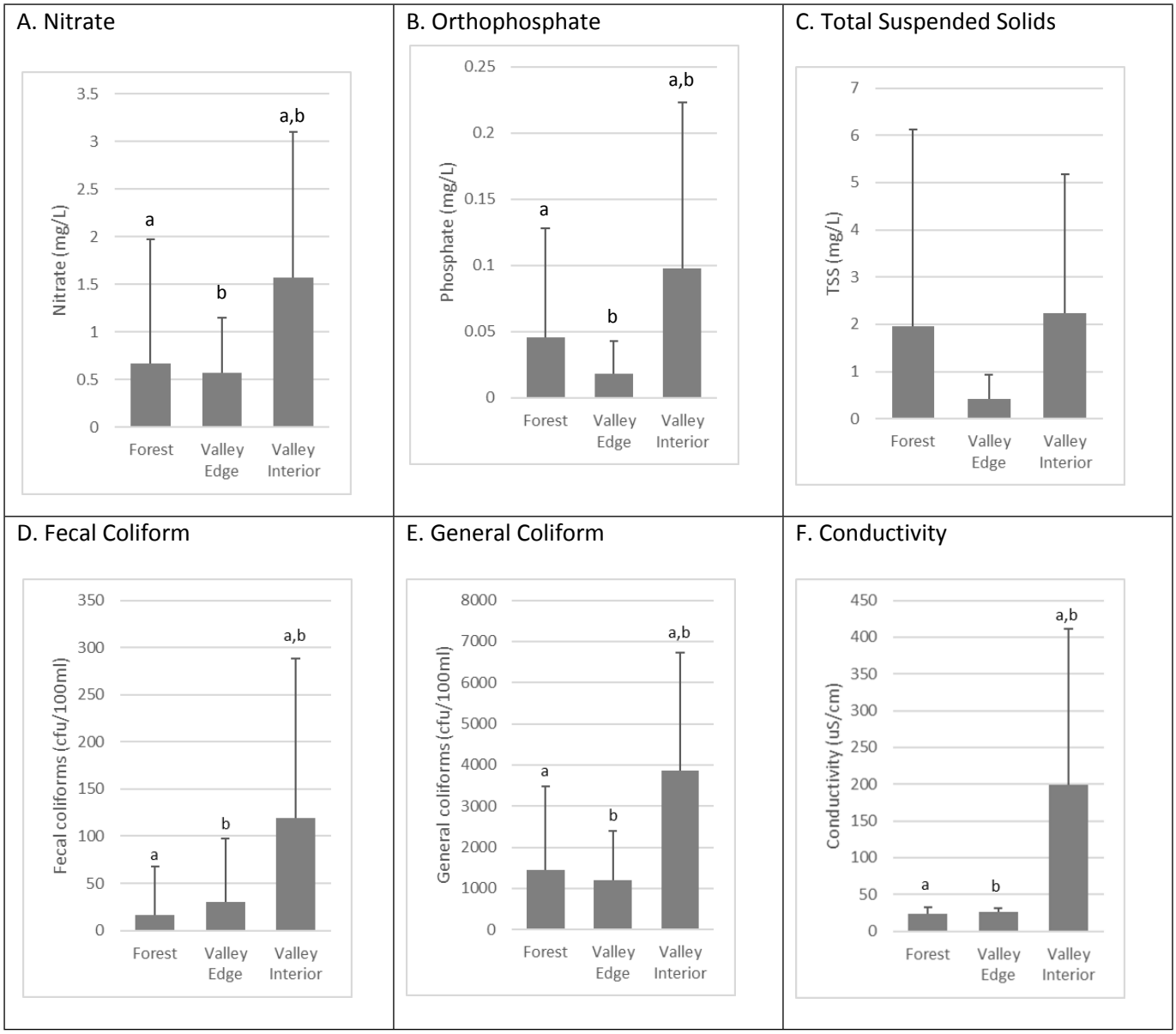


Fig. 7. Binned water quality parameters from transects, characterized by sites in forestland, sites within 8km river miles downstream from the forest/valley transition (valley edge), and sites more than 8km downstream of the transition (valley interior). Values are mean ± standard deviation; significant differences between categories indicated by letters on error bars ($p<0.05$).

Finally, although sediment, nitrate and orthophosphate were lower in mountain regions, there were still levels of these parameters (and coliforms) that would be considered impaired, indicating forested regions are still a source

273 of these pollutants at times. Orthophosphate and nitrate were in the medium to high stress level (0.05 ppm for
274 phosphate, and 1 ppm for total nitrogen) in 17.6% and 13.5% of samples, respectively, at forest sites. Comparable
275 percentages in the interior of valley samples were 45.2% of orthophosphate and 61.0% of nitrate.
276
277 **Discussion**
278
279 The current results confirm what is generally understood about water quality in naturally vegetated (in this case
280 forested) compared to agricultural areas – water quality is consistently better in watersheds which are well
281 covered with natural vegetation such as forests [5], and agricultural lands can have water quality impacts [16]. It is
282 evident, however, that within this general trend is important variability that occurs at localized areas. This
283 variability is important not only for local stream health, but also collectively drives downstream impacts. Further,
284 many waterways transect both forested and agricultural areas, and the interaction between these two is less well
285 characterized. By looking at a large public dataset, and focusing in on several representative watersheds, this
286 study identifies some distinct patterns of water quality in a large watershed with distinct forest and agricultural
287 segments.
288
289 **1. Key Water Quality Parameters in Forested vs Agricultural Land**
290
291 When analyzed across the entire Shenandoah watershed, the clearest pattern of forested vs agricultural land
292 occurred for nutrients (orthophosphate and nitrate) and conductivity. As key drivers of the downstream health of
293 aquatic ecosystems, and in this case specifically as central to the major efforts to restore Chesapeake Bay health,
294 phosphorus and nitrogen are a focus of management efforts in upstream watersheds such as the Shenandoah. In
295 fact, the Shenandoah/Potomac watershed system is one of the most impacted in Virginia for these nutrients [17].
296 In our study, both transect sampling and analysis of nutrients in the water quality portal database indicated a
297 general pattern of higher nutrient levels in agricultural regions that clearly agrees with the well-studied potential

298 of agricultural practices to impact water quality through anthropogenic activities. We did not explore specific
299 drivers of nutrient dynamics in the agricultural area, but note that the relationship between agricultural practices
300 and nutrient levels is complex. For instance, the large scale analysis of Metson et al. [18] found that there was no
301 single explanatory variable that determines riverine phosphorus levels, and other studies have found variable
302 impact of best management practices [19].
303
304 We note here three observations on nutrient trends from our data. First, our results are roughly consistent with
305 those found in other studies that focused on this region, both in average concentrations [17, 20, 21], and in the
306 presence of significant variation on the local level [8]. Second, many of these values were above estimated
307 threshold criteria for algal and benthic macroinvertebrate impairment [22], and are outside of the EPA suggested
308 criteria for ecological health [23]. Mean nutrient values for mountain and valley regions were in the low and
309 medium categories for probability of stress to aquatic organisms, respectively, according to Virginia stressor
310 analysis [17]. Third, values in areas of low impact (forested regions in the mountains) also reached levels of
311 concern at times, consistent with Smith et al [8] who found large variation in natural background of phosphorus
312 and nitrogen, with many stream and river segments in this ecoregion exceeding EPA-proposed criteria.
313
314 Conductivity was also clearly different between mountains and valley, however this reflects the strong correlation
315 with underlying geology rather than land cover differences. Although there are human activities of concern for
316 conductivity (such as road salting), studies in nearby Appalachian mountain areas point towards agricultural
317 having relatively low effect on conductivity [24], and conductivity is the parameter of least concern as a stressor in
318 the region [14,17]. In our study, conductivity then effectively acts as a tracer of water source, with low
319 conductivity indicating water originating from silicoclastic (mountain) areas, and high conductivity indicating an
320 origin or longer residence time in limestone or dolostone (valley) areas. In all cases, the mean conductivity was
321 below 300 μS/cm, generally recognized as the range of no or low probability for stress to aquatic life [17,23,25].
322 However, some individual samples fell in the high stressor categories (>500 μS/cm), and could be a driver of which

organisms make up aquatic communities in these areas. These high conductivity values occurred during low-flow periods in valley areas only, usually during mid-summer.

Two other measured parameters – sediment (as total suspended solids) and bacteria – are of major concern nationwide as drivers of water quality, and have clear associations with agricultural activity. For instance, sediment is the largest cause of invertebrate-impaired waters nationwide [25], and bacteria is the most common reason for streams receiving an impairment designation in Virginia [17]. Although these parameters also showed some patterns of higher levels in agricultural lands, the pattern was less consistent than for nutrients and conductivity. Fecal and general coliforms were higher in agricultural segments of the watershed in our transect measurements, but *E. coli* across the watershed (from the water quality portal database) did not correlate as clearly with forest vs agricultural areas. Conversely, sediments (TSS) were highly variable in the transect data and did not correlate with land cover, but TSS across the watershed was higher in drainage basins with less forest cover. Overall, this points to some higher sediment and bacteria values in forested land (see discussion below), and/or some waterways in primarily agricultural regions that are less impacted by sediments and bacteria. Thus, although forest cover and agricultural activities are critical for water quality, other factors are driving significant local variation in these parameters.

2. Water Quality Impacts in Forested Land

Although water quality was better in forested land, our data indicated that nitrogen, orthophosphate, TSS and indicator bacteria all exceeded levels of concern at particular times and locations. These results are consistent with Bricker et al [7] who likewise found similar nitrate levels in a nearby mountain area, and with Price and Leigh [26] who found TSS levels in forested areas of the southern Appalachians as high or higher than the levels throughout our study area. Smith et al's [8] nationwide study also found some sites and times with levels of concern in relatively unimpacted areas of our ecoregion.

We did not specifically investigate the source of these pollutant levels in forestlands, although our observations pointed towards three general categories of possible human-related contributors: 1) existing stable structures, 2) various types of disturbance, and 3) legacy effects. The contribution of existing stable structures is indicated by consistently high levels of measured parameters (except conductivity) in retention structures such as a small pond located in a clearing at the headwater of the North River. This pond was in a small sedimented bowl on the ridgeline, and is characteristic of numerous small retention basins purposefully maintained in the forest (usually for supporting game animals). Larger reservoirs are also present in all three watersheds for the purpose of flood control and municipal water supplies. Recent evidence suggests that ponds can serve as nutrient sinks in agricultural areas [27]; to our knowledge, there are no similar studies on the water quality role of ponds or lakes in forested areas.

Disturbance is recognized as critical for mobilization of nutrients and sediments. For instance, forests in this area are considered to show low nitrogen export unless disturbed [28]. While heavily forested, there are a variety of processes which impact forests in a way that could mobilize nutrients and sediments. These regions have a network of roads which are used for recreation, forest service activities (*e.g.* prescribed burns or firefighting), and resource extraction (*e.g*. timbering). These roads often parallel waterways, are usually unpaved, and in some areas have low-water crossings rather than bridges. Roads are a small fraction of the total area, but potentially a significant contributor to water quality, and deserve future attention to understand their role forested regions. Timber harvesting and prescribed burns are regular localized activities that can have significant impacts on streams. In addition, our observation is that some streams in the forest areas have eroded banks, either part of natural processes or as a legacy of alterations previously made, which would be a likely source of nutrients and sediment. Bare soil from both management activities or streambank erosion are known drivers of water quality and quantity in forested areas [3,29], and thus are likely contributors to the occasionally high levels of nutrients and sediments in our study.

Candidate drivers of some water quality parameters include not only present day activities, but also legacy activities. In addition to management activities occurring over the course of many decades, this area was heavily logged in the late 1800's, and then used for livestock grazing before forests were restored in the early 1900's. The role of legacy nutrients as drivers of watershed nutrients has received increased attention as a key driver of water qaulity (*e.g.* [30]) as studies demonstrate their importance in a variety of watersheds [31-33]. Legacy nutrients probably have variable residence times depending on local conditions, but are likely on the order of many decades [33,34]. Mobilization from soil is particularly important during storm events following freeze-thaw cycles [32], such as the spring rains often experienced in this central Appalachian region. Although these observations all point toward a potential role of legacy nutrients in forested land, as of the present there is no direct data to guide an understanding of how much legacy nutrients currently contribute to elevated nutrients in this area.

Finally, other "natural" processes can impact water quality in less disturbed watersheds. Atmospheric deposition is shown to be important for both nitrogen [8] and phosphorus [35] in relatively undisturbed landscapes. Insect outbreaks can dramatically increase nitrate from vegetation inputs, as shown in studies from Shenandoah National Park, which borders the eastern side of this watershed [28]. Wildlife have been shown to be significant sources of fecal coliforms in watershed analyses [36], and are likely a significant source of this parameter in our forested areas.

In summary, although a national survey of landscapes found forests with the highest water quality among all land cover types [5], a narrative that waterways in forested landscapes as invariably "clean" masks some interesting localized events, and should be distinguished from the general observation that waterways are cleaner on average. In fact, the recent study identifying forests as having the best water quality also emphasized considerable variation in sediment and nutrient levels [5]. Forestlands in this region are a complex amalgam of natural and human processes – as would be the case for most forests in the United States (and indeed globally).

Further studies should extend the general forest vs nonforest paradigm to focus on what categories of activities impact water quality more or less.

3. Patterns of Water Quality Transition as Rivers flow from Forested to Agricultural Lands

Although this area is notable for a sharp forest/agriculture transition, transect samples indicated water which flowed out of forested land did not show an immediate change in water quality parameters. Rather, there was a clear lag period as the water quality parameters remained relatively unchanged until about 8 river km downstream of the forest boundary (Figs 6,7). Although residence time (in this case, time in an agricultural area) is a known driver of water quality [16], residence alone would more likely produce a gradual degradation of water quality. Rather, the decrease in water quality was fairly abrupt, most notably for conductivity, which would track less with agricultural activities directly and more with underlying geology. When percent forest area of watersheds for each sampling point were calculated, forested area for the corresponding drainage basins also showed a sharp decrease at the 8 km mark to about 70-80%. WQP database results were similar, with parameters generally showing the largest drop in quality when forested areas dropped below 80% (Fig 3).

This is the point at which tributaries draining mostly agricultural areas become a larger part of the total drainage basin. In turn, this suggests that cumulative inputs from agriculturally derived tributaries are not substantial until the river is 8km into the agricultural area, and that the pattern may be driven by inputs from the broader tributary watershed rather than gradual inputs from agricultural activities immediately adjacent to the river. Thus, it seems that the first 8-10km remains unimpacted because the drainage area from non-forested regions is low. Once tributaries join, the water quality begins to drop. Furthermore, while the benefits of the forested region stretched well into the agricultural region, it took only 20-30% of a watershed to be nonforested in order for water quality to be impacted. Price and Leigh [26] likewise found that even moderate changes in the degree of forestation can have impacts on water quality variables. In aggregate, these data imply that 1) the value of forest in preserving

423 water quality is substantial, and extends downstream out of the forested area, but 2) that the ecological service of
424 maintaining water quality drops off once 20-30% of a total watershed was agricultural.
425
426 Although we did not focus on temperature, it is a parameter of critical concern in the area, as long-term
427 measurements in Shenandoah National Park show an increase that is expected in the presence of climate change
428 [38]. Although we measured water temperature, interpretation of single temperature readings taken at different
429 times of the day and season limit conclusions from the transects. However, a rough estimate of transect trends
430 can be estimated by standardizing individual readings as the deviation from the average of a sampling run along a
431 transect. Temperature showed a more gradual increase across the length of the transect. Temperature steadily
432 rose as water moves from headwaters to the end of the sampling transect (supplemental data), contrasting with
433 the abrupt transitions seen in other parameters. The slow increase would be expected for temperature which is
434 driven by complex energy exchanges that rely on factors (such as time with exposure to sun) beyond land cover
435 [39].
436
437 **Conclusions**
438
439 The current study confirms the crucial role of substantial forested areas regions to the water quality of
440 watersheds. While the importance of headwater regions to downstream water quality is recognized [10,40], this
441 study combined multiple datasets to explore how water quality changes with the transition of waterways from
442 forested areas, which generally protect water quality, to agricultural areas, which generally degrade water quality.
443 Key insights which add detail to the general understanding of these landscapes are: 1) substantial variability in
444 water quality in both agricultural and forest areas points towards important local factors governing pollutants, 2)
445 while forests clearly had higher water quality than agricultural areas, a narrative of pristine water in forested
446 areas does not reflect the reality of parameters reaching levels of concern at certain locations and times, and 3)
447 forested regions provide benefits substantial distances downstream of transitions to agricultural regions, but

water quality rapidly decreased when cumulative forest cover of a corresponding watershed dropped by 20 to 30%. These results contribute to a general understanding of what drives water quality in mosaiced landscapes such as the Chesapeake Bay watershed, which has shown complex and variable responses to management efforts [41].

While the current study helps draw a more complete picture of water quality across a diverse landscape, we did not investigate several other factors that are critical to understanding when and how degradation of water quality occurs – notably the contribution of storm events and seasonal cycles. Flow rates, for instance, are a known and crucial determinant of loading [10, 42, 43], and nutrients can follow seasonal trends [7]. Preisendanz et al [44] argue that "hot moments" are as important as "hot spots" – and that insufficient attention has been directed towards these temporal events as opposed to the spatial pattern. Whether the patterns seen here represent those found during peak storm flow, and across different seasons, is a further avenue of fruitful study, particularly in light of future climate changes [45]. Future increases in precipitation are highly relevant given that downstream water quality in the Chesapeake Bay is tied closely to flow rates [10], and recent estimates are that climate change threatens to increase nutrient and sediment loads in the Chesapeake Bay watershed even more than changes in agricultural intensity, population or development [46].

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Supplemental Data, Figure S1

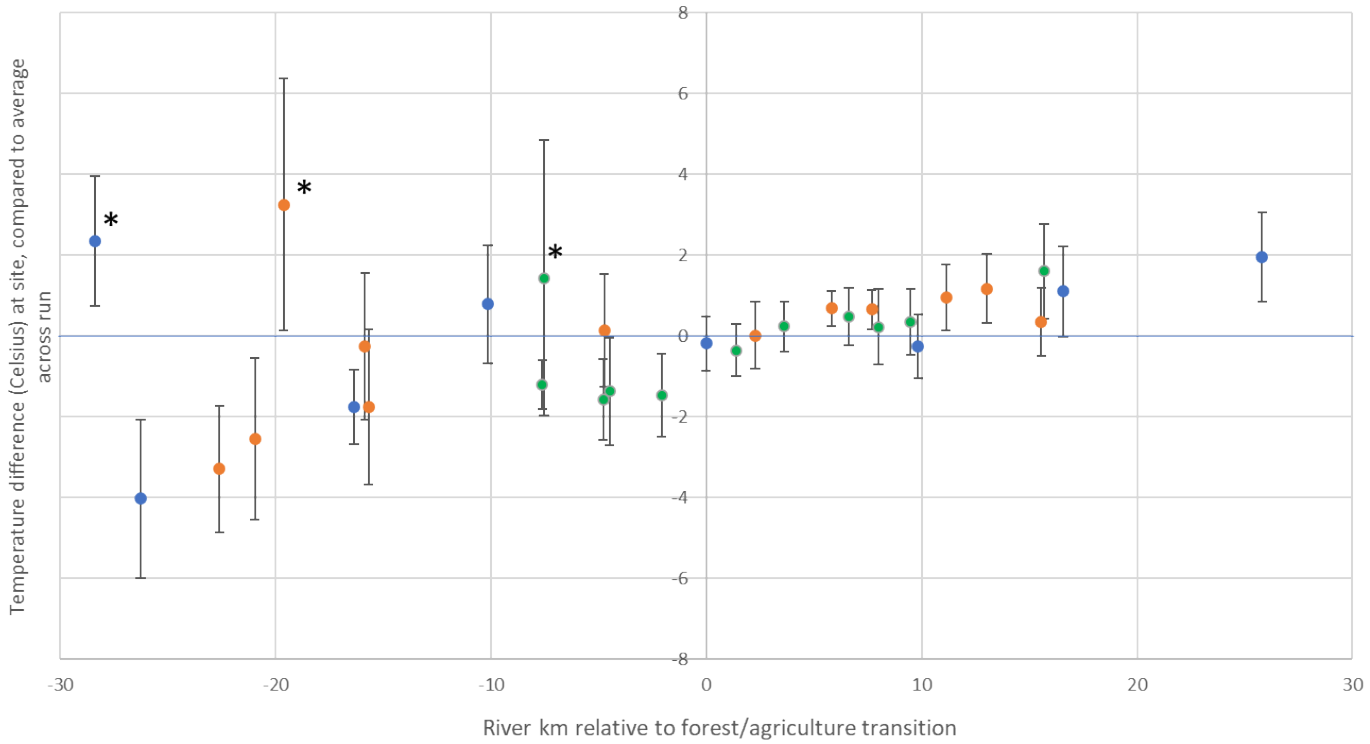

Fig. S1. Temperature differential gradient parameters along river transects, from forest headwaters to agricultural bottomland. Temperature differential for each sample calculated as site temperature minus average temperature across its run (samples for the one river and one date). Blue, orange and green points represent parameter mean ± standard deviation values for the North River, Dry River, and Briary Branch, respectively. Asterisks with higher temperature differentials are pond or reservoirs.